Plant Breeding Reviews

VOLUME 24
PART 2

LONG-TERM SELECTION: CROPS, ANIMALS, AND BACTERIA

PLANT BREEDING REVIEWS
Volume 24, Part 2

Plant Breeding Reviews is sponsored by:

American Society for Horticultural Science
Crop Science Society of America
Society of American Foresters
National Council of Commercial Plant Breeders

PLANT BREEDING REVIEWS

Volume 24

Part 2: Long-term Selection:
Crops, Animals, and Bacteria

edited by
Jules Janick
Purdue University

WILEY
John Wiley & Sons, Inc.

This book is printed on acid-free paper. ♾

Published by John Wiley & Sons, Inc., Hoboken, New Jersey
Published simultaneously in Canada

Library of Congress Cataloging-in-Publication Data:

ISBN: 0-471-46892-4
ISSN: 0730-2207

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

Contents

Contributors

A. E. Bell, Department of Animal Sciences, Purdue University, W. Lafayette, Indiana 47906.

Lutz Bünger, Animal Biology Division, Scottish Agricultural College, West Mains Road, Edinburgh, EH9 3JG, UK, l.bunger@ed.sac.ac.uk.

M. Cooper, Pioneer Hi-Bred International, A DuPont Company, Johnston, Iowa 50131.

D. N. Duvick, Department of Agronomy, Iowa State University, Ames, Iowa 50011-1010, *dnd307@aol.com*

Paul Gepts, Department of Agronomy and Range Science, University of California, 1 Shields Avenue, Davis, California 95616-8515, *plgepts@ucdavis.edu*

Irwin L. Goldman, Department of Horticulture, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706.

Arnel R. Hallauer, Department of Agronomy, Iowa State University, Ames, Iowa 50011-1010, *hallauer@iastate.edu*

William G. Hill, Institute of Cell, Animal and Population Biology, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh, EH9 3JT, UK, *w.g.hill@ed.ac.uk*

Michael Lee, Department of Agronomy, Iowa State University, Ames, Iowa 50011-1010.

Richard E. Lenski, Center for Microbial Ecology, Michigan State University, East Lansing, Michigan 48824, *lenski@msu.edu*

D. Miles, Department of Animal Sciences, Purdue University, W. Lafayette, Indiana 47906.

J. W. Miles, Centro Internacional de Agricultura Tropical, Apartado Aéreo 6713, Cali, Colombia, *j.miles@cgiar.org*

Kendall R. Lamkey, Department of Agronomy, Iowa State University, Ames, Iowa 50011-1010.

W. M. Muir, Department of Animal Sciences, Purdue University, W. Lafayette, Indiana 47906, *bmuir@purdue.edu*

S. Pandey, Centro Internacional de Mejoramiento de Maíz y Trigo, Apartado Postal 6-641, 06600 Mexico, D.F., Mexico.

Andrew J. Ross, Department of Agronomy, Iowa State University, Ames, Iowa 50011-1010.

Margaret A. Schaber, Department of Horticulture, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706.

J. S. C. Smith, Pioneer Hi-Bred International, A DuPont Company, Johnston, Iowa, 50131.

Allison E. Tiefenthaler, Department of Horticulture, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706.

William F. Tracy, Department of Agronomy, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706, *wftracy@facstaff.wisc.edu*

Preface

Selection is one of the most powerful tools available to biology. Selection is used in the plant and animal sciences to develop improved crop cultivars and livestock breeds. Selection is used in laboratory species to test many of the assumptions of the underlying quantitative genetic models and to test the limits of selection itself. The first and second international conferences on quantitative genetics held in Ames, Iowa, in 1976 and in Raleigh, North Carolina, in 1987 were essentially conferences about the theoretical and empirical aspects of selection. The power of selection is best represented by the selection responses that have been observed in two important agricultural species. U.S. maize yields increased from a pre-1930 average of 1.6 tonnes/ha (26.1 bushels/acre) to an average of 8.6 t/ha (134.7 bushels/acre) for the five-year period from 1998 to 2002, a five-fold increase over 70 years (http://www.usda.gov/nass/). Of course, not all of the increase is due to selection, but studies have consistently shown that genetics can account for 50% of the increase. Milk yield in Holsteins had increased from 5,870 kg in 1957 to 11,338 kg in 2001, representing a doubling in milk yields over 44 years (http://aipl.arsusda.gov/dynamic/trend/current/trndx.html). There is evidence that the genetic trend continues to increase with time in Holsteins.

There have been many novel uses of selection, particularly for studying gene action. Two of my favorite papers in this area are by Sprague and Miller (1950) and P. D. Keightley et al. (1996). Sprague and Miller designed a selection experiment to test the importance of dominance gene action relative to overdominance gene action for grain yield in maize. The importance of dominance and overdominance in maize had been an ongoing debate that influenced the breeding methodology used to develop improved populations and hybrids in maize. Keightley et al. took advantage of the power of divergent selection and replicated selection to generate differences in allele frequencies in high and low strains of mice to map QTLs for body size.

The impetus for the conference was to celebrate the importance of the long-term selection experiment for protein and oil in maize at the

University of Illinois, hence the title of the conference "Long-term Selection: A Celebration of 100 Generations of Selection for Oil and Protein in Maize." The Illinois experiment is certainly the longest-running selection experiment in an agriculturally important plant and has contributed to discussions about the importance of new mutations in selection response, the number of genes involved in selection response, and the theoretical and biological limits to selection response. One hundred generations of selection in an annual plant is indeed an impressive accomplishment.

The papers of this conference have been divided into two parts in *Plant Breeding Reviews*, *Volume 24*. The first part, subtitled "Long-term Selection: Maize," has three sections: Perspective and Background, The Illinois Long-term Selection Experiment, and Biological and Theoretical Models, and consists of 14 chapters, including the dedicatory chapter honoring Dr. John W. Dudley, who, along with Dr. Robert J. Lambert, has been in charge of conducting the Illinois Long-term Selection Experiment since 1966. Dr. Dudley has led the quantitative genetic analysis of the response to selection in this program. The second part, subtitled "Long-term Selection: Crops, Animals, and Bacteria," consists of 8 chapters divided into three sections as indicated in the title. The 22 papers in these two volumes clearly illustrate the importance of selection, the current status of our knowledge about mechanisms of selection, and the need for continued research on selection.

The conference steering committee was chaired by Dr. John W. Dudley. The program committee, which was chaired by Dr. James G. Coors, included Dr. Margaret Dentine, Dr. Rex Bernardo, Dr. Irwin L. Goldman, Dr. William G. Hill, Dr. Kendall R. Lamkey, and Dr. William M. Muir, was responsible for the content of the program. Dr. Rex Bernardo obtained sponsorship for the conference and Dr. Kendall R. Lamkey was in charge of getting the proceedings published. The conference would not have happened without the organizational and planning abilities of Ms. Elaine Wolff of the University of Illinois' Office of Continuing Education. I would also like to thank Drs. Coors and Dentine for their assistance in editing and getting reviews of the papers in this volume.

The steering committee would like to thank the conference sponsors for their support: NCR-167 Regional Corn Breeding Committee, University of Illinois Maize Breeding and Genetics Laboratory, University of Illinois at Urbana-Champaign Department of Crop Sciences, Illinois Council on Food and Agricultural Research (C-FAR), Syngenta Seeds,

Inc., Golden Harvest Seeds, Inc., Group Limagrain, Pioneer, A DuPont Company—DuPont Specialty Grains, and Illinois Foundation Seeds.

Kendall R. Lamkey
Department of Agronomy
Iowa State University

LITERATURE CITED

Keightley, P. D., T. Hardge, L. May, and G. Bulfield. 1996. A genetic map of quantitative trait loci for body weight in the mouse. Genetics 142:227–235.

Sprague, G. F., and P. A. Miller. 1950. A suggestion for evaluating current concepts of the genetic mechanism of heterosis of corn. Agron. J. 42:161–162.

1

Crop Domestication as a Long-term Selection Experiment

*Paul Gepts**
Department of Agronomy and Range Science
University of California
1 Shields Avenue
Davis, California 95616-8515

I. INTRODUCTION

In *The Origin of Species by Means of Natural Selection*, Charles Darwin (1859) devoted his first chapter to "Variation under Domestication." He expanded on this topic in *Variation of Plant and Animals under Domestication* (1868) and he became involved in pigeon breeding. The fact that

*Research on crop evolution in my program has been funded by several sources, notably the USDA (NRI, OICD, NPGS), AID (PSTC, Bean/Cowpea CRSP), and the McKnight Foundation. I thank two reviewers for helpful suggestions.

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-46892-4

one of the founders of evolutionary theory would pay such attention to domestication and the selection process associated with it is testimony to the exemplary value of crops and animal breeds in the study of natural selection in general. In his writings, Darwin makes several observations and raises several issues that are still relevant today, some of which have yet to be resolved. One of the benefits of considering selection under domestication was that he demonstrated that selection had heritable effects, even in the absence of any information about the histological, biochemical, and genetic foundations of heredity.

One of the major observations made by Darwin is that morphological modifications selected during domestication have been of such magnitudes that many crop plants usually cannot survive in the wild anymore without human assistance. In addition, he pointed out that selection by breeders could lead to a wide array of variation in domesticated plants and animals when compared with their wild progenitors. He also suggested that selection under human cultivation happened unconsciously or inadvertently, that is, without deliberate human action. He argued that crops are so different morphologically from their wild progenitors that humans could not have possibly identified target traits so different from those existing in the wild progenitor.

He also pondered the question as to the origin of crop plants. He was particularly interested in the number and location of domestications but stated that it would actually be very difficult to identify the centers of origin of crops. Since his time, a substantial body of information has been gathered not only on the domestication origin of crops but also on their evolution subsequent to domestication, in part through the application of a broad palette of increasingly sophisticated techniques. In addition, there have been several major contributors to the field of crop evolution studies, including A. de Candolle, who broadly laid out the types of data that can be used to trace the origin of a crop; N. Vavilov, who systematically identified the centers of domestication of crops; and J. Harlan, who also contributed to the concept of domestication centers and built close linkages between archaeology and plant science. This review will address a number of issues associated with the study of crop evolution from a long-term selection perspective.

II. THE DOMESTICATION PROCESS

Domestication is the outcome of a selection process that leads to increased adaptation of plant and animals to cultivation or rearing and utilization by humans. It is still being debated whether this selection

took place consciously by humans or if it was an inadvertent phenomenon as a by-product of human plant cultivation or animal rearing (Harlan et al. 1973; Zohary et al. 1998). Proponents of unconscious selection argue that the first farmers could not have possibly foreseen or set out to specifically select for the marked phenotypic changes that eventually arose during domestication. These changes have been so pronounced that plant taxonomists have often classified wild progenitors and domesticated descendants in different species or genera. Given these marked changes, advocates of inadvertent selection argue that early farmers could not have set out to specifically select for these changes. One could argue, however, that one need not know the end result to select intermediate steps. In a discussion of animal domestication, Zohary et al. (1998) proposed that the shift in adaptation between wild and domesticated environments was so large that cultivation or rearing would *automatically* (his italics) initiate selection for many new traits that characterize goats and sheep. He also suggested that certain traits such as the culling of young males might have been under conscious selection. This altered sex ratio in archaeological remains may be one of the earliest signs of domestication among animals.

Proponents of conscious selection argue that the first farmers were actually quite knowledgeable about their environment. They were well aware of the life cycle and some of the biological characteristics of plants and animals surrounding them well before the advent of agriculture. For example, the Cro-Magnon civilization depicted in vivid detail and color the animals that surrounded it, as can be seen in several caves in southern Europe (see the Cave of Lascaux, France: *http://www.culture.fr/culture/arcnat/lascaux/en/* and the Cave of Altamira, Spain: *http://www.mcu.es/nmuseos/altamira/colec1_1.html*). The transition from hunting-gathering to agriculture (the Neolithic revolution) is thought to have been preceded by the so-called broad-spectrum revolution (Flannery 1969; Stiner 2001). This revolution marked a switch in subsistence patterns during the Paleolithic. From large game, hunter-gatherers turned to a more diverse diet consisting of smaller animals (Poinar et al. 2001) as well as plants, particularly grains. Evidence for this transition comes from an increase in the number of species in the diet and a greater proportional evenness among prey items, an abundance of milling tools and storage facilities, and a higher frequency of plant parts (Poinar et al. 2001; Stiner 2001). In addition to increasing the familiarity of foragers with a broader range of plants and animals, the broad-spectrum revolution also led them to develop tools and techniques that would be useful in the subsequent agricultural phase. Among these techniques are methods to detoxify plant foods (Johns and Kubo 1988). Although not

all the methods listed by these authors may have been known to hunter-gatherers, some of them were probably known, as recent studies of contemporary pre-agricultural societies indicate. Thus, the biological and technological knowledge of these societies should not be underestimated. Hillman and Davies (1990) have suggested that a combination of unconscious and conscious selection may have operated in succession, with the former operating in the early stages when the frequency of mutation(s) was too low to be noticeable.

During domestication, mutations affecting specific traits of the domestication syndrome are selected until they achieve near or full fixation. Are domesticated plants more mutable and has this mutability affected their domestication? Unfortunately, there are few studies in plants that have investigated mutation rates and the magnitudes (positive or negative) of mutations (Drake et al. 1998). Particularly, there are no studies comparing the mutation process between crops and related species. As pointed out by Hill and Mbaga (1998), mutations were not thought to play a significant role in breeding programs because of the short time span and the limited response observed in some experiments. Both empirical and theoretical analyses, however, have shown that mutations can cause a significant and continued response even in small populations (see references in Hill and Mbaga 1998). One of the best examples of continued response is the long-term selection experiment for protein and oil in maize (*Zea mays*) at the University of Illinois. It has been suggested that mutations are involved in the long-term response of the Illinois experiment but the extent is unknown (Rasmusson and Phillips 1997).

In the absence of specific values for mutation parameters, Hillman and Davies (1999) assumed a mutation rate of $\mu = 10^{-6}$. At a sowing rate of 200 spikelets/m^2, observed in traditional cropping systems, such a mutation would appear in a single growing season in a 1 to 2 ha area. Assuming grain needs to provide 25% of total calorie requirements, the calorie needs of humans, and incomplete absorption and digestion, Hillman and Davies (1999) estimated that areas sown for a family of five ranged between approximately 0.5 ha and 2.8 ha. (This calculation of course assumes also that early farmers derived their foods exclusively from cultivation, which is unlikely.) These are values similar to those postulated for the occurrence of a mutation in a single growing season. Mutation rates may therefore not have been a limiting factor in the progress from selection, assuming of course that these theoretical assumptions can be confirmed with empirical data.

A comparison of the morphological and physiological differences among domesticated plants has shown that a similar set of traits has been

selected during domestication. This set has been called "the domestication syndrome" (Hammer 1984; Harlan 1992). Traits included in this syndrome (see below for a more detailed discussion) include those increasing adaptation to cultivation and desirability of human consumption and use. Harlan (1992) lists some 400 cultivated plants; there are certainly more but they may be cultivated only intermittently or on a very small scale. Among these cultivated plants, the degree of domestication varies widely. Highly domesticated plants, typified by plants such as maize, rice, common bean, and peanut, have a broad range of domestication traits and express these traits at a high level. Other crops, encompassing a wide range of domestication phenotypes, can be considered to be only partially domesticated,. On the one hand, a crop like canola (*Brassica rapa*, *B. napus*) is generally considered to be a highly domesticated crop. Yet, it still suffers annual seed losses of 20–50% due to silique shattering (Child et al. 1998). It can therefore be considered to be incompletely domesticated with respect to seed dispersal. Crops such as soybean and sesame also suffer from excessive shattering at maturity. On the other hand, the African oil palm has only been subjected to limited changes during domestication. Without having been planted, its distribution has increased indirectly through agricultural practices like slash and burn. The only major genetic change has been selection for a gene affecting kernel development inside fruits. Trees with thick-shelled kernels (called *durra* types) are generally tapped for palm wine and not for oil, whereas trees with thin-shelled kernels (*tenera*) or kernels without shell (*pisifera*) are preferred for oil harvest (Harlan 1992). In general, tree and forage crops are considered to be only partially domesticated.

There is also evidence of abandonment of domesticates. Both North America (currently the central and northeastern part of the United States) and northern China were once centers of crop domestication. In the North American center, a crop such as marshelder or sumpweed (*Iva annua*) was once domesticated (as evidenced by increased seed size), as were other crops such as sunflower (*Helianthus annuus*) and gourd (*Cucurbita pepo*). Marshelder, as a domesticate, has now disappeared, having been replaced by other crops, both local ones and those introduced from the Mesoamerican domestication center, including maize (Smith 1995a). In northern China, several hundred kilometers north from the Yangtze basin where rice was probably domesticated, two drought-tolerant millet species (broomcorn millet, *Panicum miliaceum*, and foxtail millet, *Setaria italica*) adapted to cultivation in regions with marginal rainfall were domesticated. With time, however, rice has increased in importance, whereas the importance of these millet species has decreased.

There are important corollaries to this definition of domestication. First, plant cultivation or animal rearing is a necessary but insufficient condition for domestication. Thus, each crop or animal breed will have been grown or reared for a generally undefined period (predomestication cultivation or rearing) during which selection operated. During this period, the definitive changes in phenotype normally associated with domestication may not have occurred. Second, certainly for plants, complete domestication leads to a lack of fitness in natural environments. Fully domesticated plants cannot survive on their own in the wild. One of the best examples of this situation is maize, where the husks surrounding the ear and the tight attachment of kernels to the cob prevent natural dispersal. In contrast, partially domesticated plants have conserved at least some ability to survive in natural environments. Examples of this situation are often fruit trees such as olive (Bronzini de Caraffa et al. 2002). This leads to the existence of feral populations that can be distinguished only with difficulty—if at all—from wild populations. Third, a mutualistic relationship exists between humans and their crop plants or animal breeds. The transition from hunting-gathering to agriculture was an experiment in cultural evolution that represented a drastic change in human societies and their environment (Richerson et al. 2001). In turn, agriculture became a necessary condition for the development of civilizations because it provided a surplus of food, which allowed specialization and diversification of crafts, trades, and other occupations (Maisels 1993). While fully domesticated plants and animal breeds (the latter to a lesser extent) cannot survive on their own, it can also be argued that humans would not be able to survive without their domesticates.

Agriculture has so far been able to keep pace with human population growth and provides sufficient food and other needs so that humans can tend to other activities (Cohen 1995; Smil 2001). This close relationship between humans and their domesticated plants and animals is precisely one of the aspects that makes the study of domestication such a fascinating area of study. Whereas humans have had a marked effect on domesticated plants and animals, the converse can also be said. Domesticated (and, in some cases, undomesticated) plants and animals have had a significant effect on human history (Crosby 1986; Viola and Margolis 1991; Hobhouse 1999). For example, exotic plants (at least to the Europeans of the 15th and 16th centuries) were one of the driving forces behind the explorations of new continents. In this respect, the discovery of the Americas by C. Columbus in 1492 was a significant date because it led to the Columbian exchange, the reciprocal exchange of crops between the Old and New Worlds.

Domestication is a continuing process. While in the strictest sense of the definition, domestication could refer only to the first stages of selection that coincided with the initiation of agriculture, selection by humans continues to this day. The advent of scientific plant and animal breeding has greatly accelerated the pace of change.

III. CENTERS OF AGRICULTURAL ORIGINS

Among technological developments and inventions, agriculture is perhaps one of the few, if not the only one, that originated independently in more than one location. Although the number and precise boundaries of the different centers of origin of agriculture remain to be determined, agriculture originated in at least six different areas of the world: Mesoamerica, the Andes of South America (including their piedmonts), Southwest Asia (the Fertile Crescent), Africa (Ethiopia and the Sahel), Southern China, and Southeast Asia (Fig. 1.1) (Hawkes 1983; Harlan 1992; Smartt and Simmonds 1995). Additional areas include eastern

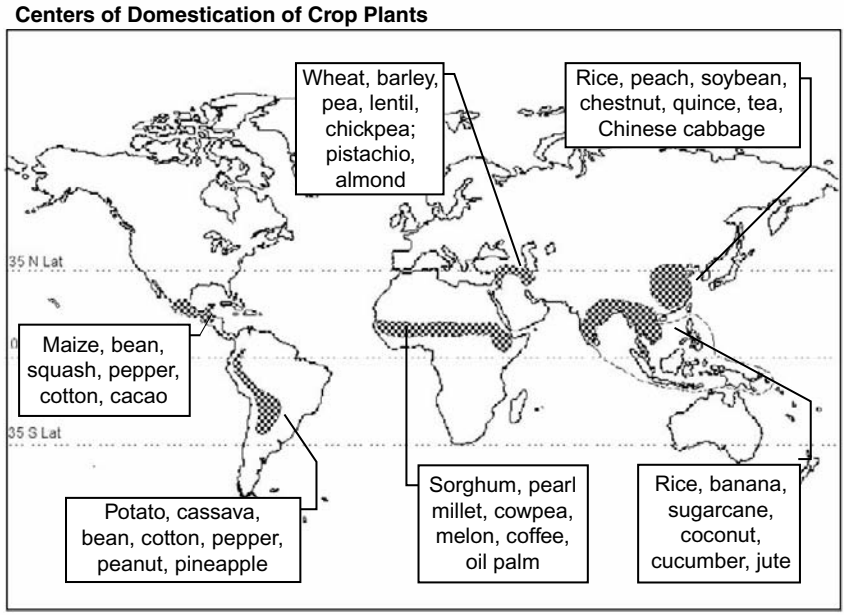

Fig. 1.1. Location of the major centers of crop domestication and some of the crops domesticated in each of them. Source: Gepts 2001.

North America, northern China, and Europe, but their impact has been much less than that of the aforementioned centers. In each of these centers, similar types of crops were domesticated. For example, in each center one or more sources of carbohydrates (cereal or root or tuber crop) and of proteins (legumes) were domesticated.

Are there commonalities between these geographically disparate regions? They are located in tropical or subtropical regions generally between 35° N. and 35° S. Lat. Their topography is generally mountainous or hilly. One can speculate that this type of environment at the time of domestication would have harbored a wider range of resources than areas that are located at higher or lower altitudes. In turn, this abundance of resources would have allowed early farmers to continue procuring food through the old methods of hunting and gathering. It would also have allowed them to more easily identify plants or animals that were predisposed to domestication.

In their natural habitat zone, Peake and Fleure (1927) proposed that the presence of a wide range of wild relatives was one of the prerequisites for a center of agricultural origin. They also suggested that an alternation of rainy and dry seasons was important and proposed that limits to migration as an alternative to agricultural intensification should exist. This could be achieved by topography or territoriality, which would prevent populations from migrating to other areas to obtain supplementary or alternative sources of food. An additional characteristic of potential centers of domestications was an absence of heavily forested areas, which would have made the conversion to agricultural lands difficult. Finally, they suggested that the existence of different groups with different traditions, cultures, and technologies would have also contributed to the development of agriculture. However, the existence of these characteristics would not per se ensure that agriculture would develop. For example, California never became a center of origin of agriculture (Bettinger 2000) although it possesses several of the distinguishing features proposed by Peake and Fleure (1927).

Elaborating on one of the characteristics of Peake and Fleure (1927), Harlan (1992) observed that most domesticated plants originated in one of two biomes, the Mediterranean and the Savannah. The Mediterranean biome is distributed on the western or southwestern edge of some continents or land masses, including the area around the Mediterranean sea, southern Africa and Australia, Chile, and California. Its main vegetation type is a shrubby or park-like grassland. Trees include conifers (cedar, pines) and evergreen broadleaf trees (such as oaks). Shrubs are often aromatic (such as rosemary, sage, and oregano). Many plants in this biome are adapted to fire. The savannah biome is also a lightly forested grass-

land that merges gradually into dry deciduous forests. It is found in Africa, South America, India, and Australia. Trees include baobab and acacia. The vegetation is also adapted to fire. Both biomes are characterized by an alternation of humid and dry seasons. In the Mediterranean biomes, rains occur primarily during the colder season, whereas in the Savannah biome rains occur mainly in the warmer season. Table 1.1 lists examples of crops arranged by their biome of origin.

The existence of a marked dry season may have constituted an impetus for the transition from hunting-gathering to agriculture. In the presence of rising populations, which put more pressure on existing food resources especially during the dry season, hunter-gatherers may have planted seeds of the grain crops they were already harvesting and consuming to increase the size of the harvest. It is significant in this respect that a majority of the basic food crops domesticated in these biomes are actually annual grains. Conservation of grains is eminently feasible over a few months of dry weather and would have provided an alternative food source, especially in those years with a marked and extended dry season.

Application of molecular and biochemical markers has allowed us to further specify potential centers of domestication. In some cases, this has

Table 1.1. Examples of origin of crops in different biomes.

Biome	Crop
Desert	Date palm
Mediterranean	Wheat, barley, rye, pea, lentil, chickpea, rapeseed
Savanna (and tropical deciduous forests)	Maize, rice, sorghum, cassava, sweet potato, bean, peanut, yams
Sea coasts	Coconut, cabbage, beet, cotton
Temperate prairies	Sunflower
Temperate steppes	Proso and foxtail millet, hemp, and *Triticum tauschii* (donor of the D genome of bread wheat)
Temperate forest	Apple, pear, cherry, grape, walnut
Tropical highland	Potato (and other root crops from the Andes: ullucu, mashua, oca, arracacha, achira, yacón, unchuca) and *arabica* coffee
Tropical rain forest	Sugarcane, banana and plantain, citrus, mango, cacao

provided astonishingly specific locations, assuming that alternative hypotheses that can account for the observed results can be dealt with. For example, the closest wild relative of domesticated maize is teosinte. Various types of teosinte exist, including diploid and tetraploid forms, as well as annual and perennial forms. Using isozyme data, Doebley et al. (1984) identified *Zea mays* ssp. *parviglumis* teosinte, a diploid, annual teosinte distributed principally in the states of Jalisco, Michoacán, Mexico and Guerrero, as the closest wild relative of domesticated maize. In particular, the populations from the Balsas river drainage in Guerrero appeared to be particularly close to domesticated maize. These findings have been recently confirmed based on sequence analyses of the *teosinte branched-1* (*tb1*) gene (Wang et al. 1999, 2001) and a microsatellite analysis of genetic diversity of maize germplasm (Matsuoka et al. 2002). The latter study was also able to identify two major dispersal routes for maize germplasm from the Mexican highlands, one to the north ending in the northeastern United States and the other to the south to the Andes via Central America. These dispersal data complement archaeological data that show maize was domesticated by 5,400 years before the present (in uncalibrated years) in highland Mexico (Piperno and Flannery 2001; Pope et al. 2001). However, in contrast with the Southwest Asian center of agricultural origins, there are only a few archaeological sites relevant to the study of agricultural origins in Mesoamerica. All of these centers are located outside the current distribution area of teosinte, the presumed wild progenitor of maize. Thus, the age of domestication of maize is likely to be even older than the finds of current archaeological sites. In addition, the data of Matsuoka et al. (2002) show a genetic and ecological gap between *Z. mays* ssp. *parviglumis* and the closest domesticated maize group from the Mexican highlands. Thus, further data are needed to clarify some of the details of the domestication area of maize.

Common bean (*Phaseolus vulgaris*) is also a domesticate from the Mesoamerican center, although—in contrast with maize—it has an additional major center of domestication in the southern Andes (southern Peru, Bolivia, or Argentina) and a potential minor one in Central America or Colombia (Gepts 1993, 1998). A more specific location for the Mesoamerican center was obtained by identifying those wild populations based on variation of phaseolin, the major seed storage protein type of beans. Prior studies had shown that the domesticated types from Mesoamerica carried a single phaseolin electrophoretic type (S phaseolin type), in contrast with the wild progenitor that displayed at least 15–20 types (Gepts et al. 1986). Because each electrophoretic pattern is the result of a complex series of steps at the molecular level, including

gene duplications, nucleotide divergence, and post-translational modifications, the likelihood of repeated origins of this same pattern is low. Hence, the presence of the same S phaseolin signals a common ancestry. One should therefore expect to be able to trace back the origin of this phaseolin type to a specific region in the distribution of wild beans in Mesoamerica. A caveat is possible gene flow between domesticated and wild beans. Although common bean is considered predominantly self-pollinated (≤2–3% outcrossing), occasionally higher levels of outcrosses have been documented (Ibarra-Pérez et al. 1997). Feral populations and cases of outcrosses between wild and domesticated beans have been documented repeatedly (Debouck et al. 1993; Freyre et al. 1996; Beebe et al. 1997). To address this issue, morphological data such as seed size and growth habit were taken into consideration to disregard those wild accessions that showed signs of past hybridization with domesticated types (Vanderborght 1983). Although one could expect—given the simple genetic control of the domestication syndrome—that some wild beans would not show any difference in spite of past hybridization, using these morphological data would have reduced the number of wild populations carrying the S phaseolin through hybridization and not common ancestry. Using this procedure, it was possible to identify a well-circumscribed area in west-central Mexico (centered around Jalisco and western Guanajuato) as the putative domestication center for common bean (Gepts 1988). It is striking that this area is located relatively close to the area proposed for the domestication of maize, although it does not match it. It remains to be determined if this lack of match truly represents a different domestication area or is an artefact due, for example, to changes in distribution of the wild relatives of common bean and maize attributable to climate changes in the last 10,000 years (Buckler et al. 1998). Even today, wild beans can be found growing on teosinte (Delgado Salinas et al. 1988). It is therefore possible that early farmers domesticated not only crops but entire cropping systems as the predominance in Latin America of the so-called milpa cropping system, which includes maize, bean, and squash, suggests. Archaeological data, however, suggests that these domestications may not have been concurrent (Kaplan and Lynch 1999).

Using AFLP analyses, Heun et al. (1997) identified a population of morphologically wild einkorn wheat that was more closely related at the DNA level to domesticated einkorn than any other wild einkorn populations. This population is located in the Karacadağ mountains in southeast Turkey near major archaeological sites relevant to the study of the origins of agriculture, such as Cayönü, Cafer Höyuk, and Nevali Cori. Lev-Yadun et al. (2000) pointed out that the distribution regions of wild

relatives of several crops, including einkorn wheat, emmer wheat, barley, lentil, pea, and bitter vetch, overlap in an area encompassed by southeast Turkey, northern Syria, and northern Iraq. As for the Mesoamerican center of origin, one could suggest that cropping systems were domesticated based on pre-existing relationships in natural vegetation. However, a more definitive answer to this question is required. For several of the crops, molecular data are as yet unavailable. In the case of barley, the proposed domestication area is located to the south in the Levant (Badr et al. 2000). One can also wonder why early farmers would have domesticated not just one cereal or legume but several of them. Presumably, specific crops were domesticated because they corresponded to a specific dietary or other need. Why then domesticate more than one cereal or legume in the same locality? Perhaps these apparently similar crops did not fulfill the same function or some were saddled with major disadvantages such as low yield in the case of einkorn wheat.

Cassava (*Manihot esculentum*) is a major source of carbohydrates grown exclusively in tropical areas of Latin America, Africa, and Asia. The genus *Manihot* consists of some 100 species distributed in the Neotropics. The presumed wild progenitor of cassava is *M. esculenta* ssp. *flabellifolia* distributed only in South America (Allem 1987; Allem et al. 2001). Sequence analysis of the single-copy nuclear gene glyceraldehyde 3-phosphate dehydrogenase (*G3pdh*) further focused the putative center of origin in west central Brazil (south and east of Amazon basin) and eastern Peru (Fig. 1.1) (Olsen and Schaal 1999).

In animals, considerable progress has recently also been made on determining origins of domestication of major livestock species (MacHugh and Bradley 2001). Different patterns are observed. The major pattern represents an East-West split, with domestications having taken place in the eastern and western halves of the Eurasian land mass. Examples of this pattern are cattle with domestications in the Near East and India (Loftus et al. 1994; Mannen et al. 1998; Troy et al. 2001), sheep (Wood and Phua 1996; Hiendleder et al. 1998), and pig (Watanobe et al. 1999; Giuffra et al. 2000). The goat has a major domestication in Southwest Asia, a minor one in India, and a poorly understood potential third origin in Eurasia (Luikart et al. 2001). In contrast, the horse does not have a well-defined center of origin (Vilà et al. 2001). In the archaeological record, the horse appears well after other livestock species. It is possible that different agricultural societies domesticated the horse from local wild horse populations after cultural dissemination of the technology to capture, break, and train these animals had occurred.

Determining the specific geographic site of domestication is not a frivolous exercise. First, it may be important to guide archaeological

studies. For example, many if not all archaeological sites in Mesoamerica are located outside the current distribution area of wild progenitors of the main crops, maize, common bean, and squash. Guiding archaeologists to other areas such as Jalisco (for common bean) or the Balsas river basin (for maize) may in the long term be rewarded by the discovery of significant sites from the standpoint of agricultural origins in those areas (Smith 1995a). Second, identification of the immediate progenitors of a crop or breed is also important for further studies aimed at studying the effect of domestication as an evolutionary process at the genetic and physiological levels. Identifying the specific progenitor of a crop (or at least its immediate descendant) and the most primitive domesticated cultivars allows a more rigorous progenitor-descendant comparison than if the comparison was conducted between any wild and domesticated population. Knowledge of the actual progenitors is too recent for this approach to have been applied as yet. Hence, most wild-to-domesticated comparisons available may show differences that do not accurately reflect changes due only to domestication but also include changes that are due to divergence within the progenitor or domesticated descendant gene pools and are unrelated to domestication. Third, determining the specific site is also important for the management of genetic resources and their utilization in breeding programs. Utilization of wild genetic resources should focus on those accessions that are not the immediate progenitor of the crop in order to introduce novel genetic diversity into the domesticated gene pool.

In all the examples mentioned, the specificity gained by the use of molecular information is impressive. One should, however, keep in mind two important caveats. First, these molecular studies are only as good as the biological and genome samples available. It is of paramount importance to establish a sufficiently representative sample. This is not a trivial operation, because the materials either have not been collected or they are unavailable for a variety of reasons. Second, similarity between a crop and its putative wild progenitor can arise in ways other than through a progenitor-descendant relationship. Gene flow through pollen, seed, or escape from cultivation have been documented numerous times not only in outcrossing or vegetatively propagated crops, but also in predominantly selfing crops. To distinguish therefore between similarity due to a progenitor-descendant relationship or to gene flow, additional precautions ought to be taken such as using markers with a well-defined map location in relation to those of domestication genes (R. Papa and P. Gepts, unpubl. data) or analyze sequence variation at domestication loci (Wang et al. 1999, 2001) and adjacent regions to determine gene identity and recombination around the domestication loci. Until

additional studies are conducted, the specific geographic locations of domestications in the examples discussed here should be considered with caution.

IV. TIME FRAME OF DOMESTICATION

The process of domestication is but one aspect of the transition from hunting-gathering to agriculture. It is generally thought that this transition has taken several millennia (Smith 1995a). One of the milestones of this transition was the domestication of crops and animals. The point at which a crop or animal can be considered to be domesticated is somewhat speculative. As mentioned earlier, there are several traits involved in the domestication syndrome. A domesticated crop or animal usually displays several of these. Yet, the archaeological record only consists of a few types of remains, usually those that have been able to withstand decomposition. Examples of these are seeds and inflorescence axes (rachis or cobs). Cereals generally offer more clues to the status of their domestication than other crops such as legumes. In addition to an increase in seed size, which can be interpreted as a sign of domestication (see next section), a tough rachis (in contrast to a brittle rachis) and free-threshing seeds (as opposed to hulled seeds) with their characteristic morphology are also useful in this respect. For legumes, in contrast, only seed size can generally be used. Seed color and pod shape (for example, the presence of marked twisting of the pod walls) are rare additional possibilities. In light of the dearth of macroscopic traits indicating domestication, other traits have been investigated and used to document the transition from wild to domesticated types (Piperno and Pearsall 1998). These include starch grains (Piperno et al. 2000) and phytoliths or silica concretions (Zhao 1998; Piperno et al. 1999). Other features strengthening the archaeological record are the presence of a sequence within an archaeological site encompassing the transition from wild to domesticated and the number of remains.

Table 1.2 shows that the earliest finds in several domestication centers are about the same age—some 10,000 years ago. The exception is Eastern North America, where the earliest remains date from some 4,300 years ago. Although there are some differences in the actual ages of the finds among these centers of agricultural origins, it is not clear to what extent these are real or a result of insufficient sampling. With the exception of the Fertile Crescent and Eastern North America, the number of archaeological sites is quite limited. For example, Hart et al. (2002) list some 25 sites in the Eastern North America region, whereas the

Table 1.2. Time frame of domestication and early spread of agriculture.

Location	Crop[z]	Age (years BP)	Source
DOMESTICATION CENTERS			
Mesoamerica	Squash	10,000	Smith 1997
	Maize	6,200	Piperno and Flannery 2001
Fertile Crescent	Einkorn wheat	9,400–9,000	Willcox 1998
	Lentil[y]	9,500–9,000	Willcox 1998
	Flax[y]	9,200–8,500	Willcox 1998
	Goat[x]	10,000	Zeder and Hesse 2000
	Pig[x]	10,000	Giuffra et al. 2000
China	Rice	9,000–8,000	Zhao 1998
Eastern United States	Squash	4,300	Asch 1995, cited by Hart et al. 2002
	Sunflower	4,300	Crites 1993
SPREAD FROM DOMESTICATION CENTERS			
Lowland Mesoamerica and Central America	Cassava, Dioscorea yam, arrowroot, maize	7,000–5,000	Piperno et al. 2000; Pope et al. 2001
Eastern North America	Maize	1,100	Smith 1989; Hart et al. 2002
Europe	Einkorn wheat	9,000–5,000	Ammerman and Cavalli-Sforza 1984

[z]Only the earliest domesticated crop remains are listed.

[y]Uncertainty as to the domestication status.

[x]Additional centers of domestication for the goat (in the Indian subcontinent) and the pig (in Eastern Asia) have been postulated.

Mesoamerican center includes only five to six sites. In addition, the Mesoamerican sites, such as those in the Tehuacán and Oaxaca valleys, are located outside the current distribution area of wild progenitors of common bean, maize, and squash. It is possible that wild progenitors have retreated from these areas because of climate changes. However, the available data on past climate in the Tehuacán and Oaxaca Valleys suggest that little cultivation or domestication occurred in areas represented by the Coxcatlán (Tehuacán valley) and Guilá Naquitz (Oaxaca valley) caves (Buckler et al. 1998).

Genetic data suggest a domestication of maize in a different locale (see previous section). It is therefore possible that the presence of domesticated remains of crops such as maize represent a late introduction in these semi-arid areas, only after early farmers had mastered cultivation in these less favorable areas. Actually, recent molecular data based on microsatellites led Matsuoka et al. (2002) to suggest a domestication time for maize of 9,188 years ago (5,689–13,093 BP), surprisingly consistent with the age of squash domestication in Mesoamerica and that of domestication in other centers of agricultural origins (Table 1.2). Further archaeological sampling is therefore needed before more definitive conclusions can be drawn as to differences in timing of domestication among the different centers. As mentioned in the previous section, genetic data may guide archaeologists to areas where significant additional sites could be identified.

Determining the speed at which crops have been domesticated, that is, the period between first cultivation and fixation of domestication genes, depends primarily on the archaeological record. The ideal situation, a sequence of remains that spans the morphological evolution from the wild to the domesticated types, is rare. In many cases, one finds either type but not both in a more or less continuous situation. Nevertheless, data available from the Fertile Crescent (Willcox 1998) suggests that at least a millennium elapsed for domestication to take place. Wang et al. (1999) calculated a selection coefficient of $s = 0.04–0.08$ and a time frame of 300–1000 years for maize domestication based on sequence data for the *tb1* gene controlling branching. In einkorn wheat, field experiments to obtain realistic estimates of selection coefficients show that the most efficient cereal grain harvest system would involve sickle reaping of plant with a tough rachis. Other systems tested involved beating and uprooting. Modeling studies showed that a gene for a tough rachis could be fixed within 20–200 years (Hillman and Davies 1990). Clearly, more data are needed to document the length of the domestication process. Genetic data show that the process could have been fairly fast, with mutation and recombination rates being possible limiting factors. Nevertheless, archaeological data are also needed to document the actual time it took. It is expected that the actual time frame will be longer than the genetic time frame, because, for example, farmers may not have cultivated wild progenitors every year, given the presence of alternative resources.

Regardless of the outcome of future studies to locate additional archaeological sites, the rough similarity of domestication dates in widely different regions of the world suggests that climate change was a major factor, although not the only one, in stimulating the transition from for-

aging to farming. The period covering the last 10,000 years, also known as the Holocene, has been characterized by a generally warmer and more stable climate than the preceding Pleistocene era (Richerson et al. 2001). The latter authors have argued that the climate change, which also included a rise in CO_2 levels and increased rainfall, provided hunter-gatherers with the conditions for further intensification of food procurement, consisting of cultivation or rearing, and eventually domestication, of highly productive (but more labor-intensive) plant and animal resources.

As mentioned earlier, the period preceding the transition from hunting-gathering was also characterized by an intensification of the use of resources, the so-called broad-spectrum revolution. Agriculture can therefore be seen as an attempt some 10,000 years ago to further increase resource availability perhaps in response to ever increasing population levels or resource depletion or a combination of both. This was made possible in part by the improved climatic conditions but also because humans had reached a higher cognitive and cultural level of advancement. Richerson et al. (2001) argue that these successive bouts of intensification were driven by a competitive ratchet-like mechanism whereby each transition to more land-efficient subsistence systems both requires and allows labor intensification correlated with population growth. In turn, "early adopters" of these novel subsistence systems tended—through sheer increase of their population—to displace non-adopters. Displacement could take place physically by short- or long-range migration into territories occupied by non-adopters (demic diffusion). It could also take place by acculturation, whereby non-adopters eventually adopt the new life style (cultural diffusion). The two types of diffusion are extremes on a continuous scale, which includes many intermediate forms.

The speed at which agriculture was adopted was generally fast. Within the Fertile Crescent, which spans several hundreds of kilometers in both North-South and East-West directions, it is difficult to identify gradients in age of the oldest remains of crops and domesticated animals. Furthermore, Ammerman and Cavalli-Sforza (1984) suggested that the introduction in Europe of agriculture from the Fertile Crescent had an important demic component. Agricultural populations spread from the Fertile Crescent in a northwesterly direction. The process involved intermating with preexisting hunter-gatherer populations and movement of the next generations of agriculturists further into Europe. Thus, agriculture spread over most of the European continent in a period of about 4,000 years between 9,000 and 5,000 BP at an average speed of about 1 km per year. The major gradient in contemporary human gene frequencies

has a Southeast-Northwest direction. Ammerman and Cavalli-Sforza (1984) argue that this gradient can directly be attributed to the migration associated with the introduction of agriculture into Europe.

V. THE DOMESTICATION SYNDROME

As already alluded to by Darwin (1859), the most intensively domesticated plants have lost their ability to survive on their own in the wild. In selecting plants to fulfill their needs for food, feed, and fiber, humans have—perhaps inadvertently—selected crops that, while they do extremely well in cultivated fields, are unable to grow and reproduce successfully for more than a few seasons in natural environments, away from the care of humans who provide adequate seed beds and reducing competition from weeds. What are the traits that have been modified as a result of selection under cultivation that have made crops so unadapted to the wild? As it turns out, many domesticated plants actually share several of these traits. Because of their repeated occurrence in widely different crops, these shared traits have been called the domestication syndrome (Hawkes 1983; Hammer 1984; Harlan 1992).

The two most important component characters of the domestication syndrome of seed-propagated crops are seed dispersal and dormancy. Domesticated types are characterized by lack of seed dispersal at maturity. This retention of seeds is realized in different ways depending on the crop. In cereal crops, a tough rachis prevents the disarticulation of the inflorescence and the release of seeds. Conversely, in wild graminaceous plants, an abscission layer is formed between each successive seed insertion site. At maturity, this layer causes the rachis to break and subsequently the dispersal of seeds. Seeds of domesticated plants display little or no dormancy compared to their wild progenitors, which usually have highly dormant seeds. On the one hand, dormancy prevents premature germination, which may be particularly important in unfavorable years, characterized, for example, by dry conditions unable to sustain the growth of seedlings. On the other hand, lack of seed dormancy promotes simultaneous germination and a more uniform population and, hence, harvest.

Domesticated plants generally have a more compact growth habit, with fewer and shorter branches. The most extreme case is maize. Teosinte, the wild relative of maize, has a highly branched plant growth habit, which contrasts markedly with the single stem of domesticated maize. The progenitor of some legume crops is a vine-like plant with long, twining branches (Fig. 1.2). This growth habit subsists in some

Fig. 1.2. Habitat of wild common bean (*Phaseolus vulgaris*) in Ecuador (see trifololiate leaves in the center of the photo). A viny growth habitat allows plants to compete with the surrounding vegetation for light. Photo: P. Gepts.

domesticates but in greatly attenuate form as in climbing or pole varieties. These same domesticates often include bush or dwarf genotypes. The most recent stage of this trend towards a more compact growth habit is provided by the development of crop ideotypes. Donald (1968) proposed these growth habits to simultaneously increase productivity of individual plants and decrease competition among plants. A consequence of this trend is an increase in the harvest index in crops, the ratio of the harvested part (e.g., grains) to the total aboveground biomass. Whereas wild plants will typically have a harvest index of around 20–30%, contemporary advanced cultivars show a harvest index of 60% or more (Evans 1993).

The presence of toxic compounds has not been a major impediment to domestication, as evidenced by several crops that still contain these compounds, although in many cases at reduced levels. In these cases, the domestication process has included not only selection for the usual

traits of the syndrome, but also the development of a detoxification process. It is possible that in certain cases this process could have been invented prior to domestication. Food processing is known among hunters and gatherers (Johns and Kubo 1988). For example, Native Californians used to grind and wash acorn to remove tannins. Examples of crops with reduced toxicity following domestication include cassava (Wilson and Dufour 2002) and lima bean (Vanderborght 1979).

A trait that has only recently received some attention as part of the domestication syndrome is the interaction between plant host and pathogens or other microorganisms, such as mycorrhizae and Rhizobium. A few preliminary studies have been conducted that suggest that these interactions have changed at the genetic level (Gouinguené et al. 2001; Rosenthal and Dirzo 1997; Benrey et al. 1998; González-Rodriguez et al. 2000; Lindig-Cisneros et al. 1997). Reciprocal selection between host and microorganism may have led to co-evolution and adaptation of the host and the micro-organism to each other. Further data are needed, however, to confirm these results.

One of the most important features of crop evolution is a change in the reproductive system of the plants involved. Usually, there is a change towards increased selfing (as in tomato or peppers; Rick 1988) or a replacement of sexual reproduction by vegetative reproduction, such as in banana/plantain (Simmonds 1966) or cassava (Elias and McKey 2000). Selfing or vegetative reproduction assures three goals. First, they would assure (re)production even under unfavorable conditions. This would be the case in particular when the crop was faced with environmental conditions unfavorable to fertilization or was disseminated into new areas without the corresponding pollinators. Second, trueness to type in the presence of outcrossing with wild relatives or other domesticated types could be maintained by farmers. Third, fruits would be more appetizing, as in the case of bananas where sterility has eliminated seeds from the fruit.

The ultimate agronomic trait, however, is yield. In addition to the harvest index already mentioned, other traits have played a role in influencing yield. Harvested organs in domesticated plants are usually much larger than those of their wild counterparts. For example, seeds of grain crops can be 5- to 10-fold larger than those of wild relatives. Because seed size is positively correlated with yield, selection for increased seed size may have led to increased yields, although yield component compensation may reduce the magnitude of this increase (Evans 1993). Heritability of seed size is usually high, thus, seed-size mediated increases in yield may have been relatively independent of environmental conditions. Other traits affecting yield are traits influencing the architecture

of the inflorescence such as reversal of sterility, which have operated in maize and barley, and increase in inflorescence size, as in maize and pearl millet (*Pennisetum glaucum*).

How then has yield fared under domestication? There are few if any historic measurements recorded of yield in wild stands, which would represent the base line for this question. Present day yield of a wild species of rice (*Oryza nivara*) in the Jeypore Tract in the state of Orissa, India, is about 1 t/ha. Stands of wild rice (*Zizania*) in North America today yield only 0.02–0.14 t/ha (Hayes et al. 1989). The wild relatives of cereals domesticated in the Fertile Crescent today yield around 0.5–0.8 t/ha (Harlan 1967; Zohary 1969). Araus et al. (2001) estimated the yield of wheat some 10,000 years ago to be around 1.5 t/ha. These numbers are similar to those deduced from cuneiform tablets, averaging about 2 t/ha around 4,400 BP (Jacobsen and Adams 1958). With time, these yields actually decreased to 1.2 t/ha by 4,100 BP and 1 t/ha by 1700 BC. Current wheat yields in the area are around 1 t/ha. This decline has been attributed to salinization of the land. This observation underscores the difficulty in distinguishing between genotypic and environmental effects in the assessment of the evolution of yield potential. In contrast, modern yields of rice are around 3,000 kg/ha in India and 6,000 kg/ha in China. Current yields of wheat are 2,000 kg/ha in Turkey, 3,000 kg/ha in Syria, and 4,000 kg/ha in the United States (FAO: *http://apps.fao.org/page/collections?subset=agriculture*). About 50% of yield increases can be attributed to genetic improvement (Fehr 1984).

Based on cob length data, Evans (1993) (his Fig. 6.7) estimated yield in maize to be around 1 t/ha some 1,000 years ago and some 0.5–0.6 t/ha around 2,000 years ago. Three thousand years ago, maize yields were approximately 0.4 t/ha. Furthermore, the initial stages of maize domestication (before 6,200 BP), which were characterized by fixation by selection of genes with major effects on the architecture of the inflorescence, may have seen initial rapid increases in seed yield. In an analysis of early (5,400 ^{14}C years) cob remains of Guilá Naquitz, Benz (2001) observed that the three samples were fixed for a tough (i.e., non-brittle) rachis and the presence of shallow fruit cupules, two domestication traits. The sample was heterogeneous, however, for the number of spikelets per cupule. Two inflorescences had one spikelet per node (and were, therefore, two-ranked), whereas the third inflorescence had two spikelets per node (and was, therefore, four-ranked). This increase in the number of seeds per inflorescence, which is positively correlated with seed yield, points to an increase in yield early on during the process of domestication. These observations suggest that the overall yield trend in maize during and after the initial domestication may have encompassed three major

phases: an initial fairly rapid increase, through conscious or inadvertent selection of major genes (see below), followed by a period of several millennia with a yield stasis or limited progress in yield potential due to inefficient farmer selection, and culminating, since the 20th century, in an era of marked progress through the application of modern plant breeding (Troyer 2000). A similar long-term trend in yield can be posited for other crops as a consequence of domestication.

Animals have also been modified considerably under domestication. The traits involved are mainly behavioral but some are also morphological (Zohary et al. 1998; Clutton-Brock 1999). For example, domesticated animals, in general and especially farm animals, are tolerant of human presence, further enhanced by imprinting of new-born animals. Human protection from predation reduced natural camouflage and allowed the appearance of contrasting color types. In addition, the size of the body, in general, and horns, in particular, and aggressive behavior have been reduced.

VI. INHERITANCE AND MOLECULAR BASIS OF THE DOMESTICATION SYNDROME

The inheritance of individual domestication traits has been based on a Mendelian approach with a segregation analysis on an individual trait basis (Ladizinsky 1985). This approach had major limitations because it was largely limited to traits with discrete segregation classes. More recently, however, the widespread availability of molecular linkage maps has allowed the conduction of genome-wide analyses based on the concept originally proposed by Sax (1923), namely to map genes for quantitative traits by establishing relationships between the continuous segregation of the quantitative trait and assess the discrete segregation of genetic markers. With this approach, one can analyze both quantitative and qualitative traits, determine the magnitude of the effect of individual genes (or at least chromosome regions), uncover the origin of the allele contributing to a trait, assess the overall proportion of phenotypic variation accounted for by the individual loci, and the linkage relationships among loci for the same or different traits. To analyze quantitative traits, replicated trials are necessary. Therefore, many studies have been performed in populations with permanent segregations such as doubled haploid or recombinant inbred populations. Disadvantages of this approach are that it tends to overestimate the effect of individual loci (called quantitative trait loci or QTLs) and that the chromosome location may be imprecise (several cMs) (Beavis 1994). Nevertheless, the chro-

mosomal location in several instances has been sufficiently precise to initiate map-based cloning experiments, for example, for domestication genes (see below). In other cases, mapped QTLs for disease resistance were co-located with major genes for resistance to the same pathogen and with resistance gene analogues (Geffroy et al. 1999; Geffroy et al. 2000). Therefore, QTL analyses are powerful and sufficiently accurate analyses to analyze complex traits such as the domestication syndrome.

The inheritance of the striking differences between crops and their wild progenitors has been studied in a wide range of crops, including both outcrossing (maize, pearl millet) and selfing species (common bean, rice), using a QTL analysis approach. With the exception of sunflower, the results appear to be quite similar among these crops (Table 1.3). The average number of QTLs per trait ranged between two and five, a relatively small number, which can be attributed to limited sensitivity of the method against genes of small effect. For many traits, however, genes with major effect (R^2 or proportion of the phenotypic variation accounted for by individual genes > 25%; Burke et al. 2002) were identified, with some genes reaching R^2 > 50%. The total genetic effect (i.e., sum of R^2 based on multiple regression) ranged between 40 and 50%, an underestimate given the sensitivity limits of QTL analysis. This suggests that

Table 1.3. Comparison of the inheritance of domestication syndromes in several crops.[z]

Crop	Mating system	Average no. QTLs or genes/trait	Average R^2 (%)	Total R^2 (%)	No. linkage groups	Source
Maize ($2n$ = 20)	Outcrossing	5.3	12 (4–42)	50 (34–61)	5	Doebley et al. 1990
Pearl millet ($2n$ = 14)	Outcrossing	2.2	29 (13–64)	57 (25–77)	4	Poncet et al. 1998, 2000
Common bean ($2n$ = 22)	Selfing	2.2	23 (12–53)	45 (18–69)	3	Koinange et al. 1996
Rice ($2n$ = 24)	Selfing	3.7	14 (7–60)	41 (16–72)	5	Xiong et al. 1999
Sunflower ($2n$ = 34)	Outcrossing	4.3	12 (3–68)	NA[y]	13	Burke et al. 2002

[z]Modified from Gepts 2002.

[y]Not available.

phenotypic variation in crosses between wild progenitors and domesticated descendants is based predominantly on genetic differences rather than environmental effects. A striking observation is that some domestication genes seem to be clustered on a relatively small number of chromosomes (Fig. 1.3). In the crops mentioned, these results have also been confirmed in additional crosses involving different parents, such as in maize (Doebley and Stec 1991), pearl millet (Poncet et al. 2002), and rice (Cai and Morishima 2000; Bres-Patry et al. 2001; Cai and Morishima 2002).

The apparent exception presented by sunflower concerns two elements of this inheritance pattern (Burke et al. 2002). First, the number of genes with major effects was much smaller than in the other crops studied and, although domestication genes showed clustering, they appeared to be distributed over a larger number of chromosomes. One can speculate that the inheritance of the domestication syndrome is a reflection of the domestication process itself. For example, the presence of genes with large effects may have facilitated the rapid selection—conscious or unconscious—of traits during domestication. Conversely, the presence of these genes may signal that initial domesticates had been

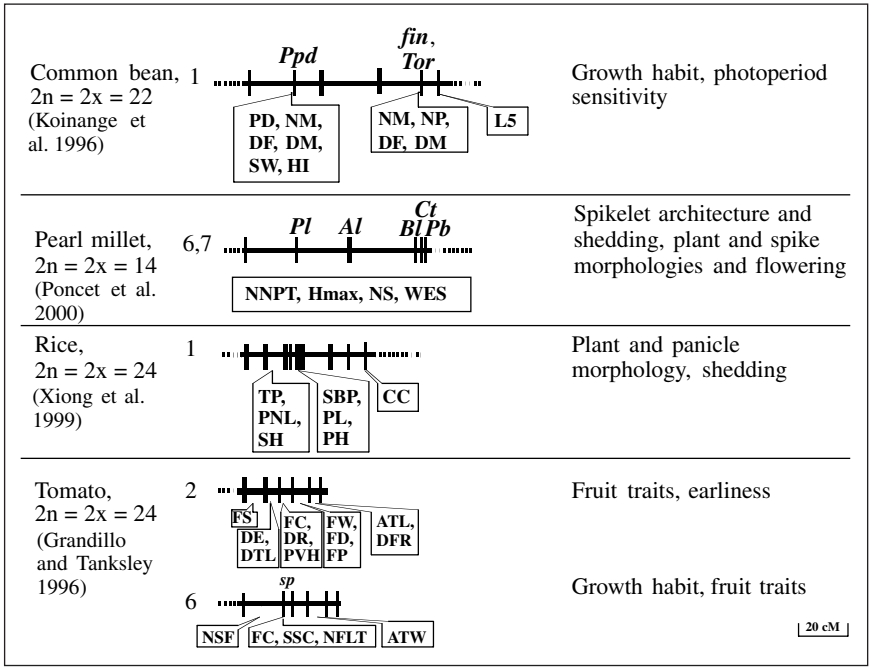

Fig. 1.3. Linkages among genes controlling the domestication syndrome in various crops.

subjected to a fairly strong selection pressure. The fact that a substantial part of the phenotypic variation can be accounted for in genetic terms suggests a relatively high broad-sense heritability that would have furthered the selection process during the early steps of domestication. Sunflower, compared to the other crops, may have undergone a slower domestication process, which did not require the presence of major genes.

Pernès (1983) suggested that linkage of domestication genes would be important in cross-pollinated crops because it would maintain the cohesion of some essential elements of the domestication syndrome when faced with repeated hybridizations of the sympatric wild progenitor. Linkage would limit recombination and aid in the recovery of domesticated types in the progeny of these crosses. This prediction was confirmed by the modeling study of Le Thierry D'Ennequin et al. (1999). They found that selection for increased fitness (increased number of domestication traits) led to selection of gametes with linked genes for domestication. The higher the outcrossing rate, the higher the proportion of parental (i.e., non-recombinant) gametes (Fig. 1.4). A similar observation was made for the migration rate. In the empirical data just reviewed, clustering, however, was observed not only in outcrossing species but also in species considered to be predominantly selfing. This suggests that these species are not as autogamous as they may seem or that they may have evolved towards autogamy as part of the domestication process. A higher level of outcrossing may have been important in the first stages of domestication to assemble the domestication syndrome. It would have been more likely that the different mutations constituting the syndrome appeared in different lineages than in the same one. Following the appearance of these mutations, they would have to be assembled into the same lineage by hybridization and recombined to achieve linkage in *cis*. Thus, linkage (but not too tight) would have facilitated the domestication process not only in outcrossing species but also in selfing ones. Linkage among domestication genes may have been made possible by clustering of genes in genomes, as shown not only by the existence of gene-rich regions in genomes (Fu et al. 2001; Weng and Lazar 2002) but also by the recent discovery of large regions (hundreds of kb) of similarly expressed but functionally unrelated genes ("expression neighborhoods" or "transcriptional territories") in the *Drosophila* genome (Spellman and Rubin 2002; Weitzmann 2002). The mechanism of the latter is not known but is likely to involve chromatin structure. The evolutionary importance may be assessed by analyzing the corresponding regions in other species. Conservation in the expression, size, and gene content of these regions would suggest a functional role.

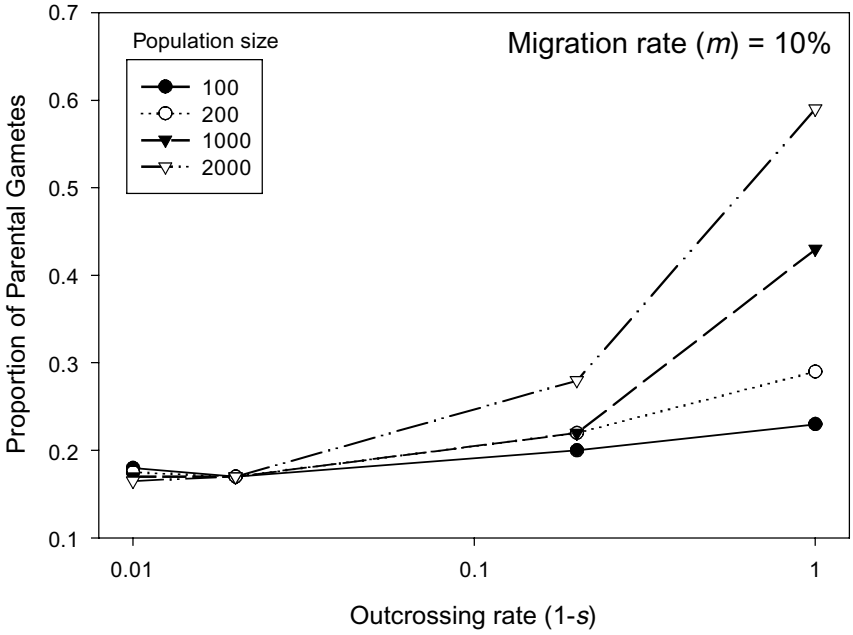

Fig. 1.4. Frequency of parental (non-recombinant) gametes as a function of outcrossing rates in domestication modeling study: linkage of domestication genes is favored in situation with high levels of outcrossing. Source: Le Thierry D'Ennequin, M., B. Toupance, T. Robert, B. Godelle, and P. Gouyon. 1999. Plant domestication: A model for studying the selection of linkage. J. Evol. Biol. 12:1138–1147. With permission, Blackwell and the authors.

Whether clusters of domestication genes belong to any expression neighborhood remains to be determined.

An additional consequence of hybridization is to transfer genes into different genetic backgrounds, which may allow expression of novel epistatic interactions that would only be active when different genes for domestication coexist within the same genome. An example is provided by Lukens and Doebley (1999), who backcrossed two unlinked teosinte alleles affecting plant growth habit (branching) into a domesticated maize background, either singly or in combination. The *tb1* allele had a strong additive effect on its own, but the second teosinte allele only had a phenotypic effect in the presence of the *tb1* allele. This led Lukens and Doebley (1999) to suggest that domestication involved not only selection on individual genes but gene complexes. They also observed that plants with the teosinte allele were phenotypically more plastic than their

counterparts with the maize allele. This observation may extend to other genes for domestication and is of concern for the current efforts to introduce additional genetic diversity from wild types. When attempting this, both the magnitude and the variance of the expression of the trait should be considered.

Polyploidy has affected the evolution of crops as well, although the effects may not be specific of domestication but rather reflect the high frequency of polyploidy among angiosperm species. Estimates of the frequency of polyploidy among angiosperms range from approximately 30% to 80% with a mode of 50% (Soltis and Soltis 2000). Hilu (1993) showed that the frequency of polyploids among crops is comparable to that of angiosperms in general. Furthermore, there were no differences in frequencies when considering taxonomic origin, habitat, life history (annual, perennial), and reproductive strategy. In addition to general attributes responsible for the success of polyploids (Soltis and Soltis 2000), some specific factors impinge upon the success of polyploids as crop plants. For example, polyploids have increased heterozygosity, which may in turn be associated with heterosis. The nature of this heterozygosity differs, however, between autopolyploids (arising through hybridization involving conspecific parents) and allopolyploids (arising from crosses involving species with diverged genomes). In the former, the increased heterozygosity stems from the polysomic inheritance, whereas in the latter, the heterozygosity results from the combination of different subgenomes into a single genome. The mode of origin of autopolyploids has an effect on the level of heterozygosity transferred to the progeny. In general, autopolyploids arising from the production of 2*n* gametes have higher levels of heterozygosity than those arising from chromosome doubling of the progeny. Furthermore, 2*n* gametes arising from first division restitution maintain a higher level of heterozygosity compared to those arising by second division restitution. Levels of heterozygosity have been correlated with potato tuber yield (Peloquin 1981). A second important characteristic is a widening of the ecological amplitude of species by polyploidization. Bread wheat (*Triticum aestivum*, with an AABBDD genome) is a cultigen, a plant type growing only under cultivation. It arose from the hybridization between emmer wheat, a domesticated tetraploid (*Triticum durum*, with the genome AABB), and a wild species, *Triticum tauschii*, with a DD genome. This hybridization took place when agriculture moved out of the Fertile Crescent into adjacent areas. In this particular case, emmer wheat moved out of the Fertile Crescent into the Caspian Sea region. The addition of the D genome broadened the adaptation of emmer wheat to include more continental climates than the Mediterranean climate to

which it was adapted. It now also became more adapted to regions with hotter summers and more severe winters (Sauer 1993).

Bread wheat serves to illustrate an additional feature of polyploidy, namely the opportunity for additional epistatic interactions between genomes. Seed proteins called glutenins give wheat flour a certain type of elasticity that entraps CO_2 bubbles resulting from fermentation of sugars by yeast. As a consequence, the dough rises and creates a lighter type of bread after baking. This property is unknown in the two parents of the hexaploid, suggesting that it arises from an interaction among genes of the two progenitors (Smith 1995b). An additional example is provided by cotton (Jiang et al. 1998). A QTL analysis conducted in a cross between Upland cotton (*Gossypium hirsutum*) and Pima cotton (*Gossypium barbadense*) (both species with AADD genomes) showed that most QTLs for fiber yield and quality originated in the D genome, in spite of the fact that only the A genome parent produces spinnable fiber. Interactions between the D genome fiber QTLs and genes in the A genome allowed the D genome gene to be expressed. Jiang et al. (1998) suggested that the reason that QTLs came predominantly from the D genome was due to fixation of "favorable" alleles in the A genome species. Absence of phenotypic expression of the fiber potential would have prevented the selection, and, therefore, fixation of these alleles in the D genome parent.

Several genes for domestication have now been cloned. These include the *tb1* gene in maize, which controls plant growth habit (Doebley et al. 1997; Wang et al. 1999, 2001). Specifically, it reduces the number and length of branches. The maize allele constitutes one of the exceptions to the rule that domesticated alleles are generally recessive. In this case, the dominance of the domesticated allele rests on increased levels of the message of the gene. The as yet unidentified lesion resides in the 5′ upstream regulatory region of the gene. The *fw2.2* gene in tomato is a QTL that increases fruit weight by up to 30% (Frary et al. 2000). The corresponding gene is expressed early in fruit development; it is expressed at a higher level in wild, small-fruited types, than in larger, domesticated types, consistent with the dominant nature of the wild allele. Sequence comparisons show that the gene may be related to the RAX gene family, which codes for, among others, proteins controlling cell division. The gene product has a structural similarity to a human oncogene.

The *Hd1* gene in rice controls response to photoperiod and is a QTL for flowering time (Yano et al. 2000). It may be promoting flowering under short day conditions and inhibiting it under long day conditions. The levels of message are similar under long and short day conditions, suggesting that other genes are also involved in photoperiod response. The HD1

protein has two zinc finger domains and is therefore likely to be a regulatory, DNA-binding protein. The DNA sequence is similar to the flowering time gene *CONSTANS* in *Arabidopsis thaliana*. In rice, the *Hd1* gene is allelic to the *Se1* gene controlling photoperiod sensitivity. The wild allele is dominant. In *A. thaliana*, the *SHATTERPROOF* genes (*SHP1* and *SHP2*) control fruit dehiscence (Liljegren et al. 2000). The two genes are redundant and can substitute for each other. They cause the differentiation of the dehiscence zone and the lignification of the adjacent cells. Their sequence includes a MADS box motif, suggesting that they are regulatory genes. Finally, the *CAULIFLOWER* gene in *Arabidopsis* and *BoCAL* gene in *Brassica oleracea* affect inflorescence structure and are responsible for the cauliflower and broccoli phenotypes (Purugganan et al. 2000). Sequence analysis reveals that this gene is also a MADS box gene and that the lesion resides in exon 5 of the gene, resulting in a premature stop codon in the middle of the K domain of the MADS-box transcriptional activator. This mutation has appeared only once in *B. oleracea* and has achieved fixation in the cauliflower accessions and near-fixation in the broccoli accessions sampled. It is, however, also observed in other taxa that do not display an altered inflorescence phenotype, suggesting that the *BoCAL* gene is not sufficient to control the cauliflower phenotype.

This brief overview of the molecular basis of domestication traits confirms the predominance of recessive mutations among domestication alleles. It may be significant here that the exception so far is the *tb1* gene in maize. As maize is a highly outcrossed species, dominant mutations would be more readily selected than recessive mutations. Conversely, in selfing species recessive mutations would be more readily selected because the frequency of homozygosity is higher compared to outcrossing species. Most of the genes involved in these morphological changes are regulatory genes, whether the lesion resides in the 5′ upstream regulatory genes or in the coding portion of these genes. Isolation of these domestication genes is a prerequisite to conduct molecular population genetic studies associated with the domestication process and to understand evolutionary factors that have affected the crop, including selective sweeps and gene flow processes.

VII. GENETIC BOTTLENECKS

A feature shared by nearly all, if not all, domesticated plants is a reduction in the genetic diversity during and after domestication. This genetic bottleneck has been measured with a variety of biochemical or molecular markers, including isozymes, seed proteins, RFLPs, RAPDs, AFLPs,

and more recently DNA sequences of specific genes. The magnitude of these bottlenecks depends on the type of markers. For example, chloroplast DNA restriction analyses (reviewed by Doebley 1992) show a marked decrease in genetic diversity between wild and domesticated types in widely different crops, including barley, sunflower, pea, sorghum, and maize (on average 75%). At the nucleotide sequence level, there have been fewer comprehensive studies. Only in maize have a large number of genes been studied. In that species, there has been on average a reduction in diversity of 30% compared with diversity in teosinte (White and Doebley 1999). Additional studies, reviewed by Buckler et al. (2001) suggest that other cereal species also are characterized by a genetic bottleneck of about 30% when considering nucleotide diversity.

Molecular data contrast with phenotypic data in that the latter show an increase in diversity. Darwin (1859) observed that the harvested organs of domesticated plants were more diverse than those of their wild relatives. The contrast between the two types of data can be reconciled by positing that the two traits are probably subject to different evolutionary factors. Molecular marker data are generally neutral and may be subject to genetic drift, whereas domestication traits (phenotypic data) are subject to selection. The stronger the selective advantage (in the cultivated environment), the higher the probability of survival of the domestication trait (Crow and Kimura 1970).

Caution should be exercised, however, because levels of diversity will vary substantially among genes as a function of position along chromosomes. There is a positive relationship between recombination and genetic diversity in *Drosophila* (Begun and Aquadro 1992), wheat (Dvorak et al. 1998), and tomato (Stephan and Langley 1998). In addition, population size plays a large role in determining the overall levels of genetic diversity. Superimposed on these differences attributable to genome organization and population levels are the effects of selection, particularly of selection during domestication. White and Doebley (1999) summarized studies in maize examining the genetic diversity at six loci, four of which were considered neutral (*adh1*, *adh2*, *te1*, and *glb1*) and two that were involved in domestication (*tb1* and *c1*). In the group of four loci, diversity in the domesticated gene pool was more than half that found in teosinte (ssp. *parviglumis*). For example, when total sites are considered, variation among domesticated maize genotypes for *adh1* was 83% of that in teosinte and 60% for the *glb1* locus. For the two domestication genes, variation contained in the domesticated gene pool was much lower. For instance, variation for *tb1* was 1–2% of that observed in teosinte.

Eyre-Walker et al. (1998) and Hilton and Gaut (1998) investigated the size and length of the genetic bottleneck that existed during maize domestication based on sequence variation and coalescent simulations for the *adh1* and *glb1*, respectively. Both studies found that the domestication bottleneck could have been of short duration and small size. Using the combined results of both studies, the bottleneck could have had a duration of 10 generations or years and involve some 10 individuals. Considering a time frame of 2,800 years, an estimate of the duration of domestication of maize based on the archaeological record, the bottleneck would have had a size of approximately 2,900 individuals, still a remarkably small number.

Hopefully, current efforts in genomics will be applied to issues in crop evolution and will not remain confined to undomesticated model systems such as arabidopsis and *Medicago truncatula*. High throughput methods can be used to evaluate sequence diversity for a larger sample of genes of known genome location in a larger number of species with contrasting life histories and domestication characteristics.

VIII. IS THERE A POTENTIAL FOR DOMESTICATION AMONG PLANT AND ANIMAL SPECIES?

There are some 250,000 angiosperm species. Of those, less than 500 have been subject to at least some attempts at domestication (Harlan 1992). Among animals, there are some 5,000 species (Myers 1999), of which less than 20 have been domesticated (Clutton-Brock 1999). Why were more species not domesticated? An admittedly incomplete list of nonmutually exclusive explanations is proposed here, which are often speculative in nature. In a general sense, for domestication to take place a number of conditions need to be satisfied from three angles: human, domesticate, and environment (Fig. 1.5). Domestication will proceed only if the conditions are satisfied in the three areas. Archaeologists study primarily the human factors and how these interact with environmental factors. Biologists focus on the plant or animal factors, although the intrinsic factors that determine whether a given plant or animal could be domesticated remain to be determined. For example, Diamond (1997) focused his analysis of domestication primarily on environmental factors influencing the various domestication areas and their subsequent influence on the development of agriculture and society.

On the plant or animal domesticate side, some species are probably more "susceptible" to domestication than others. It was F. Galton (cited

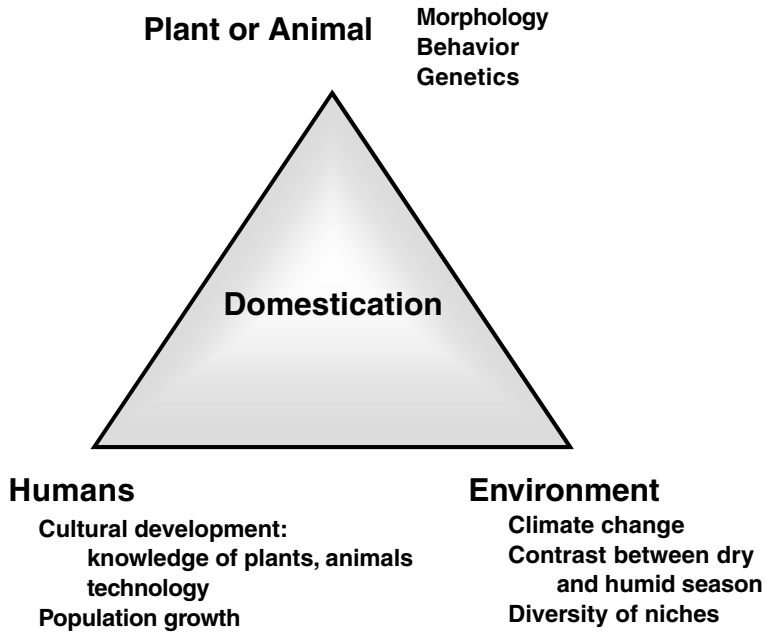

Fig. 1.5. Domestication results from the interactions of plant or animal, human and environmental factors. All three factors are required for domestication to take place.

by Clutton-Brock 1999), who in 1865 pointed out that animals should have the following characteristics (as rephrased by J. Clutton-Brock) under which they might be domesticated: (1) adaptable to different conditions, such as diet, environment, and disease pressure; (2) an inborn liking of man or at least no intense dislike or fear of humans; (3) tolerance of herding and constraint in a pen; (4) usefulness as a source of food or for other uses given the amount of effort required to rear the animals; (5) breed freely (in contrast to the difficulties encountered by zoos in maintaining some wild animal breeds or species); and (6) easy to tend by being placid, versatile in their feeding habits, and gregarious.

These characteristics in animal domestication are mainly behavioral. Although they may appear to constitute a rather unusual combination of traits that would exist only rarely among animals, which might explain the rarity of domestication, one also needs to demonstrate that other animals could not be domesticated for whatever reason. A recent study by Cameron-Beaumont et al. (2002) on potential cat domesticates is illustrative in this respect. The cat was domesticated in ancient Egypt. Some breeds of cat such as Persian and Siamese are fully domesticated,

as they satisfy the criteria of permanent isolation from the wild species and human control of breeding, territory, and food supply (Clutton-Brock 1999). Cameron-Beaumont et al. (2002) pointed out that in the cat family small felids other than the domestic cat display affiliative behavior towards human (similar to criterion 2, mentioned above). They investigated whether members of the ocelot ("small cat") lineage of the Felidae (a non-domesticated lineage) displayed affectionate behavior towards humans in captivity, such as sitting or rolling within 1 m of the keeper, head or flank rubbing, and licking of the keeper. They found that, in addition to the progenitor of the domesticate cat, other members of the Felidae displayed affiliative behavior, especially in the ocelot lineage of South America, including Geffroy's cat (*Oncifelis geoffroyi*) and the margay (*Leopardus weidii*). They concluded that ecological and geographical separation between humans and potential domesticates could explain why only some species were domesticated.

In plants, morphological features facilitating domestication are those listed in Section VI. What is not well known is to what extent different, non-domesticated species, especially related ones, display these traits or to what extent these traits could appear by mutation repeatedly in different species. Harlan (1967) pointed out that wild grass species show differences in threshing ratio (ratio grain over total biomass in the inflorescence, which includes rachis and glumes in addition to grain). Wild einkorn wheat had a threshing ratio of around 40%, whereas domesticated einkorn had a ratio of 70%. *Aegilops squarrosa*, which was never domesticated itself but is a putative donor of the B genome, had a threshing ratio of 10%. Clearly, wild einkorn is a better starting material for domestication than *A. squarrosa*.

There are several examples of crops where more than one species has been domesticated in a given genus, suggesting that to some extent phylogenetic relationships can help predict the domestication potential of a species. These include bean (*Phaseolus* spp.), pepper (*Capsicum* spp.), cotton (*Gossypium* spp.), and black and green grams, rice bean, and adzuki bean (*Vigna* spp.). However, there may be differences among these species in the degree of domestication. In the genus *Phaseolus*, the common bean (*P. vulgaris*) is by far the most strongly domesticated species when one considers the number of traits and the level of expression compared to its wild progenitor. In other domesticated *Phaseolus* species, some traits of the domestication syndrome, such as the determinate growth habit or stringless pods, are absent. This could mean that, for some reason, the traits were either never selected for or never appeared. Four of the five species, common bean (*P. vulgaris*), runner bean (*P. coccineus*), year bean (*P. polyanthus*, a hybrid species between the two

former species), and the tepary bean (*P. acutifolius*) belong to the same clade within the genus. The fifth species (lima bean) belongs to a very different clade of the genus. Thus, domestication potential may be unevenly distributed within the genus *Phaseolus*. A similar argument or analysis can be made for other genera or species of plants and animals.

Reproductive system and life history have influenced domestication. Generally, the earliest domesticates have been annual grain plants, with a selfing reproductive system. Maize, with its allogamous reproductive system, is a notable exception. Selfing and vegetative propagation may have been favored because they facilitate "true-to-typeness" after selection of a favored phenotype. It has been noted by Hancock (1992) that in any domestication there are several waves of domestication. The first wave included basic food crops, primarily annual grain crops. The second wave included vegetables and fruit trees. Later on, forages were domesticated. Fruit trees underwent limited domestication. Very often bud mutations were selected and propagated vegetatively. Thus, many fruit tree varieties differ little from other varieties and from their wild progenitors. It is also difficult to distinguish them from their wild progenitor. They can also naturalize easily and form feral populations that are difficult to distinguish from truly wild populations such as olive (Bronzini de Caraffa et al. 2002). Similar observations can be made to a lesser extent with forage crops.

Are there genetic characteristics that would favor domestication? As Darwin (1859, 1868) pointed out, genetic diversity has to be present or at least be generated by mutation during the time frame of the domestication phase. As mentioned earlier, there are few studies of mutation rates in plants and mammals, let alone comparisons among closely related domesticated vs. undomesticated species. Linkage of certain domestication genes may have been crucial to facilitate selection of the domestication syndrome (or certain crucial aspects of it). Thus, those species that have clustered domestication genes would have been easier to domesticate. Additional information on linkage from species that have not been domesticated would be required to help answer the question of whether linkage of certain genes is a prerequisite for domestication.

Paterson et al. (1995) observed in a series of cereals from distinct domestication centers (maize from Mesoamerica, sorghum from Africa, and rice in China) that some domestication traits appeared to be controlled by homologous genes. These observations were made possible by the existence of extensive synteny among grass species (Bennetzen and Freeling 1993). The traits investigated included seed size, seed shattering, and photoperiod response of flowering. Although there is some uncertainty as to the specific location of the genes because they were

analyzed by QTL analysis, corresponding locations occurred more often than just by chance. Overall, these results suggest that the same genes in different crops seem to be selected for in geographically widespread and independent domestication. Although these traits are complex and likely involve many genes, it appears that it is always the same set of genes that is selected. Why are these genes selected and not others? Additional information on these genes as well as other genes controlling the same trait will need to be obtained. In particular, mutagenesis and other experiments with homologous genes in related, undomesticated species will have to be conducted.

The cat example illustrates that human society needs to be present, predisposed towards domestication, and capable of taming or domesticating. These conditions were fulfilled in Ancient Egypt but not in lowland South America (although some plants such as peanut and cassava were domesticated in what is now part of the distribution area of some of the ocelot lineage felids). From crop studies, it is known that some initial domesticates have been abandoned. These include crops domesticated in the Eastern North American and Northern Chinese centers of crop domestication. The former gave rise to, among others, goosefoot (*Chenopodium bushianum*), marshelder or sumpweed (*Iva annua*), little barley (*Hordeum pusillum*), and sunflower (*Helianthus annuus*). Except for the latter, the other domesticates have become insignificant or have disappeared after domesticates (maize, squash, and beans) were introduced from the Mesoamerican center some 700–1,000 years ago. In northern China, broomcorn and foxtail millet were domesticated. Their importance diminished after introduction of rice, which had been domesticated further to the South in China as well. Thus, a number of species have been domesticated, but for reasons that are not well understood, and their cultivation was discontinued or sharply curtailed. It may be that they succumbed to the introduction of a dominant culture from elsewhere (including the crop plants associated with that culture). Or, alternatively, introduced crops had distinct agronomic or nutritional advantages over the native crops.

Finally, one has to ask how many crops can a society domesticate at once, especially of the same type (e.g., cereals or sources of carbohydrates; legumes or sources of protein). Lev-Yadun et al. (2000) suggested that domestication of the "founder crops" of the Fertile Crescent (einkorn, emmer, barley, pea, chickpea, lentil, and flax) had all taken place in a restricted area in southern Turkey. This assertion was based on genetic results for einkorn wheat (Heun et al. 1997) and Salamini et al. (2002) for emmer wheat showing close relationships between wild and domesticated types in that area (see previous discussion) as well as

an overlap in southern Turkey of the contemporary distribution of the wild relatives of the founder crops. Badr et al. (2000) have shown a domestication center for barley in the southern Levant (the western branch of the Fertile Crescent). Other areas that have remained inaccessible for political reasons remain to be explored, particularly in northern Syria and Iraq, so that they can either be identified or excluded as actual areas of domestication of these founder crops. It may well be that in a given area, only one cereal or legume would have been domesticated. Additional attempts at domestication would have been seen as too cumbersome and would not have been attempted as long as the original domesticate provided satisfactory returns. In *Phaseolus* beans, one of the centers of domestication of lima bean (*P. lunatus*) is located on the western slope of the Andes of Ecuador and northern Peru at mid to lower altitudes (Gutiérrez Salgado et al. 1995). This center gave rise to the so-called "Big Lima" types of lima bean. It is remarkable, however, that at slightly higher altitudes wild populations of common bean are growing, which appear never to have been domesticated (Debouck et al. 1993; Kami et al. 1995) even though they were domesticated elsewhere. This observation suggests that in any given region only a limited number of species will be domesticated in spite of the suitability of other species. It may be that there are only a limited number of species that can be domesticated at any given time.

Thus, there are a number of reasons why so few species were domesticated. Some of these are related to intrinsic characteristics of the plants or animals. Others are related to humans and the environment in which agriculture originated. It does suggest, however, that there remain other species to be domesticated.

IX. SUMMARY

There are a number of evolutionary features under cultivation or herding by humans: (1) among major cultural developments in human evolution, agriculture is perhaps one of the only ones that independently originated multiple times in widely different areas; (2) a specific area within a broader center of domestication can now be proposed using sensitive molecular marker technology; (3) a shared feature among most domesticated plants is a marked genetic bottleneck; 4) the genetic architecture of the domestication syndrome suggests that there was no genetic impediment to a fast domestication process (less than 100–200 generations); and (5) circumstantial evidence suggests that some species may be more amenable to domestication than others. Further research is

needed, however, to fully identify the biological features that render domestication possible.

Wild-to-domesticated complexes are excellent experimental systems to investigate certain evolutionary issues. There is a known time frame extending some 10,000 years. Both the progenitors and their descendants are known. This allows the integration of evolutionary and developmental genetics and a closer look at those differences at the molecular level that are responsible for the phenotypic differences between wild and domesticated types. In the past, crop evolution has been dismissed as not typical of evolution at large, because the high level of selection pressure was thought to be unusual in natural environments. While it is true that selection in nature may operate at longer time intervals, there is now plenty of evidence that strong selection also exists in natural environments (Endler 1986; Hoekstra et al. 2001; Kingsolver et al. 2001). Thus, the study of evolution under plant cultivation or animal rearing has broader implications for the study of evolution in general. Its information is also useful to further develop crop or animal biodiversity, conservation, and breeding programs.

LITERATURE CITED

Allem, A. 1987. *Manihot esculenta* is a native of the Neotropics. FAO/IBPGR Plant Genet. Res. Newsl. 71:22–24.

Allem, A., R. Mendes, A. Salomão, and M. Burle. 2001. The primary gene pool of cassava (*Manihot esculenta* Crantz subspecies *esculenta*, Euphorbiaceae). Euphytica 120: 127–132.

Ammerman, A. J., and L. L. Cavalli-Sforza. 1984. The neolithic transition and the genetics of populations in Europe. Princeton Univ. Press, Princeton, NJ.

Araus, J. L., G. A. Slafer, I. Romagosa, and M. Molist. 2001. Estimated wheat yields during the emergence of agriculture based on the carbon isotope discrimination of grains: Evidence from a 10th millennium BP site on the Euphrates. J. Archaeol. Sci. 28:341–350.

Badr, A., K. Müller, R. Schäfer-Pregl, H. El Rabey, S. Effgen, H. Ibrahim, C. Pozzi, W. Rohde, and F. Salamini. 2000. On the origin and domestication history of barley (*Hordeum vulgare*). Mol. Biol. Evol. 17:499–510.

Beavis, W. 1994. The power and deceit of QTL experiments: Lesson from comparative QTL studies. p. 250–266. In: 49th Annual Corn and Sorghum Research Conference. Am. Seed Trade Assoc., Washington, DC.

Beebe, S., O. Toro, A. González, M. Chacón, and D. Debouck. 1997. Wild-weed-crop complexes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Colombia, and their implications for conservation and breeding. Genet. Res. Crop Evol. 44:73–91.

Begun, D. J., and C. F. Aquadro. 1992. Levels of naturally occurring DNA polymorphism correlate with recombination rates in *Drosophila melanogaster*. Nature 356:519–520.

Bennetzen, J. L., and M. Freeling. 1993. Grasses as a single genetic system: genome composition, collinearity and compatibility. Trends Genet. 9:259–260.

Benrey, B., A. Callejas, L. Ríos, K. Oyama, and R. Denno. 1998. The effects of domestication of *Brassica* and *Phaseolus* on the interaction between phytophagous insects and parasitoids. Biol. Contr. 11:130–140.

Benz, B. 2001. Archaeological evidence of teosinte domestication from Guilá Naquitz. Proc. Natl. Acad. Sci. (USA) 98:2104–2106.

Bettinger, R. 2000. Holocene hunter-gatherers. p. 137–195. In: G. Feinman and T. Price (eds.), Archaeology at the millennium: a sourcebook. Plenum, New York.

Bres-Patry, C., M. Lorieux, G. Clement, M. Bangratz, and A. Ghesquiere. 2001. Heredity and genetic mapping of domestication-related traits in a temperate *japonica* weedy race. Theor. Appl. Genet. 102:118–126.

Bronzini de Caraffa, V., J. Maury, C. Gambotti, C. Breton, A. Bervillé, and J. Giannettini. 2002. Mitochondrial DNA variation and RAPD mark oleasters, olive and feral olive from Western and Eastern Mediterranean. Theor. Appl. Genet. 104:1209–1216.

Buckler, E., D. Pearsall, and T. Holtsford. 1998. Climate, plant ecology, and Central Mexican Archaic subsistence. Curr. Anthrop. 39:152-164.

Buckler, E., J. Thornsberry, and S. Kresovich. 2001. Molecular diversity, structure, and domestication of grasses. Genet. Res. 77:213–218.

Burke, J. M., S. Tang, S. J. Knapp, and L. H. Rieseberg. 2002. Genetic analysis of sunflower domestication. Genetics 161:1257–1267.

Cai, H. W., and H. Morishima. 2000. Genomic regions affecting seed shattering and seed dormancy in rice. Theor. Appl. Genet. 100:840–846.

Cai, H. W., and H. Morishima. 2002. QTL clusters reflect character association in wild and cultivated rice. Theor. Appl. Genet. 104:1217–1228.

Cameron-Beaumont, C., S. Lowe, and J. Bradshaw. 2002. Evidence suggesting preadaptation to domestication throughout the small Felidae. Biol. J. Linn. Soc. 75:361–366.

Child, R., N. Chauvaux, K. John, P. Ulvskov, and V. Van Onckelen. 1998. Ethylene biosynthesis in oilseed rape pods in relation to pod shatter. J. Exp. Bot. 49:829–838.

Clutton-Brock, J. 1999. A natural history of domesticated mammals. Cambridge Univ. Press, Cambridge, UK.

Cohen, J. 1995. How many people can the Earth support? Norton, New York.

Crites, G. 1993. Domesticated sunflower in fifth millennium B.P. temporal context: new evidence from Middle Tennessee. Am. Antiq. 5:146–148.

Crosby, A. 1986. Ecological imperialism: the biological expansion of Europe, 900–1900. Cambridge Univ. Press, Cambridge, UK.

Crow, J. F., and M. Kimura. 1970. An introduction to population genetics theory. Harper & Row, New York.

Darwin, C. 1859. On the origin of species by means of natural selection. J. Murray, London.

Darwin, C. 1868. The variation of plants and animals under domestication. J. Murray, London.

Debouck, D. G., O. Toro, O. M. Paredes, W. C. Johnson, and P. Gepts. 1993. Genetic diversity and ecological distribution of *Phaseolus vulgaris* in northwestern South America. Econ. Bot. 47:408–423.

Delgado Salinas, A., A. Bonet, and P. Gepts. 1988. The wild relative of *Phaseolus vulgaris* in Middle America. p. 163–184. In: P. Gepts (ed.), Genetic resources of *Phaseolus* beans. Kluwer, Dordrecht, the Netherlands.

Diamond, J. 1997. Guns, germs, and steel. Norton, New York.

Doebley, J. 1992. Molecular systematics and crop evolution. p. 202–222. In: P. S. Soltis et al. (eds.), Molecular systematics of plants. Chapman Hall, New York.

Doebley, J. F., M. M. Goodman, and C. W. Stuber. 1984. Isoenzymatic variation in *Zea* (Gramineae). Syst. Bot. 9:203–218.
Doebley, J., and A. Stec. 1991. Genetic analysis of the morphological differences between maize and teosinte. Genetics 129:285–295.
Doebley, J., A. Stec, and L. Hubbard. 1997. The evolution of apical dominance. Nature 386:485–488.
Doebley, J., A. Stec, J. Wendel, and M. Edwards. 1990. Genetic and morphological analysis of a maize-teosinte F_2 population: Implications for the origin of maize. Proc. Natl. Acad. Sci. (USA) 87:9888–9892.
Donald, C. 1968. The breeding of crop ideotypes. Euphytica 17:385–403.
Drake, J., B. Charlesworth, D. Charlesworth, and J. Crow. 1998. Rates of spontaneous mutation. Genetics 148:1667–1686.
Dvorak, J., M.-C. Luo, and Z.-L. Yang. 1998. Restriction fragment length polymorphism and divergence in the genomic regions of high and low recombination in self-fertilizing and cross-fertilizing *Aegilops* species. Genetics 148:423–434.
Elias, M., and D. McKey. 2000. The unmanaged reproductive ecology of domesticated plants in traditional agroecosystems: An example involving cassava and a call for data. Acta Oecologica-Int. J. Ecology 21:223–230.
Endler, J. A. 1986. Natural selection in the wild. Princeton Univ. Press, Princeton, NJ.
Evans, L. T. 1993. Crop evolution, adaptation, and yield. Cambridge Univ. Press, Cambridge, UK.
Eyre-Walker, A., R. Gaut, H. Hilton, D. Feldman, and B. Gaut. 1998. Investigation of the bottleneck leading to the domestication of maize. Proc. Natl. Acad. Sci. (USA) 95: 4441–4446.
Fehr, W. R. 1984. Genetic contributions to yield gains of five major crop plants. Crop Science Society of America, Madison, WI.
Flannery, K. 1969. Origins and ecological effects of early domestication in Iran and the Near East. p. 73–100. In: P. Ucko and G. Dimbleby (eds.), The domestication and exploitation of plants and animals. Aldine, Chicago.
Frary, A., T. Nesbitt, A. Frary, S. Grandillo, E. van der Knaap, B. Cong, J. Liu, J. Meller, R. Elber, K. Alpert, and S. Tanksley. 2000. *fw2.2*: A quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88.
Freyre, R., R. Ríos, L. Guzmán, D. Debouck, and P. Gepts. 1996. Ecogeographic distribution of *Phaseolus* spp. (Fabaceae) in Bolivia. Econ. Bot. 50:195–215.
Fu, H., W. Park, X. Yan, Z. Zheng, B. Shen, and H. K. Dooner. 2001. The highly recombinogenic *bz* locus lies in an unusually gene-rich region of the maize genome. Proc. Natl. Acad. Sci. (USA) 98:8903–8908.
Geffroy, V., D. Sicard, J. de Oliveira, M. Sévignac, S. Cohen, P. Gepts, C. Neema, and M. Dron. 1999. Identification of an ancestral resistance gene cluster involved in the coevolution process between *Phaseolus vulgaris* and its fungal pathogen *Colletotrichum lindemuthianum*. Mol. Plant-Micr. Inter. 12:774–784.
Geffroy, V., M. Sévignac, J. De Oliveira, G. Fouilloux, P. Skroch, P. Thoquet, P. Gepts, T. Langin, and M. Dron. 2000. Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localization of QTL with genes involved in specific resistance. Mol. Plant-Micr. Inter. 13:287–296.
Gepts, P. 1988. Phaseolin as an evolutionary marker. p. 215–241. In: P. Gepts (ed.), Genetic resources of *Phaseolus* beans. Kluwer, Dordrecht, the Netherlands.
Gepts, P. 1993. The use of molecular and biochemical markers in crop evolution studies. Evol. Biol. 27:51–94.

Gepts, P. 1998. Origin and evolution of common bean: Past events and recent trends. HortScience 33:1124–1130.
Gepts, P. 2001. Origins of plant agriculture and major crop plants. p. 629–637. In: M. Tolba (ed.), Our fragile world: Challenges and opportunities for sustainable development. EOLSS Publishers, Oxford, UK.
Gepts, P. 2002. A comparison between crop domestication, classical plant breeding, and genetic engineering. Crop Sci. 42:1780–1790.
Gepts, P., T. C. Osborn, K. Rashka, and F. A. Bliss. 1986. Phaseolin-protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. Econ. Bot. 40:451–468.
Giuffra, E., J. M. H. Kijas, V. Amarger, O. Carlborg, J.-T. Jeon, and L. Andersson. 2000. The origin of the domestic pig: Independent domestication and subsequent introgression. Genetics 154:1785–1791.
González-Rodriguez, A., B. Benrey, A. Castañeda, and K. Oyeda. 2000. Population genetic structure of *Acanthoscelides obtectus* and *A. obvelatus* (Coleoptera: Bruchidae) from wild and cultivated *Phaseolus* spp. (Leguminosae). Ann. Entom. Soc. Am. 93: 1100–1107.
Gouinguené, S., T. Degen, and T. Turlings. 2001. Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). Chemoecology 11:9–16.
Gutiérrez Salgado, A., P. Gepts, and D. Debouck. 1995. Evidence for two gene pools of the lima bean, *Phaseolus lunatus* L., in the Americas. Genet. Res. Crop Evol. 42:15–22.
Hammer, K. 1984. Das Domestikationssyndrom. Kulturpflanze 32:11–34.
Hancock, J. 1992. Plant evolution and the origin of crop species. Prentice-Hall, Englewood Cliffs, NJ.
Harlan, J. 1967. A wild wheat harvest. Archaeology 20:197–201.
Harlan, J. R. 1992. Crops and man. 2nd ed. Am. Soc. Agronomy, Madison, WI.
Harlan, J., J. de Wet, and G. Price. 1973. Comparative evolution of cereals. Evolution 27:311–325.
Hart, J. P., D. Asch, C. Scarry, and G. Crawford. 2002. The age of the common bean (*Phaseolus vulgaris* L.) in the northern Eastern Woodlands of North America. Antiquity 76:377–385.
Hawkes, J. G. 1983. The diversity of crop plants. Harvard Univ. Press, Cambridge, MA.
Hayes, P., R. Stucker, and G. Wandrey. 1989. The domestication of American wild rice (*Zizania palustris*, Poaceae). Econ. Bot. 43:203–214.
Heun, M., R. Schafer-Pregl, D. Klawan, R. Castagna, M. Accerbi, B. Borghi, and F. Salamini. 1997. Site of einkorn wheat domestication identified by DNA fingerprinting. Science 278:1312–1314.
Hiendleder, S., H. Lewalski, R. Wassmuth, and A. Janke. 1998. The complete mitochondrial DNA sequence of the domestic sheep (*Ovis aries*) and comparison with the other major ovine haplotype. J Mol. Evol. 47:441–448.
Hill, W., and S. Mbaga. 1998. Mutation and conflicts between artificial and natural selection for quantitative traits. Genetica 102/103:171–181.
Hillman, G. C., and M. S. Davies. 1990. Domestication rates in wild-type wheats and barley under primitive cultivation. Biol. J. Linn. Soc. 39:39–78.
Hillman, G., and S. Davies. 1999. Domestication rate in wild wheats and barley under primitive cultivation. p. 70–102. In: P. Anderson (ed.), Prehistory of agriculture: new experimental and ethnographic approaches, Vol. Monograph 40. Inst. Archaeology, Univ. California, Los Angeles.

Hilton, H., and B. Gaut. 1998. Speciation and domestication in maize and its wild relatives: evidence from the *Globulin-1* gene. Genetics 150:863–872.

Hilu, K. W. 1993. Polyploidy and the evolution of domesticated plants. Amer. J. Bot. 80:1494–1499.

Hobhouse, H. 1999. Seeds of change : Six plants that transformed mankind, 2nd ed. Papermac, London.

Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli, and J. G. Kingsolver. 2001. Strength and tempo of directional selection in the wild. Proc. Natl. Acad. Sci. (USA) 98:9157–9160.

Ibarra-Pérez, F., B. Ehdaie, and G. Waines. 1997. Estimation of outcrossing rate in common bean. Crop Sci. 37:60–65.

Jacobsen, T., and R. Adams. 1958. Salt and silt in ancient Mesopotamian agriculture. Science 128:1251–1258.

Jiang, C.-X., R. Wright, K. El-Zik. and A. Paterson. 1998. Polyploid formation created unique avenues for response to selection in *Gossypium* (cotton). Proc. Natl. Acad. Sci. (USA) 95:4419–4424.

Johns, T., and I. Kubo. 1988. A survey of traditional methods employed for the detoxification of plant foods. J. Ethnobiol. 8:81–129.

Kami, J., B. Becerra Velásquez, D. G. Debouck, and P. Gepts. 1995. Identification of presumed ancestral DNA sequences of phaseolin in *Phaseolus vulgaris*. Proc. Nat. Acad. Sci. (USA) 92:1101–1104.

Kaplan, L., and T. Lynch. 1999. *Phaseolus* (Fabaceae) in archaeology: AMS radiocarbon dates and their significance for pre-Columbian agriculture. Econ. Bot. 53:261–272.

Kingsolver, J. G., H. E. Hoekstra, J. Hoekstra, D. Berigan, S. Vignieri, C. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. Am. Nat. 157:245–261.

Ladizinsky, G. 1985. Founder effect in crop-plant evolution. Econ. Bot. 39:191–198.

Le Thierry D'Ennequin, M., B. Toupance, T. Robert, B. Godelle, and P. Gouyon. 1999. Plant domestication: A model for studying the selection of linkage. J. Evol. Biol. 12:1138–1147.

Lev-Yadun, S., A. Gopher, and S. Abbo. 2000. The cradle of agriculture. Science 288: 162–163.

Liljegren, S., G. Ditta, H. Eshed, B. Savidge, J. Bowman, and M. Yanofsky. 2000. SHATTERPROOF MADS-box genes control seed dispersal in *Arabidopsis*. Nature 404:766–770.

Lindig-Cisneros, R., B. Benrey, and F. Espinosa-García. 1997. Phytoalexins, resistance traits, and domestication status in *Phaseolus coccineus* and *Phaseolus lunatus*. J. Chem. Ecol. 23:1997–2011.

Loftus, R. T., D. E. MacHugh, D. G. Bradley, P. Sharp, and P. Cunningham. 1994. Evidence for two independent domestications of cattle. Proc. Natl. Acad. Sci. (USA) 91:2757–2761.

Luikart, G., L. Gielly, L. Excoffier, J. D. Vigne, J. Bouvet, and P. Taberlet. 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. Proc. Natl. Acad. Sci. (USA) 98:5927–5932.

Lukens, L., and J. Doebley. 1999. Epistatic and environmental interactions for quantitative trait loci involved in maize evolution. Genet. Res. 74:291–302.

MacHugh, D. E., and D. G. Bradley. 2001. Livestock genetic origins: goats buck the trend. Proc. Natl. Acad. Sci. (USA) 98:5382–5384.

Maisels, C. 1993. The emergence of civilization: from hunting and gathering to agriculture, cities, and the state in the Near East. Routledge, New York.

Mannen, H., S. Tsuji, R. T. Loftus, and D. G. Bradley. 1998. Mitochondrial DNA variation and evolution of Japanese black cattle (*Bos taurus*). Genetics 150:1169–1175.

Matsuoka, Y., Y. Vigouroux, M. M. Goodman, G. J. Sanchez, E. Buckler, and J. Doebley. 2002. A single domestication for maize shown by multilocus microsatellite genotyping. Proc. Natl. Acad. Sci. (USA) 99:6080–6084.

Myers, P. 1999. Mammalia [Online]. University of Michigan—Museum of Zoology—Animal Diversity Web, http://animaldiversity.ummz.umich.edu/chordata/mammalia.html.

Olsen, K. M., and B. Schaal. 1999. Evidence on the origin of cassava: phylogeography of *Manihot esculenta*. Proc. Natl. Acad. Sci. (USA) 96:5586–5591.

Paterson, A. H., Y. R. Lin, Z. K. Li, and K. F. Schertz. 1995. Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1714–1718.

Peake, H., and H. Fleure. 1927. The corridors of time. III. Peasants and potters. Oxford University Press, London.

Peloquin, S. J. 1981. Chromosomal and cytoplasmic manipulations. p. 117–137. In: K. Frey (ed.), Plant breeding II. Iowa State Univ. Press, Ames.

Pernès, J. 1983. La génétique de la domestication des céréales. La Recherche 14:910–919.

Piperno, D., and K. Flannery. 2001. The earliest archaeological mazie (*Zea mays* L.) from highland Mexico: new accelerator mass spectrometry dates and their implications. Proc. Natl. Acad. Sci. (USA) 98:2101–2103.

Piperno, D., and D. Pearsall. 1998. The origin of agriculture in the Neotropics. Academic Press, San Diego.

Piperno, D., D. Pearsall, R. Benfer Jr., L. Kealhofer, Z. Zhao, and Q. Jiang. 1999. Phytolith morphology. Science 283:1265.

Piperno, D., A. Ranere, I. Holst, and P. Hansell. 2000. Starch grains reveal early root crop horticulture in the Panamanian tropical forest. Nature 407:894–897.

Poinar, H. N., M. Kuch, K. D. Sobolik, I. Barnes, A. B. Stankiewicz, T. Kuder, W. G. Spaulding, V. M. Bryant, A. Cooper, and S. Paabo. 2001. A molecular analysis of dietary diversity for three archaic Native Americans. Proc. Natl. Acad. Sci. (USA) 98:4317–4322.

Poncet, V., F. Lamy, K. Devos, M. Gale, A. Sarr, and T. Robert. 2000. Genetic control of domestication traits in pearl millet (*Pennisetum glaucum* L., Poaceae). Theor. Appl. Genet. 100:147–159.

Poncet, V., F. Lamy, J. Enjalbert, H. Joly, A. Sarr, and T. Robert. 1998. Genetic analysis of the domestication syndrome in pearl millet (*Pennisetum glaucum* L., Poaceae): inheritance of the major characters. Heredity 81:648–658.

Poncet, V., E. Martel, S. Allouis, K. Devos, F. Lamy, A. Sarr, and T. Robert. 2002. Comparative analysis of QTLs affecting domestication traits between two domesticated × wild pearl millet (*Pennisetum glaucum* L., Poaceae) crosses. Theor. Appl. Genet. 104:965–975.

Pope, K. O., M. E. D. Pohl, J. G. Jones, D. L. Lentz, C. von Nagy, F. J. Vega, and I. R. Quitmyer. 2001. Origin and environmental setting of ancient agriculture in the lowlands of Mesoamerica. Science 292:1370–1373.

Purugganan, M., A. Boyles, and J. Suddith. 2000. Variation and selection at the *CAULIFLOWER* floral homeotic gene accompanying the evolution of domesticated *Brassica oleracea*. Genetics 155:855–862.

Rasmusson, D., and R. Phillips. 1997. Plant breeding progress and genetic diversity from de novo variation and elevated epistasis. Crop Sci. 37:303–310.

Richerson, P., R. Boyd, and R. Bettinger. 2001. Was agriculture impossible during the Pleistocene but mandatory during the Holocene? Am. Antiq. 66:387–411.

Rick, C. 1988. Evolution of mating systems in cultivated plants. p. 133–147. In: L. Gottlieb and S. Jain (eds.), Plant evolutionary biology. Chapman and Hall, London.
Rosenthal, J., and R. Dirzo. 1997. Effects of life history, domestication and agronomic selection on plant defence against insects: evidence from maizes and wild relatives. Evol. Ecol. 11:337–355.
Salamini, F., H. Ozkan, A. Brandolini, R. Schafer-Pregl, and W. Martin. 2002. Genetics and geography of wild cereal domestication in the Near East. Nature Rev. Genet. 3:429–441.
Sauer, J. 1993. Historical geography of plants. CRC Press, Boca Raton, FL.
Sax, K. 1923. The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552–560.
Simmonds, N. W. 1966. Bananas. 2nd ed. Longmans, London.
Smartt, J., and N. Simmonds. 1995. Evolution of crop plants. 2nd ed. Longman, Harlow, Essex, UK.
Smil, V. 2001. Feeding the world. MIT, Cambridge, MA.
Smith, B. D. 1989. Origins of agriculture in eastern North America. Science 246:1566–1571.
Smith, B. D. 1995a. The emergence of agriculture. Scientific American Library, New York.
Smith, B. D. 1997. The initial domestication of *Cucurbita pepo* in the Americas 10,000 years ago. Science 276:932–934.
Smith, C. 1995b. Crop production: evolution, history, and technology. Wiley, New York.
Soltis, P., and D. Soltis. 2000. The role of genetic and genomic attributes in the success of polyploids. Proc. Natl. Acad. Sci. (USA) 97:7051–7057.
Spellman, P. T., and G. M. Rubin. 2002. Evidence for large domains of similarly expressed genes in the Drosophila genome. J. Biol. 1:5:http://jbiol.com/content/1/1/5.
Stephan, W., and C. Langley. 1998. DNA polymorphism in *Lycopersicon* and crossing-over per physical length. Genetics 150:1585–1593.
Stiner, M. 2001. Thirty years on the "Broad Spectrum Revolution" and paleolithic demography. Proc. Natl. Acad. Sci. (USA) 98:6993–6996.
Troy, C. S., D. E. MacHugh, J. F. Bailey, D. A. Magee, R. T. Loftus, P. Cunningham, A. T. Chamberlain, B. C. Sykes, and D. G. Bradley. 2001. Genetic evidence for Near-Eastern origins of European cattle. Nature 410:1088–1091.
Troyer, A. 2000. Temperate corn—background, behavior, and breeding. In: A. Hallauer (ed.), Specialty corns, 2nd ed. CRC, Boca Raton, FL.
Vanderborght, T. 1979. Le dosage de l'acide cyanhydrique chez *Phaseolus lunatus* L. Ann. Gembloux 85:29–41.
Vanderborght, T. 1983. Evaluation of *Phaseolus vulgaris* wild types and weedy forms. Plant Genet. Res. Newslet. 54:18–25.
Vilà, C., J. A. Leonard, A. Gotherstrom, S. Marklund, K. Sandberg, K. Liden, R. K. Wayne, and H. Ellegren. 2001. Widespread origins of domestic horse lineages. Science 291:474–477.
Viola, H., and C. Margolis. 1991. Seeds of change. Smithsonian Inst. Press, Washington, DC.
Wang, R.-L., A. Stec, J. Hey, L. Lukens, and J. Doebley. 1999. The limits of selection during maize domestication. Nature 398:236–239.
Wang, R.-L., A. Stec, J. Hey, L. Lukens, and J. Doebley. 2001. Correction: The limits of selection during maize domestication. Nature 410:718.
Watanobe, T., N. Okumura, N. Ishiguro, M. Nakano, A. Matsui, M. Sahara, and M. Komatsu. 1999. Genetic relationship and distribution of the Japanese wild boar (*Sus scrofa leucomystax*) and Ryukyu wild boar (*Sus scrofa riukiuanus*) analysed by mitochondrial DNA. Molec. Ecol. 8:1509–1512.

Weitzmann, J. 2002. Transcriptional territories in the genome. J. Biol. 1:2:http://jbiol.com/content/1/1/2.

Weng, Y., and M. Lazar. 2002. Comparison of homoeologous group-6 short arm physical maps of wheat and barley reveals a similar distribution of recombinogenic and gene-rich regions. Theor. Appl. Genet. 104:1078–1085.

White, S. E., and J. F. Doebley. 1999. The molecular evolution of *terminal ear1*, a regulatory gene in the genus *Zea*. Genetics 153:1455–1462.

Willcox, G. 1998. Archaeobotanical evidence for the beginnings of agriculture in Southwest Asia. p. 25–38. In: A. Damania et al. (eds.), The origins of agriculture and crop domestication. ICARDA, Aleppo, Syria.

Wilson, W., and D. Dufour. 2002. Why "bitter" cassava? Productivity of "bitter" and "sweet" cassava in a Tukanoan Indian settlement in the northwest Amazon. Econ. Bot. 56:49–57.

Wood, N., and S. Phua. 1996. Variation in the control region sequence of the sheep mitochondrial genome. Anim. Genet. 27:25–33.

Xiong, L., K. Liu, X. Dai, C. Xu, and Q. Zhang. 1999. Identification of genetic factors controlling domestication-related traits of rice using an F_2 population of a cross between *Oryza sativa* and *O. rufipogon*. Theor. Appl. Genet. 98:243–251.

Yano, M., Y. Katayose, M. Ashikari, U. Yamanouchi, L. Monna, T. Fuse, T. Baba, K. Yamamoto, Y. Umehara, Y. Nagamura, and T. Sasaki. 2000. *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the Arabidopsis flowering time gene CONSTANS. Plant Cell 12:2473–2483.

Zeder, M., and B. Hesse. 2000. The initial domestication of goats (*Capra hyrcus*) in the Zagros mountains 10,000 years ago. Science 287:2254–2257.

Zhao, Z. 1998. The Middle Yangtze region in China is one place where rice was domesticated: phytolith evidence from the Diaotonghuan Cave, Northern Jiangxi. Antiquity 72:885–897.

Zohary, D. 1969. The progenitors of wheat and barley in relation to domestication and agricultural dispersal in the Old World. p. 47–65. In: P. Ucko and G. Dimbleby (eds.), The domestication and exploitation of plants and animals. Duckworth, London.

Zohary, D., E. Tchernov, and L. Kolska Horwitz. 1998. The role of unconscious selection in the domestication of sheep and goats. J. Zool. 245:129–135.

2

Long-term Selection in Plants in the Developing World

*J. W. Miles**
Centro Internacional de Agricultura Tropical
Apartado Aéreo 6713
Cali, Colombia

*S. Pandey**
Centro Internacional de Mejoramiento de Maíz y Trigo
Apartado Postal 6-641
06600 Mexico, D.F., Mexico

*The authors express sincere thanks to present and former colleagues at CIAT (S. E. Beebe, S. P. Singh, K. Kawano) and CIMMYT (K. D. Sayre, M. van Ginkel), and at other institutions (J. S. Beaver [U. Puerto Rico]; C. T. Hash [ICRISAT], and D. J. MacKill [IRRI]) for providing leads to relevant published material, for generously sharing valuable unpublished material (not all of which could be included), and for critically reviewing parts of the manuscript during its preparation.

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-46892-4

If there were any selective agency at work, it seems impossible to assign any limit to the complexity and beauty of the adaptive structures, which might thus be produced: for certainly the limit of possible variation of organic beings, either in a wild or domestic state, is not known.

—Charles Darwin 1844

I. INTRODUCTION

The developing world was (and is) the venue of all of the longest-term plant selection "programs." These involved the conscious and unconscious selection that produced and perfected our current crop plants from their wild progenitors. These developing world, long-term selection "projects" were conducted over thousands of years in open populations, often with continual introgression from one or more closely related wild species (Evans 1993, p. 105). And, while it appears likely that the process of domestication per se may have been "straightforward and rapid" (Evans 1993), adaptation, yield, and quality are still responding to farmer-conducted, developing world selection in plant species domesticated millennia ago. The results of these selective processes are readily observed in the large phenotypic differences between wild and domesticated types of almost any crop species, and in the often striking phenotypic diversity among surviving traditional cultivars. Some domesticates embrace a tremendous range of environmental adaptation, the result of both natural and artificial selection applied well before modern plant breeding began. However, while the genetic diversity among cultigens can often be shown to be less than that contained in the ancestral wild species, none of these selection "experiments" has evidently exhausted genetic diversity within the crop species, as response to modern selection programs generally attests.

In spite of this tradition in the developing world of long-term plant selection, we know of no "long-term selection experiments" of a strictly academic type, such as the Illinois long-term maize selection experiments, that are being or have been conducted in the developing world. There are numerous reasons for this, which we shall attempt to elucidate in this paper.

"Modern," that is, institutional-based, plant selection and breeding programs conducted during the past century or so in the developing world are almost exclusively aimed at improving the genetic potential and economic value of crop plants. These programs have generally had complex objectives, so that long-term response to selection for a single character can rarely be documented.

A significant development in crop selection and breeding in the developing world was the establishment of the international agricultural research centers (IARCs) during the 1960s and 1970s, and the subsequent creation of the Consultative Group for International Agricultural Research (CGIAR) to provide for their sustained financial support.

In this paper, we attempt to describe the institutional, economic, and social context in which plant selection and breeding programs are conducted in the developing world, highlighting in particular the special aspects of the developing world that affect genetic gain and breeding progress. Our perspective is, perhaps, overly biased towards crops and breeding programs of the international centers. In particular, we have made no attempt to document breeding efforts dedicated to tropical plantation crops [e.g., sugar cane (*Saccharum officinarum* L.), oil palm (*Elaeis guineensis* Jacq.), or rubber (*Hevea brasiliensis* Müll. Arg.)] where large genetic gains have been made. Authors affiliated with a national agricultural research institute or agricultural university in the developing world might bring a very different perspective to the task. We describe in some detail long-term breeding programs in five annual food crops, including two autogamous cereals (common bread wheat: *Triticum aestivum* L.; and rice: *Oryza sativa* L.); an allogamous cereal (maize: *Zea mays* L.); an allogamous, vegetatively propagated root crop (cassava: *Manihot esculenta* Crantz); and an autogamous grain legume (common beans: *Phaseolus vulgaris* L.).

A. The "Developing World"

For the purposes of this paper, we take the "developing world" to encompass essentially the tropics and subtropics, but also predominantly temperate countries in Latin America, Asia, and Africa. Agriculture in the developing world is extraordinarily diverse, ranging from highly advanced, intensively managed, high input, fully mechanized production systems to primitive, manual, subsistence food crop production. The very diversity of production environments, even within countries and regions, is taken as a characteristic of developing world agriculture (Kawano and Jennings 1983). Incident radiation at low latitudes is more intense, but duration of daylength is shorter than in the temperate growing season. Multiple cropping is often feasible, particularly where irrigation is available during the dry season, often requiring early maturing cultivars. Tropical climates favor, in general, a broader array of pest and disease organisms, and broader diversity within insect and pathogen species, giving them a greater capacity to overcome host plant resistance (Kawano and Jennings 1983). Crop yields range from

levels rivaling those of modern commercial agriculture in the temperate developed world, to chronically low yields of some crops owing to inherently low yield potential of traditional landrace varieties coupled with low levels of crop management. In relation to conducting plant selection programs, multiple generations of annual crops can usually be grown during a single year at the same location, or at least in the same country, if irrigation is available, thus accelerating potential genetic progress.

A common, almost defining, characteristic of the developing world is a relative political and economic instability, and in particular, a low level and lack of continuity of financial and institutional support for scientific research. This is not a propitious environment for long-term projects of any sort, including long-term plant selection experiments.

B. Crop Domestication

The selective process by which wild plant species were transformed into domesticated crops and subsequently improved for adaptation and yield are a prime case of "long-term selection." At least the early stages—the first several thousand years—of all these selection projects took place in the "developing world."

Plant domestication is clearly a result of human activities, whether conscious or inadvertent, to gain greater control over food sources. Phenotypic changes have been immense, probably owing much more to the long time spans involved than to the intensity or effectiveness of selection. We can speculate that many characters were involved, including many purely esthetic ones. Effective selection was applied to characters that affect efficiency of harvest (e.g., inflorescence size and compactness, grain retention). Yield, per se, and responsiveness to intensive crop management were probably less important selection criteria than reliability of yield in a given place. Hence, selection for tolerance to the common local stress factors, biotic or abiotic, was likely important. Broad adaptation was unimportant to any given farmer, beyond that needed to ensure some economic harvest even in unfavorable years. Breeding and selection probably had no institutional support, being mostly an individual or local community affair. The products of these selective processes were (and are) more or less heterogeneous plant populations that are able to produce a desirable product reliably under generally low-level crop management and over the normal range of year-to-year weather (and insect and pathogen) variation encountered in a particular local environment.

C. Modern Plant Breeding

Modern plant breeding is generally undertaken in an institutional context, owing to the need for advanced training of practitioners with diverse specializations. Institutional, modern plant breeding began prior to the re-discovery of Mendel's laws, and relied initially more on a Darwinian than a Mendelian intellectual heritage. The existence of institutional plant breeding programs is one indicator of the transition from "developing" to "developed" country status. One example, nineteenth-century Japan, relied on political decisions to improve agricultural productivity in the face of growing population pressure and a fixed (and limited) agricultural land base.

Modern plant breeding began, mostly in the West (Europe and North America), when plant improvement moved from the farm to formal, mostly publicly supported, agricultural research institutions that applied scientific principles to accelerate genetic gain. In large part, these programs were set up to serve economic needs as agriculture moved into a more market-oriented economy. This more deliberate approach to plant genetic improvement arose later in the developing world than in the developed countries of Europe (and European colonies in tropical Asia, Africa, and the Americas). Many of these early programs in the developing world sought to produce tropically adapted cultivars of crops for commercial agriculture practiced by European colonists, or to improve plantation crops grown for export in the European colonies. Little attention was given to the genetic improvement of food crops for local consumption by the indigenous population in the developing countries. Hence, rice breeding was being conducted in Japanese-occupied tropical Taiwan, but primarily for production for export to Japan, or wheat in British East Africa for export to the United Kingdom.

It was only following independence that plant breeders began to turn their attention to selection and breeding programs aimed at improving the productivity of food crops for local consumption, for example, for cassava in India (Koshy 1947). Following WWII, with improving health care and public health systems, the world's population began growing at an unprecedented rate, giving rise to concerns regarding the essentially stagnant growth of agricultural productivity.

The two basic approaches to increasing food production are to increase the area of agricultural land (assuming that unused lands exist that can be developed) or to increase the productivity of agricultural areas already developed. Given the rapid rate of population increase in the developing world over the past half century, most countries would

face severe environmental degradation in attempting to meet rising food demands by bringing additional agricultural land into cultivation. For instance, China produced its 1992 rice crop on only one-third as much land as would have been required at yield levels prevailing three decades earlier. Raising productivity, through a combination of genetic improvement and increased inputs, came to be seen as imperative to staving off impending massive starvation in the developing world. Hence, continuing genetic gains in crop yield are of more than merely academic interest in the developing world.

A serious impediment to achieving sustained improvement in food crop productivity in the developing world is the relative institutional instability in these countries. Any plant breeding exercise, and especially long-term selection, requires a long time frame, and in particular a perception on the part of the participants of long-term institutional support. These are frequently lacking in the developing countries, and this has undoubtedly inhibited the initiation of many programs of plant genetic improvement: It simply makes no sense to embark upon a long-term breeding and selection program if there isn't some assurance of its achieving a useful product.

D. The International Agricultural Research Centers

By the middle of the twentieth century, a number of forces were converging to set the stage for a large, productive, and sustained plant breeding effort in the developing world. Firstly, improving public health conditions and medical care were causing a sharp increase in population growth, leading to a perception in the developed world that the inability of developing countries to meet basic food needs would lead to dangerous political and social instability. While the beneficial effects of nitrogen fertilizer on crop yields had long been known, it was only at mid-century that technical developments begun decades earlier were making abundant, cheap chemical nitrogen fertilizer widely available.

Several decades earlier, institutions in the developed countries, particularly the Rockefeller Foundation, had begun to recognize the need to rapidly increase food crop productivity in the developing world (Perkins 1997). They perceived that this increase in productivity would come about only by the sustained application of agricultural research, namely plant breeding and improved agronomy, to agriculture in the developing world. A collaborative project in wheat improvement was initiated by the Rockefeller Foundation as early as 1924 with the University of Nanking in China. However, the Foundation's projects in China terminated with the outbreak of the Sino-Japanese war in the 1930s. In the

early 1940s, the Rockefeller Foundation began a sustained program of agricultural research in Mexico, and initiated a similar program during the 1950s in Colombia and later in India. These programs provided, albeit on a national scale, a sustained base for modern agricultural research in the developing world. Stable funding created a long-term perspective that would justify the initiation of major plant genetic improvement programs with basic food crops, such as rice, maize, and wheat.

In the 1950s, based on the successes of the Rockefeller Foundation's existing agricultural development projects, an entirely novel sort of institutional base for agricultural research in the developing world was conceived jointly by the Rockefeller and Ford Foundations. Seeing the need for a long-term research effort, and seeking to maximize the impact of research funding, the concept of an international research initiative developed, an institute devoted to improving rice productivity in Asia.

The International Rice Research Institute (IRRI) was founded in 1959, in the Philippines (Chandler 1982). Following a major investment in infrastructure and the assembling of a core research team, agronomic and plant breeding field work began in 1962. Additional international centers were established over the following decade in Mexico (International Maize and Wheat Improvement Center: CIMMYT), Nigeria (International Institute for Tropical Agriculture: IITA), and Colombia (International Center for Tropical Agriculture: CIAT). One of the major goals of the international centers was to provide stable, long-term institutional and financial support for agricultural research in the developing world. In the early 1970s, the Consultative Group on International Agricultural Research (CGIAR: www.cgiar.org) was established to ensure financing for a growing system of International Agricultural Research Centers (IARCs). The IARCs currently number 16 and embrace a global research agenda that extends from food crop agriculture to livestock, forestry, fisheries, and international food policy. Nine of the IARCs are engaged in plant genetic improvement programs (Table 2.1).

The so called "green revolution" that resulted from IARC research, particularly on rice and wheat, has been applauded and criticized (Evans 1993, Chapt. 2). While they were not the first institutions to engage in modern, scientific crop breeding in the developing world, the IARCs unquestionably had a significant early impact on national production figures through developing high-yielding, fertilizer-responsive germplasm and achieving its massive adoption in country after country. It has been noted (Khush 2001) that it took nearly 10,000 years from the origins of agriculture and crop domestication to achieve a world total food grain production of one thousand million tonnes in about 1960. World

Table 2.1. The CGIAR-sponsored International Agricultural Research Centers (IARCs) with plant breeding programs, and their crop focus.

Center		Crops
CIAT	International Center for Tropical Agriculture	Beans, rice, cassava, tropical forages
CIMMYT	International Center for the Improvement of Maize and Wheat	Wheat, maize, triticale
CIP	International Potato Center	Potato, sweetpotato
ICARDA	International Center for Agricultural Research in the Dry Areas	Barley, bread wheat, durum wheat, kabuli chickpea, lentil, faba bean, peas, forage legumes
ICRAF	International Centre for Research in Agroforestry	Agroforestry trees
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics	Sorghum, pearl millet, finger millet, chickpea, pigeonpea, groundnut
IITA	International Institute of Tropical Agriculture	Cassava, banana and plantain, yam (*Dioscorea* spp.), cowpea, soybean, maize
IRRI	International Rice Research Institute	Rice
WARDA	West Africa Rice Development Association	Rice

food grain production then doubled, to two thousand million tonnes, during just the next 40 years. A large part of this achievement is due directly and indirectly to the existence of the IARCs. A major component in their success lies in their providing a stable, long-term foundation, on an international scope, to developing country agricultural research, and particularly in providing a stable base for long-term plant genetic improvement. An additional component in the success of the IARCs was their deliberate and aggressive interaction with national agricultural research systems (NARS) throughout the developing world. This interaction involved providing a mechanism to facilitate massive exchange of germplasm both between the IARCs and NARS institutions, but also among the national programs themselves. This germplasm exchange undoubtedly broadened the genetic base in many national programs, providing a sound basis for accelerated genetic advance in cultivar development. Free exchange of technical information and training of

NARS scientists were additional means of stimulating the expansion of productive NARS research programs. The reach of the IARCs was (and is) indeed international.

II. LONG-TERM BREEDING/SELECTION PROGRAMS

A. Maize

1. Brief History of Domestication and Species Diversity. It is generally agreed that maize was domesticated in Mexico, probably from the closely related, weedy teosinte (one of the subspecies of *Zea mays*) (Goodman 1995). However, the putative origin of domesticated maize from teosinte by human selection faces several problems, among which are: (1) the absence of any archeological evidence of human use of teosinte prior to evidence of primitive maize; and (2) the distinctiveness of the female inflorescence of even the earliest maize known (from Tehuacán, Mexico) from that of teosinte (Goodman 1988). Whether maize was domesticated from teosinte or from an unknown wild maize that had diverged from the teosinte line prior to domestication, its evolutionary history following domestication led to wide diversification in adaptation and morphology well before becoming a subject of modern plant breeding. Maize is grown from 58° N to 40° S and from 0 to 3,808 m above sea level (Pandey and Gardner 1992). Approximately 300 races of maize have been described (Paterniani 1990). As noted by Paterniani (1990), "The great array of land races and cultivars available showing specific characteristics in almost all attributes, such as type of plant, ear, and kernel characters and adaptation, is eloquent evidence of the efficiency of the selection practiced in earlier times."

Of 600 M t of maize produced on 140 M ha worldwide during 1997–1999, 276 M t (46%) were produced in the developing world on 96 M ha (69%). The lower yields in the developing as compared with the developed world (~3 t ha^{-1} vs. ~8 t ha^{-1}) are due in no small measure to the fact that maize is predominantly a small-farmer crop in the developing world, with production of open-pollinated (OP) cultivars under very variable conditions and generally low levels of agronomic management.

Pandey and Gardner (1992) conducted a survey of 48 developing world maize breeders to assess the objectives and methods used in their programs. Recurrent selection schemes of one sort or another (mass, half-sib [HS], S1, full-sib [FS], modified-ear-to-row [MER]) are extensively practiced (Pandey and Gardner 1992), consuming a full 61% of the resources dedicated to maize breeding.

2. Early (pre-IARC) Breeding. In 1932, maize breeding began at the Instituto Agronômico de Campinas (IAC: São Paulo State) in Brazil with an inbreeding project from the local flint OP cultivar 'Cateto'. Early experimentation with inbreds and hybrids, including a population hybrid developed at the Rural University of Minas Gerais (now Federal University of Viçosa), proceeded with modest success through the 1940s and 1950s, based mainly on local, tropical Brazilian germplasm. Evaluation of temperate germplasm introduced from North America and Argentina was disappointing, as these temperate introductions lacked adaptation to Brazilian conditions (Paterniani 1990).

Maize germplasm was extensively collected in Latin America by researchers of the Rockefeller Foundation Agricultural Program (Wellhausen 1978). These collections were evaluated and described in a series of publications issued by the National Academy of Sciences between 1952 and 1961 (Wellhausen 1978). Their genetic potential was evaluated by Wellhausen (1965) through collaborative projects with a number of Latin American maize workers, leading to the identification of four basic "complexes" with high yield potential, either per se, or in hybrid combination.

More rapid progress was achieved in Brazil following the establishment of CIMMYT (in 1966) and the introduction to Brazil of diverse germplasm distributed by CIMMYT's Maize Program (Paterniani 1990). In a large evaluation of 418 hybrids developed in Brazil from 31 lines, the best single-cross was obtained from a cross between a Colombian line and a Mexican line. It yielded 55% more than the best double cross then available from exclusively Brazilian germplasm. Early maize improvement programs were started in other Brazilian states (e.g., Minas Gerais, in 1935), and a nationally coordinated maize improvement effort was established with the founding of EMBRAPA's Maize and Sorghum Research Center (CNPMS) in the mid-1970s.

3. Description of CIMMYT Maize Breeding Program. CIMMYT maize breeding began in 1966, building on activities initiated by the Rockefeller Foundation Mexican Agricultural Program, which began in 1943. Initially, CIMMYT focused exclusively on population improvement aimed at the development of improved OP cultivars, arguing that hybrid technology was inappropriate for most developing countries where seed industries are generally lacking. The initial bias against hybrids was such that CIMMYT maize composite populations were assembled without regard for heterotic patterns (Wellhausen 1978). However, in the mid-

1980s, and, significantly, at the request of several national maize improvement programs, CIMMYT began to investigate appropriate ways to exploit heterosis in commercial hybrids (Cantrell 1986).

Pandey and Gardner (1992) extensively review maize breeding strategies in the developing world, including CIMMYT's strategy. They consider breeding objectives such as morphological and physiological traits, drought tolerance, cold tolerance, tolerance of soil acidity, and disease and insect resistance, suggesting maize germplasm with useful attributes for addressing the different breeding objectives.

More than 30 gene pools adapted to different ecologies and belonging to different maturity and grain-type groups have been improved using a modification of modified-ear-to-row (MER) selection (Table 2.2) (De Leon and Pandey 1989). These broad-based populations are improved by MER in Mexico. Superior fractions of these pools are routinely merged with other existing populations and are improved using FS selection and distributed through international trials.

Several additional selection projects with more limited objectives have been conducted at CIMMYT, including selection on reduced plant height, drought tolerance, earliness, nitrogen use efficiency, quality protein, disease and insect resistance, and tolerance to soil acidity (Pandey and Gardner 1992). Reduced plant height has been pursued in tropical maize as in wheat and rice, but through a very different strategy. Single dwarfing genes (e.g., "*brachytic-2*": *br-2*) have been found in maize, but they have a number of undesirable effects on phenotype in addition to simply reducing plant height (Wellhausen 1978). Hence, while *br-2* has been used with some success in Brazil (Paterniani 1990) and Hawaii (Djisbar and Brewbaker 1987), considerable additional selection must be applied to reduce the undesirable effects of the gene. Simple recurrent selection for polygenic reduction of plant height has generally been more productive in maize than the use of single dwarfing genes, as in wheat and rice (Paterniani 1990).

4. Documentation of Gains of CIMMYT Maize Breeding Program. Genetic progress in CIMMYT's maize program is well documented. In a comparison of cycles of selection for eight tropical pools, highly significant ($P<0.01$) average gains cycle^{-1} were realized for yield, days to silk, plant height, stalk-rot score, and percentage ear rot (De Leon and Pandey 1989) (Table 2.3).

Five cycles of modified FS recurrent selection was practiced in five late tropical maize populations (Pandey et al. 1986). Highly significant

Table 2.2. Maize gene pools and populations developed and improved by CIMMYT for the tropical lowlands.

Name of material	Other name
Maize gene pools	
Tropical Early White Flint	Pool 15
Tropical Early White Dent	Pool 16
Tropical Early Yellow Flint	Pool 17
Tropical Early Yellow Dent	Pool 18
Tropical Intermediate White Flint	Pool 19
Tropical Intermediate White Dent	Pool 20
Tropical Intermediate Yellow Flint	Pool 21
Tropical Intermediate Yellow Dent	Pool 22
Tropical Late White Flint	Pool 23
Tropical Late White Dent	Pool 24
Tropical Late Yellow Flint	Pool 25
Tropical Late Yellow Dent	Pool 26
Tropical White Flint QPM	
Tropical White Dent QPM	
Tropical Yellow Flint QPM	
Tropical Yellow Dent QPM	
Maize populations	
Tuxpeño-1	Population 21
Mezcla Tropical Blanco	Population 22
Blanco Cristalino-1	Population 23
Antigua Veracruz-181	Population 24
Blanco Cristalino-3	Population 25
Mezcla Amarilla	Population 26
Amarillo Cristalino-1	Population 27
Amarillo Dentado	Population 28
Tuxpeño Caribe	Population 29
Blanco Cristalino-2	Population 30
Amarillo Cristalino-2	Population 31
ETO Blanco	Population 32
Antigua República Dominicana	Population 35
Cogollero	Population 36
Tuxpeño *o2*	Population 37
Yellow QPM	Population 39
White QPM	Population 40
Composite K *o2*	Population 41
La Posta	Population 43
Early Yellow Flint QPM	Population 61
White Flint QPM	Population 62
Blanco Dentado-1 QPM	Population 63
Blanco Dentado-2 QPM	Population 64
Yellow Flint QPM	Population 65
Yellow Dent QPM	Population 66

Table 2.3. Means of different cycles of eight tropical maize gene pools improved at CIMMYT using a modification of modified ear-to-row selection.

Pool	Cycle	Yield (t ha^{-1})	Days to silk	Plant height (cm)	Stalk rot rating (1–5)[z]	Ear rot (%)
TEWF	C0	3.62	65	172	3.9	—
	C5	4.40	66	158	3.7	—
	C8	4.36	64	158	3.3	—
	C11	4.33	62	155	3.2	—
LSD (P<0.05)		0.57	1.4	7.9	0.2	—
TEWD	C0	3.48	65	171	3.8	—
	C5	4.52	65	161	3.5	—
	C8	4.84	65	162	3.1	—
	C11	5.14	63	162	3.1	—
LSD (P<0.05)		0.38	0.8	9.0	0.2	—
TEYF	C0	2.69	65	152	4.1	—
	C5	3.73	66	157	3.9	—
	C8	3.93	64	148	3.6	—
	C11	4.36	62	151	3.3	—
LSD (P<0.05)		0.44	1.6	4.8	0.3	—
TEYD	C0	2.72	65	146	3.8	—
	C5	4.42	66	149	3.4	—
	C8	4.12	65	147	3.2	—
	C11	4.62	62	148	2.9	—
LSD (P<0.05)		0.35	1.0	8.5	0.3	—
TIYD	C0	5.46	72	199	3.5	—
	C7	5.89	69	175	3.2	—
	C12	5.83	71	177	3.1	—
	C16	6.40	70	182	2.8	—
LSD (P<0.05)		0.63	2.2	6.6	0.2	—
TLWF	C0	5.98	73	201	3.4	—
	C7	5.55	71	182	3.4	—
	C11	5.92	70	169	2.9	—
	C15	6.18	70	171	2.7	—
LSD (P<0.05)		0.46	2.0	7.5	0.2	—
TIWD	C0	4.43	73	190	—	31
	C7	4.31	70	169	—	35
	C10	4.62	69	167	—	31
	C15	5.19	67	168	—	24
LSD (P<0.05)		0.37	2.1	4.3	—	—
TLYF	C0	4.04	74	201	—	26
	C5	3.89	73	192	—	23
	C9	4.33	71	183	—	22
	C14	4.86	70	186	—	15
LSD (P<0.05)		0.44	1.0	13.4	—	—
Gain cycle^{-1} (%)[y]		2.50	–0.15	–0.35	–1.66	–0.90

[z]1 = good; 5 = poor

[y]All gains are significant at the P<0.01 level of probability.

Table 2.4. Progress from selection in five late maturity tropical maize populations improved using full-sib recurrent selection and international testing at CIMMYT.

Population	Cycle	Yield (t ha^{-1})	Days to silk	Ear height (cm)	Ears plant^{-1}
Tuxpeño-1	C0	5.98	66	112	0.96
	C2	6.04	65	110	0.97
	C5	6.34	66	114	0.96
Antigua Veracruz-181	C0	5.68	67	127	0.96
	C2	5.33	67	117	0.94
	C5	5.76	66	117	0.96
Amarillo Cristalino-1	C0	5.27	68	120	0.94
	C2	5.28	67	123	0.95
	C5	5.75	66	117	1.00
Tuxpeño Caribe	C0	6.20	67	120	0.93
	C2	6.01	66	122	0.92
	C5	6.21	65	111	0.95
Cogollero	C0	5.71	66	136	0.93
	C2	5.57	66	131	0.98
	C5	6.25	63	114	0.99
LSD (P<0.05) within population		0.31	0.8	5	0.04
Gain cycle^{-1} (%)[z]		1.31	–0.59	–1.77	0.87

[z]All gains are significant at the P<0.01 level of probability.

(P<0.01) increases in yield and ears plant^{-1}, and decreases in days to silk and ear height were achieved (Table 2.4). Five cycles of FS recurrent selection, based on international testing of the progenies in two medium maturity populations (Blanco Cristalino-1 and Mezcla Amarilla), resulted in highly significant gains for yield, days to silk, ear height, and ears plant^{-1} (Pandey et al. 1987) (Table 2.5).

Fifteen cycles of selection for reduced plant height, using modified FS selection, were evaluated by Johnson et al. (1986). Selection was strikingly effective in reducing plant height, by more than 1 m (2.4% cycle^{-1}), while lodging decreased from 43 to 5%. Yield measured at 64,000 plants ha^{-1} increased more rapidly than at 50,000 plants ha^{-1}: 4.4 vs. 3.7% cycle^{-1} (Table 2.6). Harvest index increased from 0.30 to 0.45 over the 15 cycles of selection.

Population Tuxpeño Sequía was improved for tolerance to mid-season drought over eight cycles using FS recurrent selection (Table 2.7). Yield gains averaged 0.08 t ha^{-1} (3.8%) cycle^{-1} in water-stressed environments and 0.05 t ha^{-1} (0.5%) cycle^{-1} under irrigation. Total biomass was unaffected by selection (Edmeades et al. 1999). The effects of selection for drought tolerance on performance of tropical maize were examined

Table 2.5. Gains from full-sib recurrent selection in two medium maturity maize populations at CIMMYT.

Population	Cycle	Yield (t ha^{-1})	Days to silk	Ear height (cm)	Ears plant^{-1}
Blanco Cristalino-1	C0	5.11	64	113	0.91
	C2	5.64	62	103	0.98
	C5	5.29	63	108	0.97
Mezcla Amarilla	C0	4.97	63	108	0.96
	C2	4.64	62	93	0.96
	C5	5.40	60	101	0.98
LSD (P<0.05) within population		0.28	0.7	5	0.04
Gain cycle^{-1} (%)[z]		1.22	–0.60	–0.84	0.80

[z]All gains are significant at the P<0.01 level of probability.

Table 2.6. Progress from modified full-sib selection for reduced plant height in the population Tuxpeño at CIMMYT.

		Grain yield (t ha^{-1})			
Cycle of selection	Plant height (cm)	64,000 plants ha^{-1}	50,000 plants ha^{-1}	Lodging (%)	Harvest index (%)
0	282	3.17	3.13	43	30
6	219	4.29	4.24	12	40
9	211	4.48	4.31	14	40
12	202	4.93	4.71	9	41
15	179	5.40	5.03	5	45
LSD (P<0.05)	22	0.30	0.37	7	4
Gain cycle^{-1} (%)[z]	–2.39	4.43	3.74	—	3.10

[z]All gains are significant at the P<0.01 level of probability.

Table 2.7. Yield, total biomass, and harvest index of Tuxpeño Sequía selected for drought tolerance using full-sib selection at CIMMYT.

	Yield (t ha^{-1})		Biomass (t ha^{-1})		Harvest index %	
Entry	Stress	Optimum	Stress	Optimum	Stress	Optimum
TS CO	1.75	7.48	6.31	21.02	12	40
TS C8	2.39	7.78	7.24	20.44	22	41
Gain cycle^{-1}	0.08	0.04	0.116	–0.073	1.3	0.1
% gain cycle^{-1}	3.8	0.5	1.8	–0.3	10.4	0.3
CO vs. C8	**	**	ns	ns	*	ns

under a range of N levels. Original and advanced selections (two to eight cycles of selection) of four populations improved for tolerance to drought were evaluated to study indirect responses. Selection for tolerance to drought increased grain yield by an average of 0.086 t ha^{-1} yr^{-1} with larger (but nonsignificant) gains under severe N stress (0.10 t ha^{-1} yr^{-1}). Drought-tolerant selections had greater biomass and N accumulation at maturity, the changes being greatest under severe N stress (Banziger et al. 1999).

Fifteen cycles of MER selection were practiced for early maturity in the population Compuesto Selección Precoz (Narro 1988). The cycles were evaluated in three environments at two planting densities (53,000 or 75,000 plants ha^{-1}). While no significant change was observed for yield, highly significant gains were made for days to silk (–1.0%), plant height (–1.2%), ear height (–2.0%), leaf area (–1.3%), and ears $plant^{-1}$ (0.72%) (Table 2.8).

In spite of the genetic gains documented in CIMMYT maize populations, genetic variation within populations has been maintained (Gardner et al. 1990). This is probably owing to low selection intensity, and periodic introgression of new germplasm into the populations being improved.

5. Documentation of NARS Breeding Progress. While maize breeding in the developing world began long before the founding of CIMMYT, maize breeders in the tropics and subtropics readily acknowledge the positive impact on their own breeding progress of CIMMYT work, and particularly the diverse and improved germplasm that has been made available

Table 2.8. Progress from modified ear-to-row selection for early maturity in the population Compuesto Selección Precoz at CIMMYT.

Cycle of selection	Yield (t ha^{-1})	Days to silk	Plant height (cm)	Ear height (cm)	Leaf area (cm^2 $plant^{-1}$)	Ears $plant^{-1}$
0	3.2	71	209	112	6300	0.74
3	2.8	67	193	96	5816	0.73
6	3.4	65	192	94	5738	0.79
9	3.1	64	188	91	5530	0.77
12	3.4	60	176	82	5340	0.81
15	3.2	58	172	75	5097	0.81
LSD (P<0.05)	0.40	2.3	5.6	3.3	219	0.05
Gain $cycle^{-1}$ (%)	ns	–1.0**	–1.2**	–2.0**	–1.3**	0.72**

**Significant at P<0.01 level of probability.

through CIMMYT's Maize Program (e.g., Paterniani 1990). Recurrent selection is very widely practiced by developing country maize breeders (Pandey and Gardner 1992), including both intrapopulation (mass, HS, MER, FS, and S1 selection) and interpopulation, reciprocal selection schemes (reciprocal HS, reciprocal FS).

A broad array of breeding objectives is listed for tropical maize: plant and ear height, prolificacy, reduced tassel size, disease and insect resistance, drought tolerance, Al tolerance, nutritional quality, tolerance to low temperature, N use efficiency, and early maturity. In most cases, where sustained selection has been applied, phenotypic modification of populations has been readily achieved.

Mass selection is the simplest method logistically and has been widely used. Darrah (1986) reported yield gains of 2.3% cycle^{-1} in the KCA population after 10 cycles of mass selection. Araujo et al. (1989) used stratified mass selection in a dent, and in a flint population for low corn earworm damage, high ear weight, husk compaction, and extension of husk beyond the ear. In the dent population, ear damage was reduced by 5.53%, and husk compaction and husk extension increased by 0.75 and 0.78% cycle^{-1}. In the flint population, the corresponding gains cycle^{-1} were 1.60, 1.66, and 2.33%. Ten cycles of mass selection for prolificacy in the population BC10 resulted in increases of 2.6% for yield, 4.4% for number of ears m^{-2}, and 3.2% for number of grains m^{-2} (Subandi 1990). However, plants became slightly taller (2.7 cm cycle^{-1}) and later (0.1 d cycle^{-1}).

Physiological and biochemical parameters were compared between C_0 and C_{22} of the population Zacatecas 58 (Sarquis et al. 1998), where stratified mass selection was practiced for yield. Plants of C_{22} were 0.5 m taller, had almost twice the amount of leaf area at anthesis (2600 versus 1330 cm^2 plant^{-1}), accumulated more than twice the amount of aboveground dry matter (294 versus 133 g plant^{-1}), had a 30% higher harvest index, and yielded 2.7 times more than C_0. Photosynthetic rate was greater in C_{22}, but dark respiration rate was 20% lower than in C_0.

Song et al. (1999) conducted 11 cycles of mass selection for kernel oil content in Zhongzong-2 maize synthetic. The oil content of the final cycle population, designated as BHO, was 11.25% of kernel dry weight—1.39 times higher than in C_0. Average gain cycle^{-1} was 0.54% and the realized heritability was 0.42. The increase in oil content did not affect kernel weight and was positively correlated with protein content and negatively correlated with starch content. Improvement in oil content was associated with increased seed protein and lysine contents, but lower plant height, grain weight, ear weight, grain weight ear^{-1}, ear diameter, and total grain yield.

There are fewer reports in the literature on the long-term use of the more complex recurrent selection methods in the improvement of maize in developing countries. Darrah (1986) conducted 10 cycles of modified ear-to-row selection in KCA with gains of 2.9% cycle^{-1}. Yield improved 3.6 or 0.9% cycle^{-1} over five cycles of FS or S1 recurrent selection, respectively, in the same population. De Carvalho et al. (2000) conducted five cycles of selection among and within HS families (one complete cycle of selection yr^{-1}) in the population BR 5011-Sertanejo, obtaining a yield increase of 12.7% cycle^{-1}, with no reduction of genetic variability at the end of the fifth cycle of selection.

Response to selection for tolerance of soil acidity was studied using an altered version of MER followed by FS selection (Granados et al. 1993). For the first 16 cycles, 180 HS families were evaluated each cycle under 45 or 80% aluminum (Al) saturation. One to three ears from each of the best 30% of the families were selected each cycle. Following 16 cycles of MER selection, FS selection was initiated. Two hundred fifty FS families were evaluated in five to six acid soil environments (ASEs) and one normal soil environment (NSE) each cycle and the best 25% selected. Gain from selection on yield, measured in six ASEs and five NSEs, averaged 0.040 t ha^{-1} cycle^{-1} (1.49%) with MER or 0.250 t ha^{-1} cycle^{-1} (8.10%) with FS selection. Across the six ASEs, yield improvement of 0.040 t ha^{-1} cycle^{-1} (1.99%) with MER and 0.310 t ha^{-1} cycle^{-1} (13.96%) with FS selection were obtained. Yield measured in the NSEs also improved, by 0.050 t ha^{-1} cycle^{-1} (1.10%) with MER and 0.150 t ha^{-1} cycle^{-1} (3.31%) with FS selection.

Cycles 0, 2, 4, and 6 of CIMMYT maize Population 43 (La Posta), improved by FS family selection for grain yield and other traits, were evaluated at 0, 80, or 160 kg ha^{-1} N at 6 locations in Ghana (Sallah et al. 1998). Grain yields across environments and cycles averaged 3.0, 4.7, or 5.2 t ha^{-1} at 0, 80, or 160 kg ha^{-1} N, respectively. Progress cycle^{-1} of selection for grain yield, days to mid-silk, plant height, and lodging were 1.6, –0.6, –1.5, –1.1% at 0 kg ha^{-1} N; 2.1, –0.8, –1.5, –3.5% at 80 kg ha^{-1} N; and 1.8, –0.7, –1.4, –2.8% at 160 kg ha^{-1} N, respectively. These results indicate that selection was effective for improving all traits at 80 and 160 kg ha^{-1} N, and that recurrent selection for improved agronomic performance had little effect on the N fertilizer response of the population.

Five cycles of FS selection were practiced for resistance to head smut and ear rot, for reduced plant height, and for root and stalk quality in the population PABGT-CE (Ramírez-Díaz et al. 2000). Plant height was reduced by 7.48 cm and ear height by 5.83 cm cycle^{-1}. Percent ear rot was reduced 0.97% cycle^{-1}. However, grain yield decreased 0.130 t ha^{-1}

cycle^{-1} (not significant), probably because of reductions in plant height and lodging resistance. Ochieng and Kamidi (1992) obtained no improvement in the populations KSII and Ec573 per se following eight cycles of reciprocal recurrent selection, but reported a 3.56% improvement cycle^{-1} in the population cross.

A more recent phenomenon in maize breeding in the developing world is the increasing participation of the private sector. It is estimated that 20% of maize grown in the developing world, particularly Latin America (Morris and Pereira 1999) and Asia (Gerpacio 2001), is from hybrid cultivars developed by the private seed sector. The private sector has promoted biotechnology products, such as hybrids containing herbicide- and insect-resistance genes imported from developed countries. These products target maize producers in the commercial sector who can afford them. It is anticipated that genetic improvement programs conducted with public funds will continue to serve producers in the more marginal agricultural sectors in the developing world, as they do not represent attractive markets for private seed companies.

6. Impact on National Productivity. Maize is still extensively grown as a subsistence crop in the developing world. Only about half of the area planted to maize in the developing world is in improved cultivars. Of improved maize, nearly three-quarters is hybrid cultivars, the remainder being improved open-pollinated cultivars. The relatively low documented adoption of improved open-pollinated maize is probably owing to the reluctance of commercial seed producers to commercialize OP cultivars (Wellhausen 1978). There may, however, be much more use of improved maize germplasm than is documented, through intentional or unintentional introgression of improved germplasm into farmers' landraces, and the informal farmer-to-farmer exchange of seed. Wellhausen (1978), for instance, reports massive replacement of traditional maize cultivars, at least in the lowland tropics, following the wide distribution of improved CIMMYT populations.

Average maize yield increases are about the same in the developing as in the developed world (nearly 2.2% yr^{-1}), but of course from a far lower base (~3 t ha^{-1} vs. ~8 t ha^{-1}) (Pingali and Pandey 2001). However, a smaller proportion of the yield improvement in the developing world is attributable to genetic improvement, as large gains are readily achieved from use of improved agronomy (fertilizer and weed control), in the absence of which large genetic gains are rarely expressed anyway. Much of the gain that is realized is apparently under conditions of more intensive management in the tropics and in temperate regions of the

developing world (e.g., Argentina, China). As with other crops, important gains in on-farm maize yield under the low-management conditions of subsistence farmers in the tropics are difficult to achieve (Paterniani 1990).

B. Rice

1. Brief History of Domestication and Species Diversity. Current evidence suggests a diffuse origin of domesticated rice (Chang 1995), or perhaps more than one independent domestication (Khush and Virk 2002). There are two recognized subspecies: the predominately tropical "*indica*," and the predominately temperate "*japonica*." These may originate from independent domestications, or they may be a result of isolation and selection subsequent to domestication (Chang 1995). A tropically adapted type of *japonica*, known as "*javanica*," is also recognized (Glaszmann 1987). Until about 100 years ago, genetic modification of rice was entirely in the hands of farmers. Over a broad geographical area, they developed a tremendous diversity of types.

2. Early (pre-IARC) Breeding. Modern, institutional plant breeding on government experiment stations began with comparisons of farmers' cultivars in the late nineteenth century in Japan, and somewhat later China and India. Deliberate hybridization programs in Japan began releasing named cultivars as early as 1906 (Chang 1995). These resulted in a fairly rapid extension of rice growing to 45° N latitude. Outside of Japan, hybridization programs had little impact until about 1950. On-farm yield increases resulting from the release of selections among and within farmer cultivars has been documented for China, where adoption of superior selections on collective farms was assured by government decree (Huang et al. 1998). The area in China planted to superior selections rose from 6% in 1952 to 82% just six years later, leading to a 2.2% annual increase in rice yields.

Rice breeding on the tropical Chinese island of Taiwan was conducted by Japanese researchers during Japan's occupation of Taiwan in the 1930s and early 1940s. This work was largely directed towards developing tropically adapted, short statured *japonica* cultivars—the "ponlai" cultivars (Chang 1995). The first semi-dwarf *indica* rice developed was 'Taichung Native 1', released in Taiwan in the mid-1950s. It was probably inspired by the concept of short statured, fertilizer responsive, non-lodging plant type for cereals that Japanese wheat breeders had developed as early as the late nineteenth century.

Breeders in mainland China began breeding semi-dwarf rice cultivars at about the same time as on Taiwan, but this work was not widely known outside of China at the time. Significant adoption was achieved, with consequent impact on Chinese rice production (Huang et al. 1998).

3. Description of IRRI Breeding Program. Four decades ago, when rice breeding at IRRI began, the concept of high-yielding, fertilizer responsive, short statured, non-lodging plant type was already well established for rice. This concept oriented the initial IRRI crosses between semi-dwarf Taiwanese material and tall, traditional cultivars: high yielding ability and responsiveness to N fertilization were the prime breeding objective. Selection in the first hybrid populations led to the development of 'IR8' in 1966 (Chandler 1982). Compared with traditional tall rice cultivars, 'IR8' represented a major advance in rice yield potential (up to 11 t ha^{-1}) and in responsiveness to applied nitrogen (up to 150 kg ha^{-1}) (Chang 1995).

Based on the early success of 'IR8', IRRI and collaborating national rice research programs have continued for four decades to give high priority to plant breeding to improve rice productivity and quality. Breeding objectives broadened beginning in the 1970s to include required resistances to predominant diseases and insects. These included fungal diseases such as blast (*Pyricularia grisea* (Cooke) Sacc.), bacterial blight (*Xanthomonas oryzae* pv. *oryzae* (ex Ishiyama) Swings et al.), sheath blight (*Rhizoctonia solani* Kühn); the viral diseases tungro and grassy stunt; and insect pests such as brown planthopper (*Nilaparvata lugens* (Stål)), green leafhopper (*Nephotettix virescens* (Distant)), stem borers, of which several species are important, including pink (*Sesamia nonagrioides* Lefebre), striped (*Chile suppresalis* Walker), and yellow (*Scirpophaga incertulas* Walker), and gall midge (*Orseolia oryzae* Wood-Mason). Agronomic characteristics included improved grain quality, and early maturity. The general strategy for disease and insect resistance was to find sources of resistance in IRRI's large germplasm collection—generally simply inherited, qualitative resistances—and introgress these into the crossing program. Successes were achieved, leading to rapid shifts in cultivar adoption. Vertical resistance "breakdown" due to the evolution of new races of pathogens or biotypes of insects is anticipated and new resistance genes are continually sought. For some diseases (e.g., sheath blight), useful levels of resistance have not been found (Khush and Virk 2002).

The yield advantage of 'IR8' was fully realized only in intensely managed, irrigated lowland, rice production systems. The target environment of IRRI's breeding program was broadened, and projects were developed

to include rainfed lowland, upland (or dryland), deep-water, and tidal swamp production environments.

4. Documentation of Gains of IRRI Breeding Program. Peng et al. (2000) recently compared IRRI advanced lines and cultivars released in the Philippines since 1966, for yield and components of yield. In spite of attention to a diversity of characters, gain in yield per se, calculated in relation to the yield of 'IR8', was estimated at approximately 1% yr^{-1}—of the same general magnitude as gains reported for breeding programs in other autogamous crops. Yield gains before 1980 were attributable to increase in harvest index (HI), while gains after 1980 were due to increase in total biomass.

Genetic advance towards the broad array of breeding objectives has been documented, particularly for pest and disease resistance (Khush and Virk 2002), earliness, and grain quality. These genetic gains are further attested to by the wide adoption of succeeding IRRI and national program releases (with the replacement of previous cultivars).

One effect of the attention to disease and pest resistance has been the development of genotypes with greatly reduced year-to-year variation in yield (Kush and Virk 2002). In a series of replicated yield trials conducted at IRRI between 1973 and 1986, the yield of 'IR8' ranged between less than 3, to 8 t ha^{-1}, while yields of multiple disease and insect resistant lines 'IR36' and 'IR42' were more consistent, in the range of 6 to 8 t ha^{-1}.

Genetic gains in yield potential for production systems other than the favored intensively managed, irrigated category, were disappointing (Chang 1995), owing to a general environmental limit on yield potential and the wide diversity of local environmental constraints on yield, even within recognized production system types. The diversion of resources to multiple objectives has undoubtedly diminished genetic gains for yield potential in the highly productive target environment of intensively managed irrigated rice.

Cultivars and lines released from IRRI's breeding program were regularly included in national breeding programs, and thus have an impact beyond their direct utility. These germplasm exchanges worked in both directions, with the consequent enrichment of IRRI's elite germplasm base. Hence, the genetic successes of IRRI's breeding program is in no sense a reflection of genetic gain in a closed population under methodical selection, at least in the conventional sense.

5. Documentation of NARS Breeding Progress. Documentation of genetic gain is available from several Brazilian rice breeding programs

(Breseghello et al. 1999; Santos et al. 1999; Soares et al. 1999; Atroch and Nunes 2000). These cover relatively short time spans (7 to 22 years) and genetic gains in yield are estimated, not from direct comparison, but from advanced yield trials conducted over the period, by one or another of several methods where yield comparisons between succeeding years are corrected for a "year effect" by subtracting the mean difference of genotypes common to the two years' trials. Overall estimated yearly gains in these studies range from 0.98 to 3.37% yr^{-1}. A common pattern in these reports is of yearly gain diminishing over the period, which is generally attributed to either diminishing weight given to yield as other selection criteria increase in importance or to the large initial genetic gains as low-yielding, traditional cultivars are replaced by modern, high-yielding cultivars. The IRRI data, from direct comparison of cultivars and lines released over a 30-year period (Peng et al. 2000), do not suggest a diminution in yearly gain.

6. Impact on National Productivity. 'IR8' had much greater impact on global rice production than the earlier Chinese and Taiwanese semi-dwarf rices. IRRI, as an international center, had the interest and resources aggressively to promote 'IR8', along with the requisite package of agronomic practices, widely in tropical Asia.

Total world rice production has doubled since the first IRRI line was released in 1966, and in most of the major rice producing countries in Asia, production has kept up with or exceeded population growth (Khush and Virk 2002). This doubling of total production has been much more dependent on yield increase (71%) than on area sown to rice, which increased only 17%.

C. Bread Wheat

1. Brief History of Domestication and Species Diversity. Hexaploid bread wheat is the result of chance interspecific and intergeneric hybridization, followed by polyploidization, that occurred in a two-stage process: diploid to tetraploid about 10,000 yr before present (BP) and tetraploid to hexaploid about 8,000 yr BP. Three complete genomes are present in modern bread wheat, but in the presence of a dominant allele at the *Ph1* locus, intergenome chromosome pairing is suppressed and the plant behaves as a diploid. Interaction among alleles at homeologous loci confers what may be termed stable internal heterosis. A wide diversity of germplasm was developed prior to modern plant breeding; wheat is grown from 67° N, through the tropics (at higher elevations, up to 3,000–3,500 m above sea level), to 45° S.

2. Early (pre-IARC) Breeding. Organized plant selection programs began in several developing countries from the early years of the last century, for example, in India (Rao et al. 2001) and in China (Sun et al. 2001). These were based, at least initially, on isolating pure lines from heterogeneous landraces. Some of these selections were highly successful, not only in their country of origin, but internationally (Rao et al. 2001).

3. Description of CIMMYT Wheat Breeding Program. The CIMMYT wheat breeding program evolved from the bilateral Rockefeller Foundation program established in Mexico in 1943 (Perkins 1997), and has provided a stable institutional base for wheat breeding in the tropics and sub-tropics for more than 50 years (Rajaram and van Ginkel 2001). The early focus of the Rockefeller Foundation's Mexican wheat improvement program was on resistance to stem rust (*Puccinia graminis* f. sp. *tritici*). With the introduction of derivatives of the Japanese semidwarf cultivar, 'Norin 10' (via USDA wheat breeder O. A. Vogel) in 1953 (Perkins 1997), a strategy of seeking short-statured, lodging resistant, high-yielding, nitrogen responsive wheat cultivars was established.

Currently CIMMYT wheat breeding involves a massive hybridization program (~8,000 crosses yr^{-1}), exploiting a broad and constantly renewed genetic base (500–800 parental lines in any given year), combined with extensive international testing (Rajaram and van Ginkel 2001). CIMMYT-derived wheat germplasm is used heavily in national wheat breeding programs.

Attention has been placed on resistance or tolerance to biotic and abiotic stresses. In contrast to IRRI's strategy, based on qualitative resistance, at CIMMYT a deliberate strategy has been followed to exploit "horizontal" or "non-specific" resistance to diseases and insect pests, with resistance genes derived from diverse sources. The strategy appears to have been effective in achieving durable resistance; even for leaf rust (*Puccinia recondita* f. sp. *tritici*), a notoriously variable pathogen, "no major epidemic has been observed for over 20 years" (Rajaram and van Ginkel 2001).

The breeding strategy followed for drought tolerance emphasizes combining adaptation (and high yield) under stress conditions with the ability to respond to favorable conditions (Braun et al. 1996). This has been achieved by alternating selection generations between drought stressed and favorable (irrigated) conditions.

Multiple generations $year^{-1}$ of field trials are often easily achieved in plant breeding programs conducted in the tropics. From the early years of the Rockefeller Foundation's wheat improvement program, in order to accelerate breeding progress two generations were routinely grown at

contrasting sites in Mexico, an irrigated, winter site at sea level and a high rainfall, disease prone summer site at more than 2,600 m (Braun et al. 1992). This practice had the inadvertent effect of producing germplasm with broad adaptation conferred in large measure by photoperiod insensitivity and a broad disease resistance spectrum.

4. Documentation of Gains of CIMMYT Wheat Breeding Program. While concern about "yield barriers" is expressed (Reynolds et al. 1996), wheat cultivars released in Mexico, for example, show steady improvement in yielding ability (0.88% yr^{-1}, over nearly three decades) when compared directly in the same high-yield environment (Sayre et al. 1997). Attempts have been made to discover the specific traits responsible for this yield improvement in the hopes of facilitating future gains. Sayre et al. (1997) found a high correlation between grain yield and HI ($r = 0.81$) and kernel number m^{-2} ($r = 0.84$), but not with kernel weight or other yield components, nor with crop duration. Unlike Waddington et al. (1986), they found no evidence of an association between grain yield and total crop biomass. Using the same set of wheat genotypes, Fischer et al. (1998) found a high correlation between grain yield and stomatal conductance ($r = 0.94$), maximum photosynthetic rate ($r = 0.85$), and canopy temperature depression ($r = 0.76$) and suggested that one or more of these characters may be useful as indirect selection criteria for grain yield, at least under favorable conditions.

5. Documentation of NARS Breeding Progress. The genetic impact of long-term wheat breeding programs in several developing countries have been documented (Bonjean and Angus 2001). Most of these national wheat improvement programs have made heavy use of CIMMYT-derived germplasm.

6. Impact on National Productivity. Wheat yields for the developing countries as a whole have increased at a rate of 2% $year^{-1}$ between 1961 and 1994 (Rajaram and van Ginkel 2001). This increase has been achieved by a combination of adoption of modern, high yield potential wheat cultivars with improved management.

D. Common Beans

1. Brief History of Domestication and Species Diversity. Wild *P. vulgaris* has a wide natural distribution in the New World tropics and subtropics, from Mexico to northwestern Argentina. At least two independent domestication events, one in Mesoamerica and another in the Andes, are

postulated, supported by morphological, isozyme, and RFLP evidence (Debouck and Smartt 1995). These independent domestications gave rise to two major common bean genepools. Archeological evidence of fully domesticated beans goes back to 6,000 to 7,000 yr BP in Mexico, and 8,000 to 10,000 BP in Peru. The true antiquity of bean domestication is unknown, but these dates set lower limits.

Farmer selection throughout the Americas led to a vast diversity of bean cultivars, which differ in growth habit, seed size, shape, and color. Bean germplasm moved extensively even prior to the European conquest of the Americas, as evidenced by Mesoamerican types throughout the Caribbean, northwestern South America and as far south as modern Brazil, and Andean types in the Caribbean. The movement of bean germplasm accelerated after 1492. Domesticated beans can be crossed with wild *P. vulgaris* without serious infertility barriers. Barriers between some cultivars of the Mesoamerican and of the Andean gene pools have been encountered, but these appear to have a very specific genetic basis (Singh 2001).

2. Early (pre-IARC) Breeding. Several countries, mostly in Latin America, have a long tradition of common bean breeding. The earliest record of common bean breeding as an organized, state-supported activity in Latin America is in Colombia, where work began in 1929 (Voysest Voysest 2000). This work was expanded in the 1950s, following the initiation of a Rockefeller Foundation project in Colombia, such that bean breeding work was being carried out at experiment stations from 1,000 to 2,700 m. Several broadly adapted, high-yielding black-seeded bean cultivars ('ICA Huasanó', 'ICA Tuí', and 'ICA Pijao') were developed during the 1960s. These lines were widely tested internationally through CIAT bean networks and 'ICA Pijao' was released in at least eight different countries, including one (Mozambique) in Africa. Bean breeding in Brazil dates from the initiation of a program at the IAC in 1930. IAC, as well as other state and university bean breeding programs and (from the mid-1970s) EMBRAPA's Rice and Bean Center (CNPAF) have continued to be very productive in developing a long list of improved cultivars (Voysest Voysest 2000).

Bean breeding on a more intensive scale was supported by the Interamerican Institute of Agricultural Sciences (IICA) with the establishment of a research and teaching center at Turrialba, Costa Rica in 1942, and by the Rockefeller agricultural projects in Mexico and Colombia during the 1940s and '50s. A Central American cooperative program for the improvement of food crops (PCCMCA, its Spanish acronym) was established in 1955, followed seven years later by the establishment of a

cooperative program specifically dedicated to bean improvement (PCCMF) in the region. The PCCMF organized regional trials and was responsible for the diffusion and adoption of bean selections throughout the Central American region.

3. Description of CIAT Bean Breeding Program. Eight years after the founding of CIAT in 1967, a team of researchers was organized around the topic of bean improvement. Early efforts involved assembling a diverse collection of bean germplasm, which was evaluated and distributed to collaborating bean researchers in national research programs through a regional trials network (Bean Yield and Adaptation Nursery: IBYAN). A bean hybridization program began in 1975, and within three years, CIAT-developed finished lines were being distributed through IBYAN. Over the 20 years of its existence (1976–1995), IBYAN distributed a total of 1,500 lines to national program collaborators, of which 120 were selected and released directly as named cultivars in one or more countries.

A large collection of common bean germplasm was assembled by CIAT, now totaling approximately 26,000 accessions. These have been routinely evaluated and lines possessing useful attributes have been distributed directly and incorporated into the hybridization projects.

The focus of bean breeding, either at CIAT or in national programs, has never been strictly on yield potential per se. Although ample genetic variation for yield has been demonstrated in tropical bean germplasm (Nienhuis and Singh 1988), the wide gap between average on-farm yields in tropical countries (often no more than 0.70 t ha^{-1}) and yield potential of existing bush bean cultivars (at least 3 t ha^{-1}) argued strongly in favor of addressing biotic and abiotic constraints to realizing demonstrated yield potential. Stringent consumer demand for specific grain types further slowed progress. These factors, coupled with a focus on small farmer production systems at a low level of management, has restricted the potential to achieve genetic gain for grain yield per se.

Bean breeding at CIAT was subdivided, first by bean gene pool (Mesoamerican or Andean), then by grain type and target production region (CIAT 2000), such that several parallel breeding programs were conducted simultaneously.

Much of CIAT's bean breeding work is dedicated to developing, in more or less isolated populations, resistance or tolerance to specific biotic or abiotic constraints (e.g., anthracnose resistance, or drought tolerance). Promising parental genotypes for specific traits are then incorporated into crosses for varietal development, either for selection by CIAT breeders or by national programs. A major effort is directed at

developing commercial lines with high levels of resistance to bean golden mosaic and other virus diseases (Morales 2000).

4. Documentation of Gains of CIAT Bean Breeding Program. Quantitative documentation of the genetic gains for yield (or any other trait) accruing to more than 25 years of bean improvement work at CIAT (1976–2001: probably between 50 and 75 generations) is scarce. Progress in bean breeding has generally been reported in descriptive terms of combinations of resistances to different insects and diseases, rarely with accompanying direct quantitative comparisons for yield (Singh 1999; Morales 2000; J. S. Beaver, 2002, unpublished).

Amézquita and Voysest (1987) attempted to document progress in breeding for resistance to five bean diseases using data from CIAT preliminary and advanced nurseries. However, the time period (8 yr: 1979–86) and the structure of the trials are not really adequate to establish a long-term trend in breeding progress, which, in any case, does not appear to be very sustained, even over the short time under study.

The success of the CIAT bean program in resistance breeding is best illustrated with the case of a widely grown genotype, DOR 364, which was the first red-seeded cultivar resistant to bean golden yellow mosaic virus (BGYMV) in Central America. In a 1989 trial, it yielded 1.238 t ha^{-1} under disease pressure when the best local cultivar yielded only 0.298 t ha^{-1} (Orozco et al. 1990). As white fly vector populations increased over time and began to overcome DOR 364's resistance, it was necessary to accumulate more resistance genes to meet the challenge of BGYMV. One especially effective gene, *bgm-1*, recovered from a Mexican accession, G2402 ('Garrapatos'), significantly increased resistance in combination with genes in DOR 364. Subsequent lines such as DOR 482 received a disease rating of 2 (on a 1=immune to 9=totally susceptible scale), while DOR 364 received a rating of 4 (Orozco and Beebe 1990). This higher level of resistance has been maintained and is now widely deployed in breeding lines developed within the region.

The gains in breeding for BGYMV resistance and the incorporation of resistance genes into a wider array of genetic backgrounds has led to recent improvements in yield. Many crosses made in Central America (especially at the Panamerican School in Zamorano, Honduras) combine parents that are resistant but that offer sufficient diversity in yield to permit genetic gain. It is now possible to isolate lines that yield 15–20% more than DOR 364 even in the absence of disease pressure (PROFRIJOL 2001). Marker-assisted selection is being utilized to recover key BGYMV resistance genes (CIAT 2000).

Several authors report on-farm yield advantages from "improved" over traditional bean cultivars. Viana Ruano (1998) reports a yield advantage of 0.205 t ha^{-1} for improved bean cultivars over traditional cultivars in Central America in a small farmer, low input context. Although modest in absolute terms, this represents a substantial percentage improvement (29%) since yield levels are low (0.918 vs. 0.713 t ha^{-1}). Johnson et al. (2002a) quantifies on-farm yield gains owing to substitution of "CIAT-related" (i.e., improved) bean cultivars for traditional cultivars in 13 countries of Latin America and the Caribbean. These gains range from 0.100 to 0.350 and average 0.210 t ha^{-1} for the region, strikingly similar to the improvement found in Central America alone. However, it is not possible from this analysis to separate genetic gain from increases due to agronomic management.

Although most effort was dedicated to disease resistance breeding, attempts to increase yield in the CIAT bean breeding program focused on exploiting genetic diversity among bean races, especially races Durango and Mesoamerica, as practiced by Kelly and Adams (1987) to improve yield of pinto bean cultivars in Michigan. In the tropical context, the line A774 best illustrates this effort. A774 is a small cream-colored bean of the type popular in northeast Brazil. Its parentage consists of 15.6% Durango genotypes combined with race Mesoamerica. In trials across ten sites in Brazil, it outyielded local checks by 34% (CIAT 1991).

5. Documentation of NARS Breeding Progress. Bean yield gains attributed to breeding progress of 1.9% yr^{-1} over an 18-year period (1972/73 to 1990/91) are reported for a Brazilian state program (Abreu et al. 1994). Gains were estimated from yields measured in advanced cultivar evaluation trials conducted over the period, where the regression of trial means over years was adjusted for the regression of a check cultivar included in all trials.

A long list of bean cultivars have been released by Latin American NARS (Voysest Voysest 2000). Documentation of adoption is difficult in the absence of an organized seed industry.

6. Impact on National Productivity. Johnson et al. (2002a) estimate a total incremental bean production owing to CIAT bean genetic improvement programs of 380,000 t in 13 countries in Latin America and the Caribbean, and an additional 88,000 t in 7 African countries. This represents less than 10% of the combined production (5,106,660 t in 1998) of Africa and Latin America (Johnson 2002a). However, the production

increments reported are admittedly low, as several important bean-producing countries (e.g., Mexico) are excluded owing to lack of reliable data on adoption. In any case, the total increment (468,000 t) does not necessarily reflect only genetic gain. Improvements in on-farm bean yield are constrained in the developing world by the marginal land on which beans are commonly grown and the generally low level of management given to bean crops (J. S. Beaver, pers. commun., April 2002).

E. Cassava

1. Brief History of Domestication and Species Diversity. The tropical root crop cassava (manioc, tapioca) is an important source of calories in human diets, ranking fourth in the tropics (after rice, sugar, and maize). The plant is monoecious and normally insect-pollinated and outcrossing. Selected genotypes are commercially propagated by vegetative stem cuttings.

Manihot esculenta is an American domesticate. It is unknown in the wild, and its wild progenitor species is not known with certainty (Byrne 1984). It was likely developed from a wild form through selection for root yield and ease of vegetative propagation. A very wide diversity exists among cultivated clones, developed through many generations of adaptation to environmental conditions and conscious or unconscious selection for agronomic and culinary characters. Diversity may have been enhanced through introgression from one or more of the wild *Manihot* species, which are more or less cross-compatible with *M. esculenta* (Jennings and Hershey 1984). In many cultivated types, uncooked roots contain toxic levels of HCN, which may confer protection against diseases or predators. Toxicity is eliminated by traditional preparation methods, including fermentation. In fact, the additional N contained in bitter cassava probably makes a significant contribution to the protein content of fermented products after transformation by the microorganisms involved (Rogers and Appan 1970).

2. Early (pre-IARC) Breeding. Institutional-based cassava breeding has a long history, with programs dating from the first half of the twentieth century in Brazil, Ghana, Tanzania, Madagascar, Nigeria, and India. All except Brazil were at the time tropical colonies of European countries (Byrne 1984). Koshy (1947) describes intra- and interspecific *Manihot* hybrids (*M. esculenta* × *M. glaziovii*), and outlines a cassava improvement strategy including polyploidization, interploidy hybridization (to produce triploid cultivars), and reports results of forced selfing.

Developing resistance (or tolerance) to the devastating cassava mosaic disease (CMD: caused by a white-fly-transmitted geminivirus) was the motivation of early cassava breeding programs in East Africa (Hahn and Theberge 1987). An additional objective from the start was to breed higher-yielding clones. Useful resistance to CMD was transferred to *M. esculenta* from *M. glaziovii* by hybridization and backcrossing in a program in East Africa (Nichols 1947), and derivatives of this material formed the basis of subsequent breeding work at IITA (Hahn et al. 1980; Beck 1982).

3. Description of CIAT and IITA Cassava Breeding Programs. IITA and CIAT were established almost simultaneously in the late 1960s in Ibadan, Nigeria, and in Cali, Colombia, respectively. In recognition of cassava's important role as a basic food crop, especially for the poor in the low-land tropics, both Centers focused attention on its improvement, IITA for tropical Africa and CIAT for tropical America and Asia. CIAT, based in tropical America, took responsibility for acquiring and maintaining the world cassava germplasm collection. The general organization and strategy of these programs is described in Jennings and Hershey (1984). K. Kawano (unpublished) thoroughly reviews three decades of cassava genetic improvement in his program, which began at CIAT headquarters in Colombia and subsequently transferred to a southeast Asian regional program based in Thailand. The target environment of early cassava genetic improvement was not, as was the case with rice and wheat for instance, highly modified production environments at a high level of crop management. The objective was rather to identify genotypes that would produce better yields than traditional, farmer-selected clones, under "low input conditions in less favorable environments." Later breeding work has focused on cassava grown for industrial use under better crop management, particularly in Asia.

Large germplasm collections, mainly of cultivated *M. esculenta* were assembled and evaluated (Hershey 1987). The general breeding strategy developed at both IITA and CIAT was basically intrapopulation recurrent population improvement, seeking simultaneous improvement in a series of important traits (Byrne 1984). There seems to be no strategy (e.g., reciprocal recurrent selection) deliberately to exploit heterosis, which would be easy to capture in this vegetatively propagated crop.

A key development in the CIAT breeding program was the evolution in thinking about testing sites (Kawano 1987). Early hybrids selected in the very favorable environment of the CIAT headquarters farm failed completely when subsequently transferred to the more rigorous

environment of the Colombian north coast. Target environments were eventually rationalized into "edapho-climatic zones" (ECZs) by CIAT breeders (Hershey 1984). It was only after the main selection process was moved to sites more representative of real, on-farm production conditions that useful hybrid clones were identified. Three trial sites—at CIAT headquarters, on the north (Caribbean) coastal region, and in the Llanos Orientales (Eastern Plains)—were routinely used in the Colombian program.

Owing to cassava's inherently low multiplication rate, a system of sequential trial stages, beginning with unreplicated seedlings and progressing to multilocational, replicated, bordered yield test plots (described in detail in Kawano et al. 1998) was adopted to evaluate new clones. Clones judged to be inferior at each stage are culled based on progressively more reliable data as the volume of propagating material permits larger plots, greater replication, and the possibility to test in multiple locations. Both Centers have developed more or less systematic schemes for recurrent selection, though it takes 6–8 yr to complete a single selection cycle owing to the time lag between the origin of a new clone as a single seedling and the stage when replicated, multilocational yield trials can be conducted. At CIAT, multiple cycles run concurrently since a new cohort of hybrid seedlings is planted each year. Whenever a superior hybrid clone is identified as a potentially useful parent, it is recycled into the hybridization program. IITA's program in Africa focused heavily on disease resistance and, more recently, on insect resistance in addition to yield. CIAT's program was able to place more weight on yield owing to the absence of any single devastating insect or disease problem comparable with CMD in Africa. Moderate to high heritability estimates (by parent:offspring regression) for a number of plant and root traits (summarized in Kawano 1987) suggested that rapid genetic gains should be possible.

From 1,950 germplasm accessions evaluated at CIAT in 1973, 230 clones were selected to enter the initial cycle of hybridization in 1974. These 230 clones form the main genetic base of subsequent breeding, although additional germplasm accessions were introgressed into the breeding program over the years.

Cassava is an important cash crop in Thailand and other Asian countries, but national yield figures were stagnant through the 1970s (Sinthuprama and Tiraporn 1987). An important strategic decision was taken by CIAT in the early 1980s to place an experienced, senior cassava breeder in Thailand to support the Thai program (and other Asian national programs), and also to facilitate exploitation in Asia of CIAT's extensive cassava genetic resource base. It was anticipated that a major

genetic impact on cassava production could be achieved in Thailand for several reasons, mainly because cassava is grown as a cash crop rather than a subsistence food crop, meaning better agronomic management of the crop and fewer quality restrictions. Further, geographic isolation of the American crop species from a wide range of the biological constraints on cassava productivity would simplify the breeding objectives (Kawano 1987).

4. Documentation of Gains of CIAT Cassava Breeding Program. K. Kawano (unpublished) documents a doubling of yield (relative to the mean of three standard checks), attributable to recombination and selection in the decade between 1973 and 1983 in the CIAT program in Colombia. This gain was owing to improvement in HI more than to increase in biomass. Essentially all the gain was achieved in the first 5–6 years of the period.

Iglesias and Hershey (1994) reviewed progress achieved during the 1980s in the Colombian breeding program for a number of traits in two important ECZs (the seasonally dry, and the acid soil savannas). Genetic progress was measured against a common set of check clones. Yield in one ECZ increased, but no gain was realized in the other over the entire decade. This inconsistent breeding progress is attributed to various factors, including insect and pathogen buildup at the testing sites over time, and changes of test sites.

Modest cassava breeding activities were carried out in Thailand during the 1970s, but without producing a clone superior to the standard, farmer-selected 'Rayong 1' (Boonsue and Sinthuprama 1975). A greatly increased influx of exotic germplasm, principally from CIAT, during the late 1970s, and a larger hybridization program beginning in the early 1980s led to a 15-year period of continual genetic progress and development of new commercial cultivars, documented by K. Kawano (unpublished). Dry matter yield increased by 50% (3.3% yr^{-1}) between 1982 and 1997. Yield increase over the period in the Thai program was attributable to increase in total biomass production, with essentially no change in HI, in contrast to the results documented during the early years of the breeding program in Colombia, which began from an essentially unimproved germplasm base.

It would appear that the breeding program initiated in Colombia, after initial rapid progress in yield improvement, quickly reached some sort of plateau, probably owing to a growing complexity of selection criteria (increasing pressure of diseases and insect pests and a shift to more diverse and more stressful testing sites). That essentially the same germplasm, after transfer to Thailand, sustained 15 years of continual

genetic gain in yield seems to be owing to greater weight being given to yield and less attention needing to be given to biotic stresses—a number of cassava diseases and insect pests are unknown in Asia—and quality considerations—cassava is principally used as an industrial source of starch and animal feed in Asia (K. Kawano, unpublished).

5. Documentation of NARS Breeding Progress. A number of national cassava breeding programs exist, the oldest continuous efforts being in Brazil and India. The São Paulo State agronomic research institute at Campinas (IAC) began breeding cassava in 1940, to overcome production constraints of a large commercial cassava industry in the state (Fukuda and Porto 1991). A series of high-yielding, disease-resistant clones have been released over the years and their adoption has been a significant component in São Paulo's having the highest average yields of any state in Brazil (nearly double the Brazilian national average). With the establishment of Brazil's national agricultural research institution, EMBRAPA, in 1974, a national cassava improvement program was established at Cruz das Almas, in Bahia State. However, its impact to date on national production figures has apparently not been very great, as national average yields have hovered around 12 t ha^{-1} for at least 30 years (Fukuda 1996).

In India, cassava breeding began in Kerala State, on the southwest coast, in the 1940s (Abraham et al. 2001). Koshy (1947), working at the Travancore State University, reports early hybridization work, outlines a comprehensive genetic improvement strategy, including interspecific hybridization and polyploidization. In 1963, the Central Tuber Crops Research Institute was established, and has actively pursued cassava genetic improvement and the release and dissemination of new hybrid clones, several of which have been widely adopted for cassava production for industrial use. It is interesting to note, however, that a clone introduced to India from Malaysia, which was selected and released in 1956, is reported (Abraham et al. 2001) still "even after 44 years, . . . to be the best table variety with unmatched culinary quality." No bred cultivar of comparable quality "is as stable in yield performance" as this Malaysian clone. Active breeding programs exist also in a number of other countries, notably in Asia (Howeler and Tan 2001).

6. Impact on National Productivity. Given that the bulk of cassava is grown as a subsistence crop by small farmers, reliable assessment of adoption of improved clones and their impact on commercial yields is difficult, a problem acutely appreciated by cassava breeders (Jennings and Hershey 1984). As noted by Johnson et al. (2002b), "In the absence

of empirical surveys, the data [on adoption] come from expert opinion from a variety of sources . . .” Furthermore, many production constraints under subsistence agriculture limit expression of full potential of genetically improved cultivars, even when they are adopted.

Where cassava is grown as a commercial crop, for example, to supply raw material for industrial processing, more reliable production statistics are possible. However, even in countries such as Thailand, where long-term production figures are available, and in spite of documented improvement in yield potential of bred cultivars (K. Kawano, unpublished), no trend in national yield averages is evident over a 40-year period (1961–2000) (Sriroth et al. 2001). Other countries [e.g., India (Abraham et al. 2001) and China (Tian et al. 2001)] do show sustained improvement in average commercial yield, though the areas involved are much smaller than in Thailand.

III. CONCLUSIONS AND PROSPECTS

Plant genetic improvement is a human activity that inherently takes place in a continuous temporal context. Plant breeding not only needs long-term institutional and financial support; its practitioners must perceive a continuity of future support. In the developing world, stable institutional support or the perception of such stability are often lacking.

Long-term selection experiments in closed plant populations and with purely academic aims are rare to the point of non-existence in the developing world. Selection experiments with the more utilitarian aim of the improvement of economic crop plants have been conducted both within national agricultural research and development institutions and, for the past 3–4 decades, in the International Agricultural Research Centers.

Realizing genetic gain in a crop improvement program is vastly more complex than demonstrating genetic gain in an academic long-term selection experiment, for expression of genetic gain depends intimately on the environment where the improved genotype is grown. Sustained genetic gain has been demonstrated for several IARC crop improvement programs under controlled, experimental conditions. In some cases, national production figures show even greater gains owing to a synergistic combination of the improved genotypes with improved crop management. In other cases, even where demonstrable genetic gain has been achieved, average national on-farm yield has remained stagnant (e.g., cassava in Thailand). Much depends, of course, on the general economic environment in which developing country farmers must survive. In many situations, there is no economic incentive for a farmer to make

the investments needed to aspire to higher yields. It has proven difficult to demonstrate major genetic yield gains on-farm in the absence of improved agronomy.

While the IARCs breeding and selection programs built on prior knowledge generated in both the developed and developing world, they brought an unprecedented level of financial support, and especially *stability* of institutional support, to agricultural research in general and plant genetic improvement in particular in the developing world. As K. Kawano and J. Cock (unpublished, 2002) observe: " It is worth noting in the present age of short-term projects that this successful program [CIAT's cassava research program] was developed over three decades." Unfortunately, the IARCs as well as developing country agricultural research institutions, like most publicly funded institutions, have not fared well in recent decades. As noted by Reynolds et al. (1999), "While demand for wheat is growing faster than gains in genetic yield potential are being realized, investment into conventional breeding by national programs and development agencies is being reduced." Increasingly, plant breeding research is supported by short-term, "special project" funding that simply is not conducive to the creation of expectation of long-term, stable support that is necessary for successful selection programs. The change over the past 2–3 decades is well reflected in the difference in tone between the references in Wellhausen (1978) to active maize breeding programs in a number of developing countries (including Venezuela, Colombia, Peru, Brazil, Thailand, Philippines, India, and Egypt) and the lament only 14 years later, in Pandey and Gardner (1992), that "little advanced technology development is expected to take place in the developing countries, due mainly to declining budgets for agricultural research." The situation has only worsened over the most recent decade.

The IARCs, by their international nature, also brought a vastly broadened geographical focus to tropical crop improvement. This broadened focus was highly advantageous in the acquisition, maintenance, and use of plant genetic resources. A broadened focus, however, complicates a plant breeding program in setting its goals, as the environments in which a particular crop species are grown and the crop quality demands differ widely across the developing world. Hence, to achieve any genetic progress at all, most IARC breeding programs have been subdivided on one or more criteria, and priorities established and revised over time. This subdivision has, in some cases, led to the creation of programs on a single crop at more than one center (e.g., cassava at CIAT and IITA, wheat at CIMMYT and ICARDA, rice at IRRI, CIAT, and WARDA).

One of the major frustrations of the IARC breeding programs is the difficulty in achieving genetic improvement under the conditions of low-input, subsistence agriculture that is still common in the developing world. A general conclusion of IARC breeding programs seems to be that genetic gain, at least for yield, is easier to realize in high-input (e.g., irrigated rice), than in low-input production systems (e.g., upland rice). Early gains in cassava yield from breeding at CIAT headquarters disappeared almost completely when the selected clones were grown in more difficult, commercial cassava-growing regions, even within Colombia. For wheat, however, Ortiz-Monasterio et al. (1997) showed genetic gain for yield of Mexican cultivars released between 1950 and 1985, even when measured under conditions of low N fertilization (i.e., at 2.0 to 2.5 t ha^{-1} yield levels).

Attention to stringent quality demands (e.g., for bean grain type or culinary quality in rice and cassava) inevitably diminishes gain that can be achieved in other attributes such as yield. These demands must, however, be met: a cultivar that fails to fulfill consumer quality standards is unlikely to be grown at all.

The IARCs have clearly had a major impact on food production in the developing world, particularly for the major cereal grain crops (Evans 1993). Much of this increased food production is owing to genetic improvement in yield potential achieved by hybridization and selection over the past 3–4 decades, combined with improved crop management and particularly N fertilization. Thus, over the past four decades, basic food production has increased even faster than population in the developing world (Evans 1993).

At least for wheat, rice, maize, and cassava, there is no indication that genetic gain for yield potential per se, under high levels of crop management, is exhausted (Sayre et al. 1997; Reynolds et al. 1999, Peng et al. 1999, 2000; Gardner et al. 1990; K. Kawano, unpublished). In addition to the plant breeding programs conducted directly by the IARCs, the Centers have made an impact on crop improvement in the developing world by facilitating access to diverse germplasm and by both practical and academic training programs for developing country agricultural scientists.

A number of factors impinge on the effectiveness of long-term selection programs in the developing world, and diminished level and shorter term funding is only one of these. Diversion of funding into biotechnologies of doubtful short-term applicability diminishes funds for conventional plant breeding in the developing as in the developed world. A number of genetically transformed plants have been developed and

their potential utility is being studied at the IARCs [e.g., *Bt* gene for rice stem borer resistance (IRRI 1997) and a chitinase gene for sheath blast resistance (Potrykus et al. 1996)]. Some of these genetically modified plants have been developed by IARC scientists (CIAT 2001). However, even if these genetic transformants prove useful, none can be freely released to farmers owing to the complex intellectual property issues and food safety regulations involved. In a recent analysis of the impact of intellectual property restrictions on the free exchange of plant germplasm, protection of even conventionally derived plant germplasm, is predicted to have a disproportionately negative impact on developing country economies (Evenson 1999).

While it can be (and is) argued that current and projected food shortages are caused by the lack of purchasing power of the poor, rather than insufficient food production (e.g., Latham 2000), nevertheless genetic gain in yield potential, as well as sustained genetic gains in stress resistance/tolerance has led to an astonishing increase in world food production in developing countries over the past half-century, especially where genetic gains are accompanied by improved cultural practices and use of inputs. A world food crisis, if not avoided, has at the least been postponed for the past four decades as food production has increased faster than population growth in the developing world. A significant portion of this increase can be attributed to sustained support of the international agricultural research institutions.

LITERATURE CITED

Abraham, K., S. G. Nair, and S. K. Naskar. 2001. Cassava breeding and varietal dissemination in India: major achievements during the past 25–30 years. p. 174–184. In: R. H. Howeler and S. L. Tan (eds.), Cassava's potential in the 21st Century: Present situation and future research and development needs. Proc. 6th Regional Workshop, Ho Chi Minh City, Vietnam. 21–25 Feb. 2000. CIAT, Cali, Colombia.

Abreu, A. de F. B., M. A. P. Ramalho, J. B. dos Santos, and L. A. Martins. 1994. Progresso do melhoramento genético do feijoeiro nas décadas de setenta e oitenta nas regiões sul e Alto Paranaíba em Minas Gerais. Pesq. Agropec. Bras., Brasília 29:105–112.

Amézquita, M. C., and O. Voysest. 1987. Progress in disease resistance and yield potential: An analysis of VEF-EP nurseries 1979–1986. CIAT, Cali, Colombia.

Araujo, S. M. C. de, J. A. Osuna, and D. A. Banzatto. 1989. Evaluation of six cycles of stratified mass selection for resistance to earworm, *Heliothis zea* (Boddie 1850), in two maize populations. Ciencia e Cultura 41:1208–1212.

Atroch, A. L., and G. H. de S. Nunes. 2000. Progresso genético em arroz de várzea úmida no estado do Amapá. Pesq. Agropec. Bras., Brasília 35:767–771.

Banziger, M., G. O. Edmeades, and H. R. Lafitte. 1999. Selection for drought tolerance increases maize yields across a range of nitrogen levels. Crop Sci. 39:1035–1040.

Beck, B. D. A. 1982. Historical perspectives of cassava breeding in Africa. p. 13–18, 122–128. In: Root crops in Eastern Africa, Kigali, Rwanda, 1980. Proc. Workshop. Intl. Develop. Res. Centre, Ottawa, Canada.

Bonjean, A. P., and W. J. Angus (eds.). 2001. The world wheat book: A history of wheat breeding. Lavoisier Publ., Paris.

Boonsue, B., and S. Sinthuprama. 1975. Thailand. p. 26–28. In: B. Nestel and R. MacIntyre (eds.), The international exchange and testing of cassava germplasm: proceedings of an interdisciplinary workshop, Palmira, Colombia, 1975. Intl. Develop. Res. Centre, Ottawa, Canada.

Braun, H. J., W. H. Pfeiffer, and W. G. Pollmer. 1992. Environments for selecting widely adapted spring wheat. Crop Sci. 32:1420–1427.

Braun, H. J., S. Rajaram, and M. van Ginkel. 1996. CIMMYT's approach to breeding for wide adaptation. Euphytica 92:147–153.

Breseghello, F., P. H. N. Rangel, and O. P. de Morais. 1999. Ganho de produtividade pelo melhoramento genético do arroz irrigado no nordeste do Brasil. Pesq. Agropec. Bras., Brasília 34:399–407.

Byrne, D. 1984. Breeding cassava. Plant Breed. Rev. 2:73–134.

Cantrell, R. P. 1986. The past experience and future course of the CIMMYT maize program. Rep. Annu. Corn and Sorghum Res. Conf. 41:134–154.

Chandler, R. F., Jr. 1982. An adventure in applied science: A history of the International Rice Research Institute. IRRI, Manila.

Chang, T. T. 1995. Rice. p. 147–155. In: J. Smartt, and N. W. Simmonds (eds.), Evolution of crop plants, 2nd ed. Longman Scientific & Technical, Burnt Mill, Harlow, Essex, UK.

CIAT. 1991. 1990 Annu. Rep., Bean Program. CIAT, Cali, Colombia.

CIAT. 2000. Annual report, Project IP-1: Bean improvement for sustainable productivity, input use efficiency and poverty alleviation. CIAT, Cali, Colombia.

CIAT. 2001. Annu. Rep. 2000: IP-4, Improved rice germplasm for Latin America and the Caribbean. CIAT, Cali, Colombia.

Darrah, L. L. 1986. Evaluation of population improvement in the Kenya maize breeding methods study. p. 160–176. In: B. Gelaw (ed.), To feed ourselves. Proc. 1st Eastern, Central, and Southern Africa Regional Maize Workshop, Lusaka, Zambia, 10–17 March 1985. CIMMYT, Mexico.

Darwin, C. [1844] 1909. The essay of 1844. p. 57–254. In: F. Darwin (ed.), The foundations of the origin of species: Two essays written in 1842 and 1844. The Univ. Press, Cambridge, UK.

Debouck, D. G., and J. Smartt. 1995. Beans. p. 287–294. In: J. Smartt and N. W. Simmonds (eds.), Evolution of crop plants, 2nd ed. Longman Scientific & Technical, Burnt Mill, Harlow, Essex, UK.

De Carvalho, H. W., M. D. D. Leal, M. X. Dos Santos, and C. A. P. Pacheco. 2000. Genetic potential of BR 5011-Sertanejo maize cultivar in the coastal plainlands of the northern region of Brazil. Pesq. Agropec. Bras., Brasília 35:1169–1176.

De Leon, C., and S. Pandey. 1989. Improvement of resistance to ear and stalk rots and agronomic traits in tropical maize gene pools. Crop Sci. 29:12–17.

Djisbar, A., and J. L. Brewbaker. 1987. Effects of the *brachytic-2* gene on maize yield and its components. Maydica 32:107–123.

Edmeades, G. O., J. Bolaños, S. C. Chapman, H. R. Lafitte, and M. Banziger. 1999. Selection improves drought tolerance in tropical maize populations: I. Gains in biomass, grain yield, and harvest index. Crop Sci. 39:1306–1315.

Evans, L. T. 1993. Crop evolution, adaptation and yield. Cambridge Univ. Press, Cambridge, UK.

Evenson, R. E. 1999. Intellectual property rights, access to plant germplasm, and crop production scenarios in 2020. Crop Sci. 39:1630–1635.
Fischer, R. A., D. Rees, K. D. Sayre, Z.-M. Lu, A. G. Condon, and A. Larque Saavedra. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci. 38:1467–1475.
Fukuda, W. M. G. 1996. Mandioca: estratégia para um programa de melhoramento genético. Documentos CNPMF No. 71. EMBRAPA, Cruz das Almas, Bahia, Brazil.
Fukuda, W. M. G., and M. C. M. Porto. 1991. A mandioca no Brasil. p. 15–42. In: C. H. Hershey (ed.), Mejoramiento genético de la yuca en América Latina. CIAT, Cali, Colombia.
Gardner, C. O., M. A. Thomas-Compton, W. A. Compton, and B. E. Johnson. 1990. Final Report to USAID, UNL/IANR/ARD Project NEB 12-159, Univ. Nebraska, Lincoln.
Gerpacio, R. V. (ed.). 2001. Impact of public- and private sector maize breeding research in Asia, 1966–1997/98. CIMMYT, Mexico.
Glaszmann, J. C. 1987. Isozymes and classification of Asian rice varieties. Theor. Appl. Genet. 74:21–30.
Goodman, M. M. 1988. The history and evolution of maize. CRC Critical Rev. Plant Sci. 7:197–220.
Goodman, M. M. 1995. Maize. p. 192–202. In: J. Smartt and N. W. Simmonds (eds.), Evolution of crop plants, 2nd ed. Longman Scientific & Technical, Burnt Mill, Harlow, Essex, UK.
Granados, G., S. Pandey, and H. Ceballos. 1993. Response to selection for tolerance to acid soils in a tropical maize population. Crop Sci. 33:936–940.
Hahn, S. K., E. R. Terry, and K. Leuschner. 1980. Breeding cassava for resistance to cassava mosaic disease. Euphytica 29:673–683.
Hahn, S. K., and R. L. Theberge. 1987. Techniques and advances in breeding cassava for disease resistance in Africa. p. 105–115. In: C. H. Hershey (ed.), Cassava breeding: A multidisciplinary review. Proc. workshop held in the Philippines, 4–7 March 1985. CIAT, Cali, Colombia.
Hershey, C. H. 1984. Breeding cassava for adaptation to stress conditions: Development of a methodology. p. 303–314. In: Symposium of the International Society of Tropical Root Crops, 6th. Lima, Peru. CIP, Lima, Peru.
Hershey, C. H. 1987. Cassava germplasm resources. p. 1–24. In: C. H. Hershey (ed.), Cassava breeding: a multidisciplinary review. Proc. workshop held in the Philippines, 4–7 March 1985. CIAT, Cali, Colombia.
Howeler, R. H., and S. L. Tan (eds.). 2001. Cassava's potential in the 21st Century: Present situation and future research and development needs. Proc. 6th Regional Workshop, Ho Chi Minh City, Vietnam. 21–25 Feb. 2000. CIAT, Cali, Colombia.
Huang, J., S. Rozelle, and J. Y. Lin. 1998. Impacts of research and technological change on China's rice production. p. 263–278. In: Impact of rice research. Proc. Intl. Conf. on the Impact of Rice Research, 3–5 June 1996, Bangkok, Thailand. Thailand Development Research Institute, Bangkok, Thailand, and International Rice Research Institute, Manila, Philippines.
Iglesias, C. A., and C. H. Hershey. 1994. Cassava breeding at CIAT: Heritability estimates and genetic progress in the 1980s. p. 149–163. In: F. Ofori (ed.), Proc. 9th Symp. Intl. Soc. Tropical Root Crops. Accra (Ghana). 1991. Intl. Soc. Tropical Root Crops, Accra, Ghana.
IRRI. 1997. *Bt* rice: Research and policy issues. IRRI Informa. Series no. 5. IRRI, Manila.
Jennings, D. L., and C. H. Hershey. 1984. Cassava breeding: A decade of progress from international programmes. p. 89–116. In: G. E. Russell (ed.), Progress in plant breeding. Butterworths, London.

Johnson, E. C., K. Fischer, G. O. Edmeades, and A. F. E. Palmer. 1986. Recurrent selection for reduced plant height in lowland tropical maize. Crop Sci. 26:253–260.

Johnson, N. L., D. Pachico, and C. S. Wortmann. 2002a. The economic impact of crop genetic improvement in beans. p. 257–274. In: R. E. Evenson and D. Gollin (eds.), Crop variety improvement and its effects on productivity: The impact of international agricultural research. CAB Intl., Wallingford, Oxon, UK.

Johnson, N. L., V. M. Manyong, A. G. O. Dixon, and D. Pachico. 2002b. The impact of IARC genetic improvement programs on cassava. p. 337–355. In: R. E. Evenson and D. Gollin (eds.), Crop variety improvement and its effects on productivity: The impact of international agricultural research. CAB Intl., Wallingford, Oxon, UK.

Kawano, K. 1987. Inherent and environmental factors related to cassava varietal selection. p. 207–226. In: C. H. Hershey (ed.), Cassava breeding: A multidisciplinary review. Proc. workshop held in the Philippines, 4–7 March 1985. CIAT, Cali, Colombia.

Kawano, K., and P. R. Jennings. 1983. Tropical crop breeding: Achievements and challenges. p. 81–99. In: W. H. Smith and S. J. Banta (eds.), Symposium on potential productivity of field crops under different environments. IRRI, Manila, Philippines.

Kawano, K., K. Narintaraporn, P. Narintaraporn, S. Sarakarn, A. Limsila, J. Limsila, D. Suparhan, V. Sarawat, and W. Watananonta. 1998. Yield improvement in a multistage breeding program for cassava. Crop Sci. 38:325–332.

Kelly, J. D., and M. W. Adams. 1987. Phenotypic recurrent selection in ideotype breeding of Pinto beans. Euphytica 36:69–80.

Khush, G. S. 2001. Green revolution: The way forward. Nature Reviews–Genetics 2: 815–822.

Khush, G. S., and P. S. Virk. 2002. Rice improvement: Past, present, and future. p. 17–42. In: M. S. Kang (ed.), Crop improvement: Challenges in the twenty-first century. Haworth Press, New York.

Koshy, T. K. 1947. The tapioca plant and methods for evolving improved strains for cultivation. Proc. Indian Acad. Sci. (Section B) 26(2):32–59.

Latham, J. R. 2000. There's enough food for everyone, but the poor can't afford to buy it. Nature 404:222.

Morales, F. J. 2000. Métodos de control de begomovirus del frijol. p. 133–154. In: F. J. Morales-Garzón (ed.), El mosaico dorado y otras enfermedades del frijol común causadas por geminivirus transmitidos por mosca blanca en la América Latina. CIAT, Cali, Colombia.

Morris, M. L., and M. A. López Pereira. 1999. Impacts of maize breeding research in Latin America, 1966–1997. CIMMYT, Mexico.

Narro, L. N. 1988. Evaluation of half-sib selection in a maize population. M.S. thesis, Iowa State Univ., Ames.

Nichols, A. F. W. 1947. Breeding cassava for virus resistance. East Africa Agr. J. 17: 184–194.

Nienhuis, J., and S. P. Singh. 1988. Genetics of seed yield and its components in common beans (*Phaseolus vulgaris* L.) of middle-American origin. II. Genetic variance, heritability and expected response from selection. Plant Breed. 101:155–163.

Ochieng, J. A. W., and R. E. Kamidi. 1992. Response to eight cycles of reciprocal recurrent selection in Kitale Synthetic II, Ecuador 573 and their variety cross. J. Genetics Plant Breed. 46:315–319.

Orozco S., S. H., and S. E. Beebe. 1990. Vivero de adaptación centroamericano VIDAC 1989–1990. Programa Cooperativo Regional de Frijol para Centroamérica, México y el Caribe (PROFRIJOL), Guatemala City; Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia; Corporación Suiza al Desarrollo (COSUDE); Programas

Nacionales Miembros del Programa Cooperativo Centroamericano de Mejoramiento de Cultivos y Animales (PCCMCA).

Orozco S., S. H., J. M. Herrera, and C. A. Pérez. 1990. Vivero centroamericano de adaptación y rendimiento VICAR 1989. Programa Cooperativo Regional de Frijol para Centroamérica, México y el Caribe (PROFRIJOL), Guatemala City; Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia; Corporación Suiza al Desarrollo (COSUDE); Programas Nacionales Miembros del Programa Cooperativo Centroamericano de Mejoramiento de Cultivos y Animales (PCCMCA).

Ortiz-Monasterio, J. I., K. D. Sayre, S. Rajaram, and M. McMahon. 1997. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Sci. 37: 898–904.

Pandey, S., A. O. Diallo, T. M. T. Islam, and J. Deutsch. 1986. Progress from selection in eight tropical maize populations using international testing. Crop Sci. 26:879–884.

Pandey, S., A. O. Diallo, T. M. T. Islam, and J. Deutsch. 1987. Response to full-sib selection in four medium maturity maize populations. Crop Sci. 27:617–622.

Pandey, S., and C. O. Gardner. 1992. Recurrent selection for population, variety, and hybrid improvement in tropical maize. Adv. Agron. 48:1–87.

Paterniani, E. 1990. Maize breeding in the tropics. CRC Crit. Rev. Plant Sci. 9:125–154.

Peng, S., K. G. Cassman, S. S. Virmani, J. Sheehy, and G. S. Khush. 1999. Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. Crop Sci. 39:1552–1559.

Peng, S., R. C. Laza, R. M. Visperas, A. L. Sanico, K. G. Cassman, and G. S. Khush. 2000. Grain yield of rice cultivars and lines developed in the Philippines since 1966. Crop Sci. 40:307–314.

Perkins, J. H. 1997. Geopolitics and the green revolution: Wheat, genes, and the cold war. Oxford Univ. Press, New York.

Pingali, P., and S. Pandey. 2001. Meeting world maize needs: Technological opportunities and priorities for the public sector. p. 1–20. In: P. Pingali (ed.), CIMMYT 1999–2000 world maize facts and trends. CIMMYT, Mexico.

Potrykus, I., G. A. Armstrong, P. Beyer, S. Bieri, P. K. Burkhardt, H. Ding Chen, G. C. Ghosh Biswas, S. K. Datta, J. Fütterer, A. Klöti, G. Spangenberg, R. Terada, J. Wünn, and H. Zhao. 1996. Transgenic *indica* rice for the benefit of less developed countries: toward fungal, insect, and viral resistance and accumulation of β-carotene in the endosperm. p. 179–187. In: Rice genetics III. Proc. 3rd Intl. Rice Genet. Symp., 16–20 Oct. 1995. Manila (Philippines). IRRI, Manila.

PROFRIJOL (Programa Cooperativo Regional de Frijol para Centroamérica, México y el Caribe). 2001. Informe técnico por países POA 2000–2001. Guatemala City, Guatemala.

Rajaram, S., and M. van Ginkel. 2001. Mexico: 50 years of international wheat breeding. p. 579–608. In: A. P. Bonjean and W. J. Angus (eds.), The world wheat book: A history of wheat breeding. Lavoisier Publ., Paris.

Ramírez-Díaz, J. L., J. Ron-Parra, J. J. Sánchez-González, and M. Chuela-Bonaparte. 2000. Recurrent selection in the subtropical maize population PABGT-CE. Agrociencia 34:33–39.

Rao, S., G. Singh, and M. Chatterjee. 2001. Indian wheat pool. p. 773–816. In: A. P. Bonjean and W. J. Angus (eds.), The world wheat book: A history of wheat breeding. Lavoisier Publ., Paris.

Reynolds, M. P., S. Rajaram, and A. McNab (eds.). 1996. Increasing yield potential in wheat: Breaking the barriers. CIMMYT, Mexico.

Reynolds, M. P., S. Rajaram, and K. D. Sayre. 1999. Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand. Crop Sci. 39:1611–1621.

Rogers, D. J., and S. G. Appan. 1970. Untapped genetic resources for cassava improvement. p. 72–75. In: D. L. Plucknett (ed.), Proc. 2nd Symp. Intl. Soc. Tropical Root and Tuber Crops. Honolulu and Kappa, Kauai, Hawaii, 1970. Univ. Hawaii, Honolulu.

Sallah, P. Y. K., N. J. Ehlke, and J. L. Geadelmann. 1998. Progress from selection in La Posta maize population evaluated under three nitrogen fertilizer levels. African Crop Sci. J. 6:241–248.

Santos, P. G., P. C. Soares, A. A. Soares, O. P. Morais, and V. M. de O. Cornélio. 1999. Avaliaçao do progresso genético obtido em 22 anos no melhoramento do arroz irrigado em Minas Gerais. Pesq. Agropec. Bras., Brasília 34:1889–1896.

Sarquis, J. I., H. González, E. Sánchez de Jiménez, and J. R. Dunlap, Jr. 1998. Physiological traits associated with mass selection for improved yield in a maize population. Field Crops Res. 56:239–246.

Sayre, K. D., S. Rajaram, and R. A. Fischer. 1997. Yield potential progress in short bread wheats in northwest Mexico. Crop Sci. 37:36–42.

Singh, S. P. 1999. Improvement of small-seeded race Mesoamerica cultivars. p. 255–274. In: S. P. Singh (ed.), Common bean improvement in the twenty-first century. Kluwer Acad. Publ., Dordrecht, The Netherlands.

Singh, S. P. 2001. Broadening the genetic basis of common bean cultivars: A review. Crop Sci. 41:1659–1675.

Sinthuprama, S., and C. Tiraporn. 1987. Cassava varietal improvement in Thailand. p. 227–239. In: C. H. Hershey (ed.), Cassava breeding: a multidisciplinary review. Proc. workshop held in the Philippines, 4–7 March 1985. CIAT, Cali, Colombia.

Soares, A. A., P. G. Santos, O. P. de Morais, P. C. de Soares, M. de S. Reis, and M. A. de Souza. 1999. Progresso genético obtido pelo melhoramento do arroz de sequeiro em 21 anos de pesquisa em Minas Gerais. Pesq. Agropec. Bras., Brasília 34:415–424.

Song, T. M., F. Kong, C. J. Li, and G. H. Song. 1999. Eleven cycles of single kernel phenotypic recurrent selection for percent oil in Zhongzong no. 2 maize synthetic. J. Genet. Breed. 53:31–35.

Sriroth, K., C. Rojanaridpiched, V. Vichukit, P. Suriyapan, and C. G. Oats. 2001. Present situation and future potential of cassava in Thailand. p. 25–46. In: R. H. Howeler and S. L. Tan (eds.), Cassava's potential in the 21st century: Present situation and future research and development needs. Proc. 6th Regional Workshop, Ho Chi Minh City, Vietnam. 21–25 Feb. 2000. CIAT, Cali, Colombia.

Subandi. 1990. Ten cycles of selection for prolificacy in a composite variety of maize. Indones. J. Crop Sci. 5:1–11.

Sun, B. Q., A. M. Zhang, and A. P. Bonjean. 2001. Chinese wheat pool. p. 667–701. In: A. P. Bonjean and W. J. Angus (eds.), The world wheat book: A history of wheat breeding. Lavoisier Publ., Paris.

Tian, Y., X. Lin, and S. Jin. 2001. Present situation and future potential of cassava in China. p. 71–83. In: R. H. Howeler and S. L. Tan (eds.), Cassava's potential in the 21st Century: Present situation and future research and development needs. Proc. 6th Regional Workshop, Ho Chi Minh City, Vietnam. 21–25 Feb. 2000. CIAT, Cali, Colombia.

Viana Ruano, A. 1998. Flujo de germoplasma e impacto del PROFRIJOL en Centroamérica. Profrijol, Guatemala City.

Voysest Voysest, O. 2000. Mejoramiento genético del frijol (*Phaseolus vulgaris* L.): Legado de variedades de América Latina 1930–1999. CIAT, Cali, Colombia.

Waddington, S. R., J. K. Ransom, M. Osmanzai, and D. A. Saunders. 1986. Improvement in the yield potential of bread wheat adapted to northwest Mexico. Crop Sci. 26:698–703.
Wellhausen, E. J. 1965. Exotic germ plasm for improvement of Corn Belt maize. p. 31–45. In: Proc. 20th Annu. Hybrid Corn Ind. Res. Conf.
Wellhausen, E. J. 1978. Recent developments in maize breeding in the tropics. p. 59–84. In: D. B. Walden (ed.), Maize breeding and genetics. Wiley, New York.

3

Trends in Productivity of U.S. Crops and Long-term Selection

William F. Tracy
Department of Agronomy
College of Agricultural and Life Sciences
University of Wisconsin-Madison, Madison, Wisconsin 53706

Irwin L. Goldman, Allison E. Tiefenthaler, and Margaret A. Schaber
Department of Horticulture
College of Agricultural and Life Sciences
University of Wisconsin-Madison, Madison, Wisconsin 53706

I. INTRODUCTION

Twentieth-century U.S. agriculture was marked by large increases in crop production. For most crops, U.S. average productivity (yield per hectare) at least doubled and in one case septupled (Table 3.1). Most of the changes occurred over a period of 70 years. There are many underlying causes of increased crop productivity. Changes in agricultural technologies including increased inputs and irrigation and improved crop genetics, pest protection, and management practices had a large role in

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-46892-4

Table 3.1. Percent yield for the five-year rolling mean in 1931 and 1998 relative to the first five-year mean that data was collected by the USDA and linear regression coefficients (b) and R^2 values for trend line from 1868 through 1930 and 1931 through 1998. Trend lines are based on five-year rolling means.

Crop	Center year of first five-year mean	Percent yield for the five-year rolling mean in 1931 and 1998 relative to the first five-year mean		*b* and R^2 value for trend line from 1868 through 1930		*b* and R^2 value for trend line from 1931 through 1998	
		1931	1998	*b*	R^2	*b*	R^2
Alfalfa	1920	90.2	157.2			1.13	0.95
Oats	1868	101.2	218.4	0.26	0.51	1.94	0.95
Rye	1868	98.6	250.0	0.23	0.29	2.97	0.91
Barley	1868	89.0	261.4	0.03	0.01	2.84	0.97
Winter wheat	1909	99.5	294.3			3.11	0.97
Spring wheat	1920	95.5	310.3			3.59	0.95
Soybeans	1924	116.2	318.4			2.73	0.97
Peanuts	1907	88.4	337.9			4.79	0.89
Sweet corn	1929	100.0	342.7			3.97	0.99
Sweet potato	1868	109.9	367.9			4.41	0.97
Cotton	1868	118.1	416.5	0.01	0.00	4.57	0.94
Green peas	1929	100.0	452.2			3.91	0.87
Sorghum	1929	100.0	486.8			7.19	0.94
Maize	1868	95.0	521.9	0.01	0.00	6.91	0.98
Rice	1895	192.6	546.6			6.20	0.95
Potato	1868	124.9	692.2	0.59	0.74	8.65	0.99

increasing productivity (Warren 1998). But when comparing trends based on national averages over long periods of time, other factors such as changes in cropping regions, crop utilization, and crop economics must be considered. The contribution of these various factors to changes in productivity vary greatly among crop species. On the occasion of the centennial celebration of the Illinois Long-term Selection Experiment, we review some of these long-term trends and offer some thoughts on the impact of long-term selection on crop productivity in the United States.

II. METHODOLOGY

We used data on yield per acre as collected by the USDA National Agricultural Statistical Service (USDA 1900–2001). The National Agricultural Statistical Service has been collecting data on productivity (yield

per acre) of certain crops since 1866. Over time, other crops were added as they became more prominent in U.S. agriculture. The data are collected by surveying growers. The number of individuals surveyed varies according to the number of growers of that crop. In 2001, responses from more than 10,000 growers were used to estimate maize (*Zea mays* L.) productivity (pers. commun., Bob McEwen, Sampling and Estimation Research Section, USDA National Agricultural Statistical Service). Crops grown on fewer hectares than those on which maize was grown would have fewer respondents. In 2001, only 500 growers reported on rye (*Secale cereale* L.) (pers. commun., Bob McEwen, Sampling and Estimation Research Section, USDA National Agricultural Statistical Service). This data set is useful because of the long-term nature of the survey and the number of species included.

In this report we surveyed 12 field crops and four vegetables (Table 3.1). We included data from the year the USDA first started recording average yield per acre for a specific crop until 2000. To directly compare productivity trends among crops, we calculated percent change over time. We used a five-year rolling mean to avoid under- or overestimating changes due to exceptionally good or poor years. We calculated the mean productivity of the first five years. We calculated a five-year mean centered on each year. Then for each year we calculated the percent change relative to the mean of the first five years.

Dramatic increases in crop yields for most crops began in the 1930s, so we estimated b and R^2 values for linear trends of the rolling mean of percent yield increase between 1931 and 1998. To determine if variability changed over time, for each year we calculated the percent deviation of that year from the rolling five-year mean centered on that year.

III. LONG-TERM TRENDS

A. Crop Productivity

The upward trend in maize productivity has been well publicized and is a good starting point for discussion. Maize productivity did not change from 1866 through the 1930s ($b = 0.01$, $R^2 = 0.00$) (Table 3.1, Fig. 3.1A). Note that Fig. 3.1A is based on raw yield data, not on a rolling mean. Beginning in the late 1930s, maize yields began to increase and despite yearly fluctuations due to climate and economic factors maintained a strong linear trend through 2000, the last year in this analysis ($b = 1.76$, $R^2 = 0.94$). Using a five-year rolling mean smoothes the curve and reveals a strong positive linear trend (Fig. 3.1B). The increasing productivity of

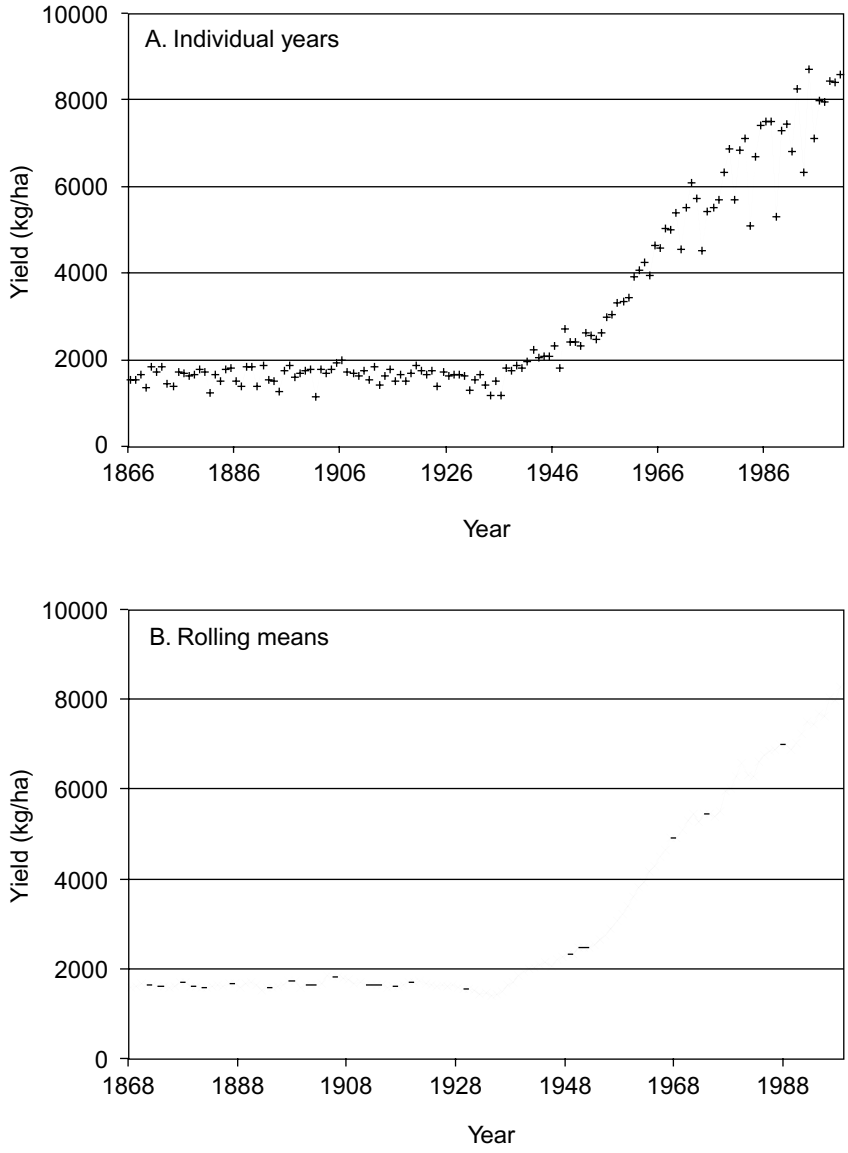

Fig. 3.1. A. Annual average U.S. maize yields (kg ha^{-1}) from 1866 to 2000. B. Rolling five-year mean of U.S. maize yields (kg ha^{-1}) from 1868 to 1998.

corn has been remarkably linear, but a few minor deviations from linearity are apparent. A small but distinct plateau is apparent in the first half of the 1970s. Three distinct factors probably played a role in creating this plateau: the southern corn leaf blight epidemic in 1971–1972, exceptionally dry weather in the Corn Belt in 1974, and the Arab oil embargo in 1973–1974. Two other brief deviations also appear around 1983 and 1988, both years with below-normal rainfall for much of the Corn Belt.

Among the six crops for which data collection began in 1866, productivity trends from 1868 to 1998 are similar. Between 1868 and 1930 productivity changed little or trended up slightly (Table 3.1, Fig. 3.1B and 3.2). Potato productivity began rising in the early 1900s and by 1931 had reached 125% of the rolling mean centered on 1868. In the late 1930s and 1940s, productivity for all crops began to climb rapidly. The size of the increases varied. Oat (*Avena sativa* L.) productivity in 1998 was 218% of 1868 productivity, while potato productivity was 692% of 1868 (Table 3.1). All trend lines were strongly linear (Table 3.1) but some

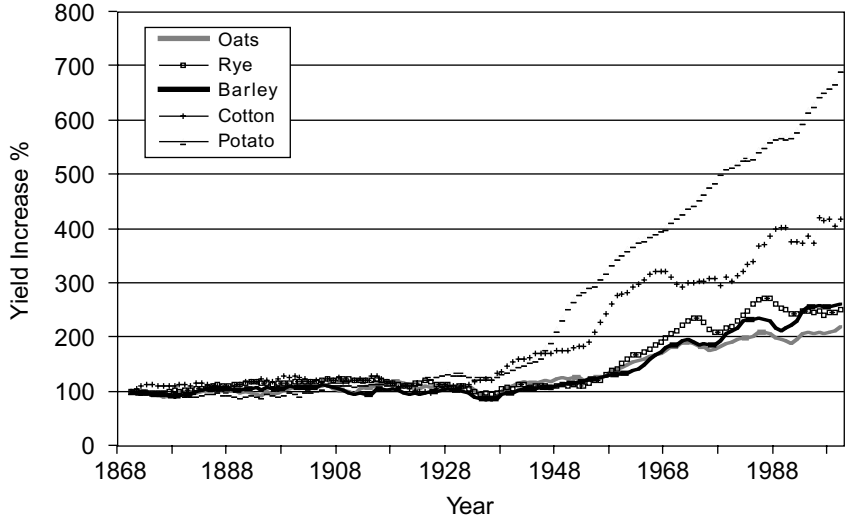

Fig. 3.2. Percent increase in yield for the five-year mean centered on a given year relative to the mean of the first five years of data collection, for potato, cotton, barley, rye, and oats grown in the United States from 1868 to 1998.

exhibited a steady increase, others an apparent periodicity, still others had fairly long periods of stasis interspersed with brief periods of rapid increases.

Similar trends were observed among the crops for which data collection began after 1866. For all such crops except rice (*Oryza sativa* L.), little change occurred from the beginning of data collection until the 1930s. Rice productivity nearly doubled between 1895 and 1930 (Table 3.1). Then in the 1930s and 1940s productivity of all crops began to increase. Alfalfa productivity increased by only 57% from 1922 to 1998, but for the other crops increases ranged from two- to seven-fold (Table 3.1).

Since the upward climb for most crops began in the 1930s, it is instructive to focus on changes between 1931 and 1998. In 1998, productivity of small grains as a mean of the first five years ranged from 218% (oats) to 310% (spring wheat, *Triticum aestivum* L.) (Fig. 3.3A, 3.3B). The statistical significance and biological reasons for differences in rates of gain are unknown, but clearly differences of this size have economic implications. If relative cost of production for oats and spring wheat had not changed over time, the value of spring wheat relative to oats would have increased over time.

The undulating shape of all of these curves is striking. Part of this is an artifact of the five-year means, as years with very low yield depress the means of surrounding years. But five-year means were calculated for the other crops and the resulting trends do not have such a pronounced undulation. Generally, small grains are not irrigated and are often grown in more marginal environments than other crops in this survey, and this may result in greater swings in productivity. The similar timing of the curves suggests that economic forces may also be at play. Low maize prices reduce the demand for the small grains. In response, farmers would reduce inputs and productivity would decrease. High maize prices have the opposite effect.

Three legumes in our survey have remarkably different trends. Alfalfa increased only 57% between 1920 and 1998, with most of the increase occurring between 1950 and 1980 (Fig. 3.3C, Table 3.1). The productivity increases for soybean (*Glycine max* L.) and peanut (*Arachis hypogaea* L.) were similar to each other and higher than that of alfalfa (Table 3.1). They exceeded the magnitude of gain for any of the small grains, yet the trends for soybean and peanut are quite different. Soybean steadily increased over time (Fig. 3.3C). Peanut, on the other hand, did not begin its upward trend until the late 1940s and, by the early 1980s, peanut

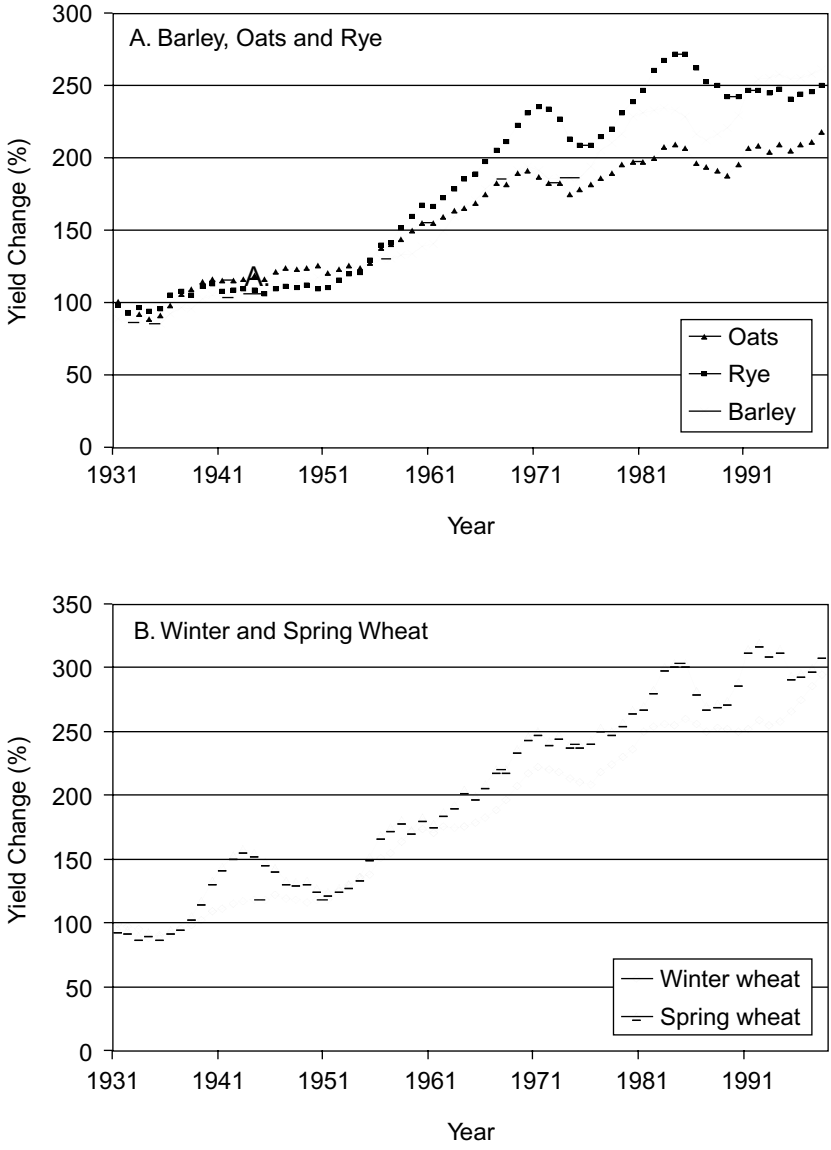

Fig. 3.3. Percent change in yield for the five-year mean centered on a given year relative to the mean of the first five years of data collection for 16 U.S. crops. Data shown are for the period between 1931 and 1998. A. Oats, rye, and barley. B. Winter and spring wheat. C. Soybean, peanut, and alfalfa. D. Cotton, rice, and sorghum. E. Peas, sweet corn, and sweet potato. F. Potato and maize.

(*continued*)

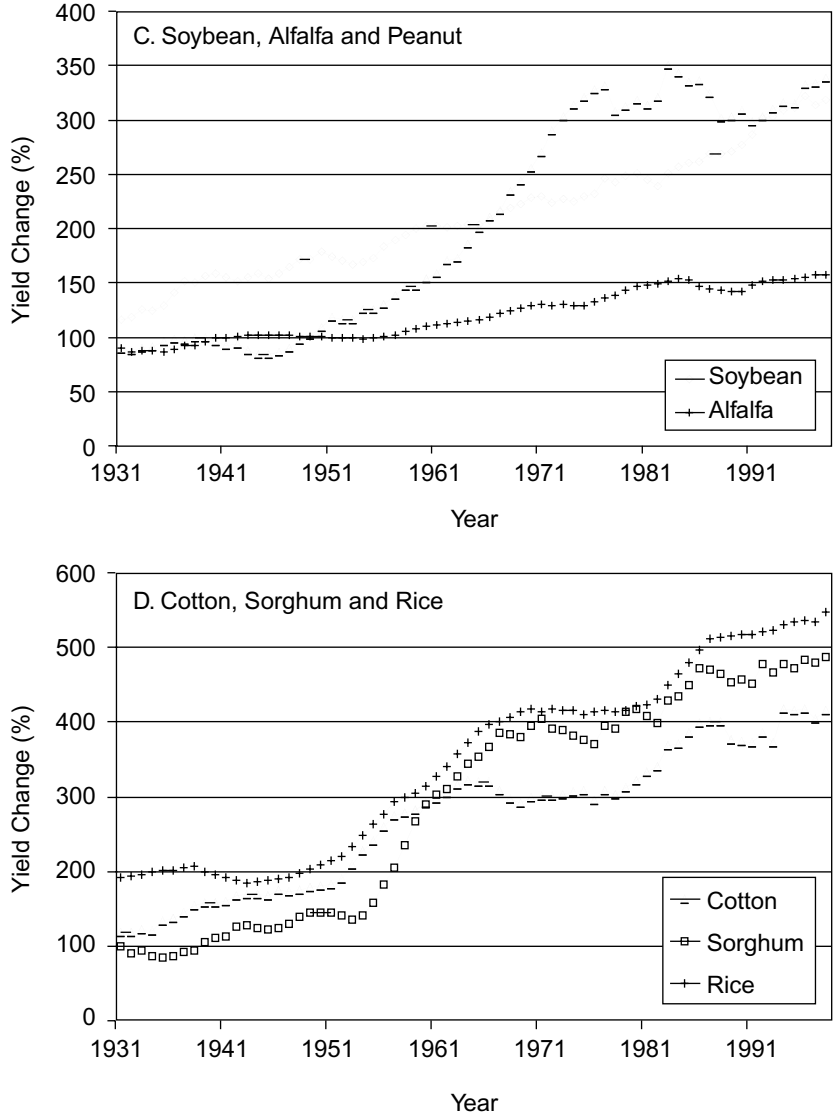

Fig. 3.3. (*Continued*)

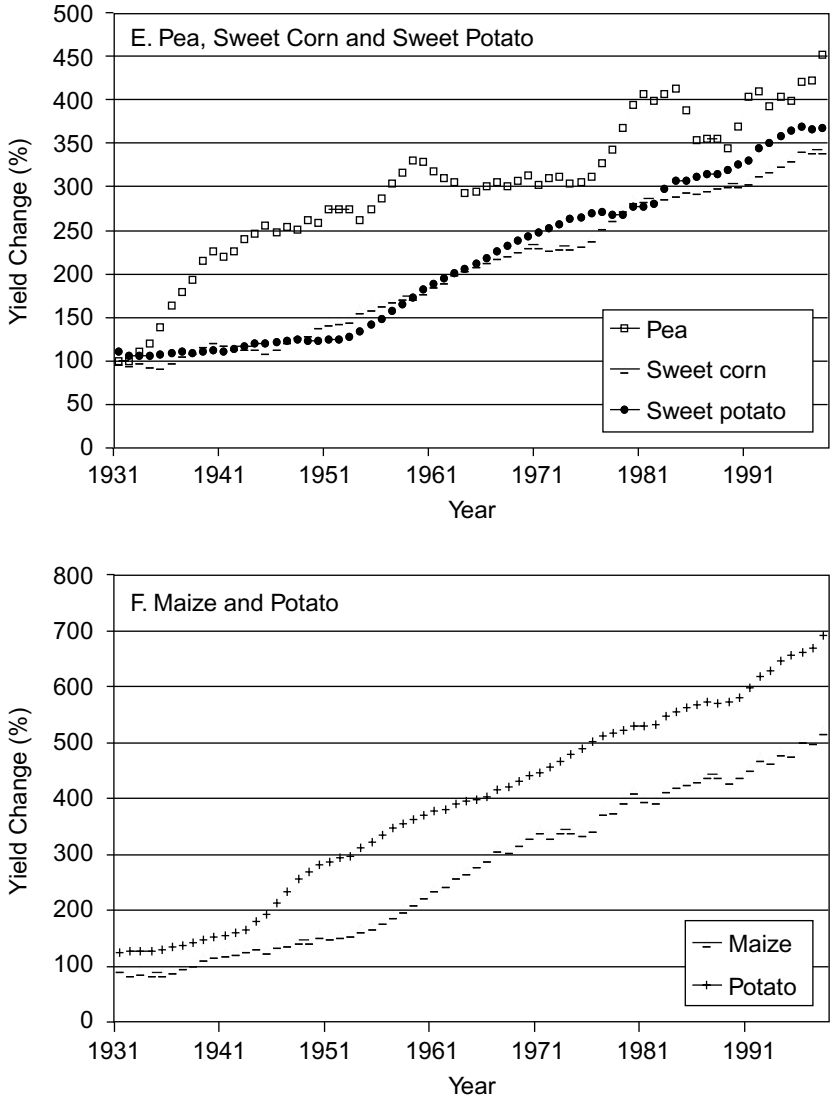

Fig. 3.3. (*Continued*)

productivity plateaued and has remained at 1980s levels ever since. Peanut yields tripled in 40 years, whereas it took more than 70 years for soybeans to make gains of the same magnitude.

Cotton (*Gossypium hirsutum* L.), rice, and sorghum (*Sorghum bicolor* (L.) Moench) have remarkable similarities in their productivity trends, despite significant differences in biology and cultural requirements (Fig. 3.3D). Productivity of all three either plateaued or increased very gradually from 1931 through the mid-1950s. Then productivity began to increase fairly rapidly until the mid-1960s, at which time productivity plateaued for 15 to 20 years followed by a brief period of increase. Productivity of these three crops has changed little over the last ten years. Given the biological differences among these species, it is tempting to hypothesize a large role for economic factors in determining the shape of these curves. Changes in availability and effectiveness of pesticides may be important in affecting changes in productivity of these crops. Despite the series of plateaus, the increases in these three crops were among the greatest of the crops examined, ranging from 4- to 5.5-fold (Fig. 3.3D, Table 3.1).

The four crops grouped as vegetable crops really have very little in common, other than being harvested and consumed at high moisture content. Sweet corn for processing and sweet potatoes (*Ipomoea batatas* (L.) Lam.) are grown in entirely different regions. The breeding systems are different, as is the investment by the private seed industry in their improvement (Frey 1996). Yet their productivity curves are nearly identical (Fig. 3.3E). Pea (*Pisum sativum* L.) productivity has increased by 4.5-fold. Grown in the same region, often by the same growers, pea and sweet corn have very different productivity trends. The sweet corn trend is strongly linear with a few minor plateaus, while pea productivity is marked by a series of lengthy plateaus interspersed with short rapid gains. Potato had the largest productivity increase of any of the crops surveyed. In 1998, productivity of potato was 6.9 times that of 1868. The productivity trends for potato and maize between 1931 and 1998 were remarkably linear (Table 3.1, Fig. 3.3F).

The above comparison of productivity trends was based on percent increases over time and overlooked the large differences in dry matter production among crops. Dry matter production of alfalfa, the crop with the smallest percent change (0.57-fold), increased by 3000 kg ha^{-1} approximately the same amount as sorghum, a crop with nearly a five-fold increase (Fig. 3.4). The increase in maize dry matter production slightly exceeds that of potato (Fig. 3.4).

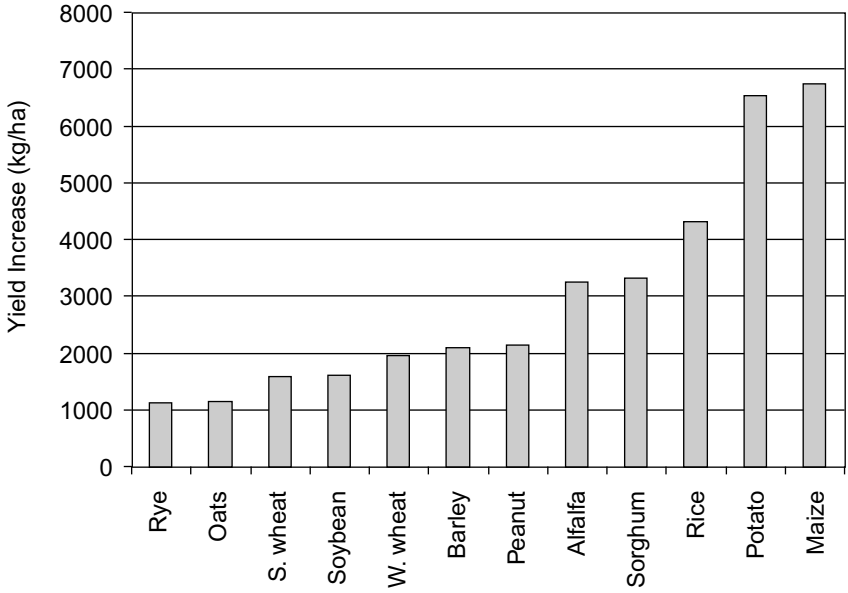

Fig. 3.4. Increase in dry matter production (kg ha^{-1}) between 1931 and 1998 for 12 crops.

B. Variability of Crop Productivity

When raw yield data are plotted, there appears to be an increase in the variability of crop yields over time (Fig. 3.1). Some have commented that the apparent increase in variability indicates lack of stability of modern crop cultivars and/or production systems. Others have indicated that the apparent variability is a simple arithmetic artifact resulting from the relationship between the size of the mean and the size of the deviation. Small means necessarily have small deviations and as the means increase so will the size of the deviation.

For maize, there seems to be no change in the amount of variation from 1868 through 1998 (Fig. 3.5A). In 1901, a 29.8% reduction in productivity relative to the five-year mean was the largest reduction during 130 years. The next largest reduction (24.2%) was in 1988. In 1901 average maize yield was 1128 kg ha^{-1}, while it was 5268 kg ha^{-1} in 1984. There appears to be a relatively stable period between 1950 and 1970 during which time deviations ranged between +6 and –8%.

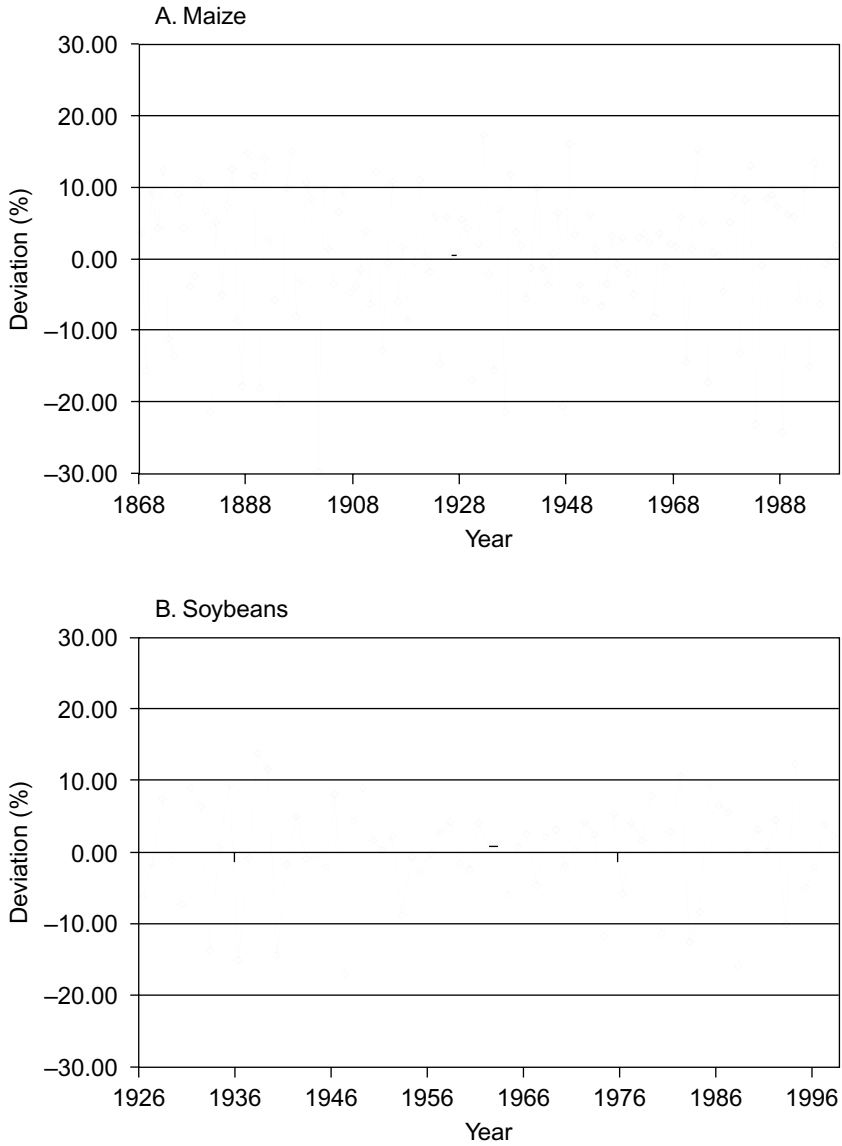

Fig. 3.5. Percent deviation of yearly average yield from the five-year mean centered on that year. A. Maize from 1868 to 1998. B. Soybean from 1926 to 1998. C. Rice from 1897 to 1998. D. Potato from 1868 through 1998.

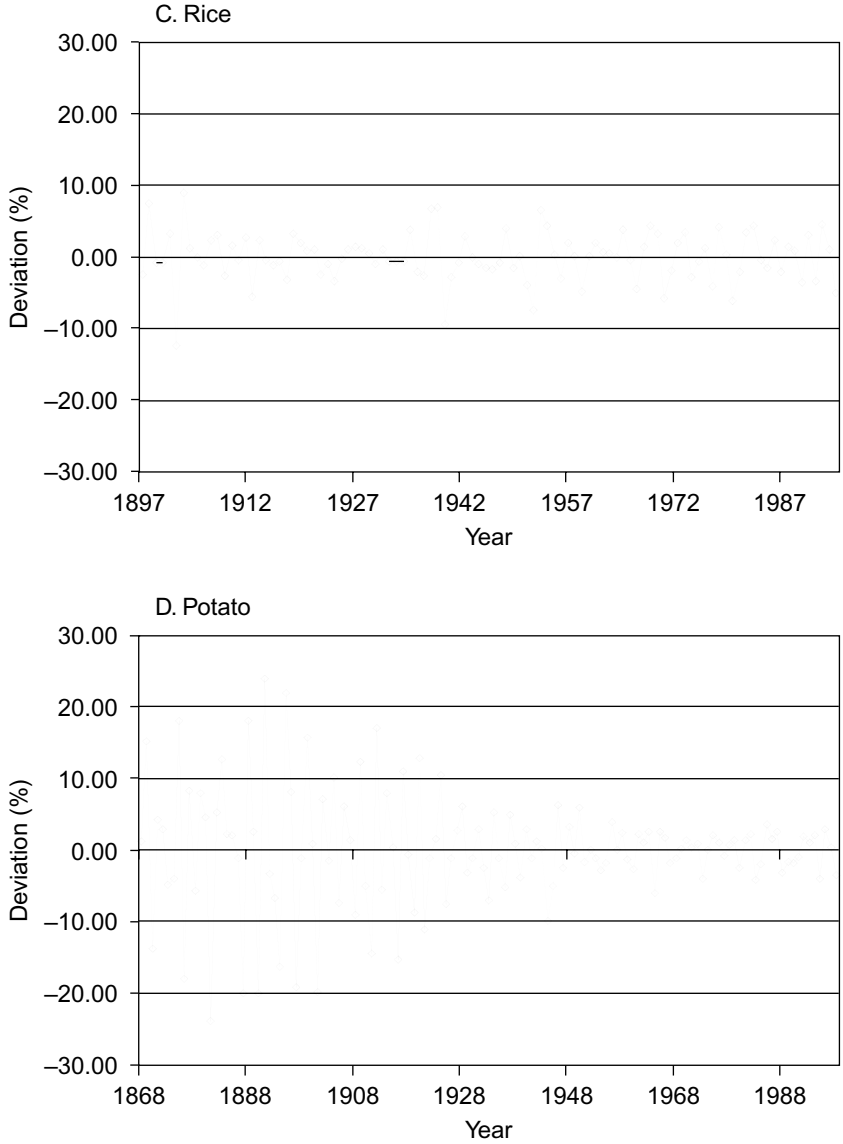

Fig. 3.5. (*Continued*)

Much of the soybean crop is grown in the same region as maize, and the soybean deviation data reflect the maize data. The worst year for soybeans was 1947 (fourth worst for maize over the same time period), followed by 1988. Soybean data also indicate the period of relative stability in the middle of the twentieth century (Fig. 3.5B).

We analyzed the pattern of deviation for all crops in this survey, and all of the crops had patterns similar to those of maize and soybean (Fig. 3.5A,B). With the exception of potato, none of the crops showed any change in variation over time (data not shown). The fact that variation has not been increasing over time is welcome but, at the same time it is disappointing that over 100 years of scientific plant breeding and agronomy has not decreased on-farm variation.

While many factors affect changes in productivity over time, climatic factors (especially rainfall) have the largest role in determining the pattern and magnitude of annual deviations. This is borne out be the data for rice (Fig. 3.5C). Since all rice grown in the United States is paddy rice, the effect of rainfall would be reduced relative to crops that are largely rainfed such as maize and soybean. With the exception of a deviation of –12.8% in 1902, maximum deviations for rice ranged from +8.9% to –9.5% (Fig. 3.5C). In the same time period, maize had five deviations greater than 20%, all negative. Between 1958 and 1998, rice yields deviated from the five-year means by greater than 5% only twice (5.8%, 1973; 6.1%, 1983), while over that same period maize yields deviated from the rolling mean by more than 10% nine times.

Unlike the other 15 crops, potato showed a change in the magnitude of deviation over time. The size of yearly yield deviations clearly decreased (Fig. 3.5D). From 1868 through the first years of the twentieth century, variation from the five-year mean was as great as in any other crop. Then sometime in 1910 or 1920 the magnitude of the deviations began to decrease. By the 1960s the variation in potato yields was as small as those observed in rice. This striking pattern may be evidence for the importance of water availability in crop productivity variation. Over the time covered by this plot, the bulk of potato hectarage shifted from the rainfed areas of the eastern United States to the irrigated areas of the West.

IV. CAUSES OF INCREASED PRODUCTIVITY

The USDA data do not allow any inferences regarding the causes of increased productivity. Careful experiments are required to determine the contributions of different factors to increasing crop yields. In many experiments, cultivars from different eras are grown under one set of management conditions, usually the current conditions, yields are determined, and linear regression is calculated. The regression coefficient is used as an estimate of genetic gain.

Such studies have been done on some agronomic crops but are rare for vegetables. Recent estimates for genetic gain in soybean range from 0.5 to 1.0% yr^{-1} and 14 to 22 kg ha^{-1} yr^{-1} (Voldeng et al. 1997; Specht et al. 1999; Ustun et al. 2001). Ustun et al. (2001) found that current cultivars also have better yield stability than older cultivars. Specht and Williams (1984) estimated that 50% of the gain in on-farm yields was due to genetic improvement. In wheat, genetic gains of 11.3 to 18.8 kg ha^{-1} yr^{-1} and 0.2 to 1.4% yr^{-1} have been reported (Cox et al. 1988; Donmez et al. 2001; Khalil et al. 2002a). Large genetic contributions to increasing crop productivity have been estimated in barley (73%), cotton (74%), and sorghum (39%) (Wych and Rasmusson 1983; Meredith and Bridge 1984; Miller and Kebede 1984).

Genetic effects can be overestimated in such studies due to changes in abiotic or biotic factors. In some studies, a portion of the apparent yield increase is actually due to greater susceptibility of older cultivars to current pest populations. As a result of pest pressure, yields of older cultivars are reduced relative to that of current, more resistant cultivars. But if the pests are controlled, no differences in yield are detectable (Peng et al. 1999; Riday and Brummer 2002). In a study of indica rice cultivars developed by the International Rice Research Institute, Peng et al. (1999) found no increases in yield potential over a 30-year period. When cultivars from different eras were tested side-by-side, newer cultivars yielded more. But this was apparently due to greater susceptibility of the older cultivars to current disease populations (Peng et al. 1999).

Comparing cultivars under only one set of management conditions is another source of potential overestimation of genetic gain, because genetic effects are confounded with the genetic by management interaction. When modern maize cultivars are grown under 1930s management, much of the yield gain evaporates (Duvick 1984; Duvick and

Cassman 1999). In maize, genetically improved cultivars are required for modern management practices to be effective and modern management practices are required for new cultivars to attain maximum yield. To estimate the genetic, management, and genetic × management interaction effects, cultivars representing different eras must be evaluated under multiple production practices, reflecting current management practices as well as older practices. Experiments must be conducted under a number of location-year combinations and must account for the interaction among factors in addition to individual factors. Experiments designed to determine the causes of crop yield changes that meet all the conditions required to partition genetic effects from management effects are relatively rare.

Among the most extensive studies of this kind in the United States are those of Duvick and colleagues (Duvick 1984, 1992; Duvick and Cassman 1999). Duvick (1984) evaluated the relative effects of genetic and management changes on maize hybrids grown from 1930 to 1980 in central Iowa. Duvick observed a strong linear increase in yield for the duration of this time period. He attributed 56% of the annual average gain in Iowa maize yields to genetic improvement. Much of the genetic change in maize has been adaptation to increased inputs and stress associated with high input management systems. Modern maize is more resistant to drought stress and stalk lodging than maize from the 1930s. Drought stress and lodging are associated with high population densities (30,000 plants ha^{-1} in the 1930s; 80,000 plants ha^{-1} in the 1980s) (Duvick 1999). High levels of available nitrogen also increase stalk lodging in grain crops. In maize, genetic changes were required for adaptation to new management systems. Increased resistance to the stress applied by intensive management systems is the basis for the majority of genetic gains for yield in maize and most other crops (Cassman 1999; Evans and Fischer 1999). In comparing worldwide yield changes in wheat, maize, and rice, Cassman (1999) asserts that only wheat has shown genetic gains in both yield potential and stress resistance.

Potato, the crop with the highest percentage gains in our survey, offers a striking contrast to maize. While commercial maize hybrids are replaced by new hybrids in three to five years (Duvick and Cassman 1999), potato cultivars may maintain a large market share for decades, indicating that genetic changes on a national basis must be very slow. ‘Russet Burbank’ was released in 1914 and is still widely grown today (Douches et al. 1996). Controlled studies indicate that genetic changes have contributed little to increased yields (Douches et al. 1996). Genetic changes were associated with early maturity and improved tuber appear-

ance. Thus, the primary cause of increased yields was improved management practices, including improved pest control and increased fertilizer application and irrigation.

At first glance, it is remarkable that nineteenth-century potato cultivars are able to tolerate very high inputs, especially large amounts of nitrogen fertilizer. In cereals, high soil nitrogen results in increased stalk lodging. Pre-1940s cultivars of maize, wheat, and rice lodge severely at modern nitrogen levels. In response to increased nitrogen inputs, maize breeders improved stalk and root strength (Duvick 1984). Green revolution wheat and rice breeders introduced semi-dwarf cultivars, which can tolerate higher levels of nitrogen (Evans and Fischer 1999). Potato's harvestable biomass is below ground; therefore, increased nitrogen fertility can increase yield without increasing lodging.

Relative to the other crops, alfalfa yields have increased little (57% from 1920 to 1998). Holland and Bingham (1994) estimated a gain of approximately 0.18% yr^{-1}. But Riday and Brummer (2002) suggest that the figure may be inflated due to changes in disease populations. Limited gains in alfalfa may be due to both the biology and use of alfalfa. Because alfalfa fixes nitrogen, yields were not increased by the increased application of nitrogen fertilizer, as has been the case in many other crops. Essentially all aboveground plant parts are harvested, so there are fewer genetic options in terms of changes in harvest index or plant habit. Unlike many other crops, alfalfa is not managed for maximum yield production, because feed value decreases as yield increases (Undersander et al. 1994).

V. LONG-TERM SELECTION AND CROP PRODUCTIVITY

Long-term selection as practiced in the Illinois Long-term Selection program features a closed population with cyclic selection practiced over many generations. Selection in each cycle is for the same trait or group of traits. Selected progeny make up the parental generation for the next cycle. Long-term selection in a strict sense is seldom practiced in most crop cultivar development programs. Cultivar development programs in some crops approximate scientific long-term selection experiments, but in other crops have few features of long-term selection.

Maize breeding in the United States has many features of long-term selection. While not a completely closed population, the majority of parental material is derived from the most elite germplasm of the previous generation and very little outside germplasm has been incorporated

into the pool (Goodman 1999). Traditionally, breeders from different companies access germplasm by using competitors' hybrids as parental material. Most breeders have very similar selection criteria. Studies clearly indicate long-term trends in changes of yield and yield components and these changes have a genetic basis (Duvick 1984; Duvick and Cassman 1999). Thus the U.S. maize breeding industry over most of the twentieth century could be considered a large-scale demonstration of long-term selection.

Sweet corn represents an interesting contrast to maize. While the two crops are conspecific, the breeding objectives are quite different. Most U.S. maize is grown for animal feed and industrial uses. Harvestable yield is the main selection criterion and relatively little attention is given to grain quality. Sweet corn is consumed as a fresh or processed vegetable. Many different factors determine overall table quality, and some of these have pleiotropic effects on other characteristics such as yield, germination, and pest resistance. A large portion of sweet corn breeding effort is devoted to improving established inbreds by backcrossing individual genes for quality or disease resistance (Marshall and Tracy 2003). Thus, sweet corn breeding in the aggregate bears little resemblance to long-term selection in a closed randomly-mated population. Sweet corn breeders may also cycle germplasm from other companies through their program, but this may actually result in the antithesis of long-term selection. Different sweet corn breeders often have very different selection criteria. One might focus on fresh quality and disease resistance while another selects for processing quality and yield.

Backcrossing is a key feature of breeding programs for many crop species. Incorporating single genes for pest resistance, plant architecture, photoperiod response, quality, and other traits is a common feature in the breeding of many crops. U.S. hybrid maize breeding, characterized by little backcrossing, may be the exception to the importance of backcrossing in cultivar development. However, with the advent of transformation technology, backcrossing has now assumed greater importance in maize cultivar development programs.

Other crop species may incorporate features of long-term selection but for traits other than yield. Among the primary long-term objectives for many crops may be improved quality or stress or pest resistance, which in some cases may be antagonistic to yield (Hill et al. 1988; Douches et al. 1996). Milling quality for wheat (Khalil et al. 2002b), malting quality for barley (Wych and Rasmusson 1983), and oil and protein composition for soybean (Ustun et al. 2001) are key considerations in those crops. Alfalfa breeders routinely select for multiple pest resistance (Hill

et al. 1988), and potato breeding has been characterized by selection for early maturity and improved tuber appearance (Douches et al. 1996). Thus long-term selection may be a characteristic of cultivar development in many crops but would not be observed in our survey of yield trends in the twentieth century. In some cases, the changes in other traits, despite concurrent selection for increased yield, may have slowed upward yield trends due to improved management.

The Illinois Long-term Selection Experiment clearly demonstrates the power of phenotypic selection and the importance of the indirect effects of selection. Current trends in germplasm ownership and transformation technology may make it more difficult for breeders to incorporate features of long-term selection into their breeding programs. Given the potential power of long-term selection, it is important that plant breeders develop methods that integrate the creative power of long-term selection with the conservative backcross breeding method characteristic of transformation programs. Ways of exchanging germplasm that will make available the raw material for crop improvement while protecting the investment of the developer of that germplasm will also need to be developed to maximize genetic gain.

LITERATURE CITED

Cassman, K. G. 1999. Ecological intensification of cereal production systems: Yield potential, soil quality, and precision agriculture. Proc. Natl. Acad. Sci. (USA) 96:5952–5959.

Cox, T. S., J. P. Shroyer, L. Ben-Hui, R. G. Sears, and T. J. Martin. 1988. Genetic improvement in agronomic traits of hard red winter wheat cultivars from 1919 to 1987. Crop Sci. 28:756–760.

Donmez, E., R. G. Sears, J. P. Shroyer, and G. M. Paulsen. 2001. Genetic gains in yield attributes of winter wheat in the Great Plains. Crop Sci. 41:1412–1419.

Douches, D., D. Maas, K. Jastrzebski, and R. W. Chase. 1996. Assessment of potato breeding progress in the USA over the last century. Crop Sci. 36:1544–1552.

Duvick, D. N. 1984. Genetic contributions to yield gains of US hybrid maize, 1930–1980. p. 15–41. In: W. R. Fehr (ed.), Genetic contributions to yield gains of five major crop plants. CSSA Special Publication 7. ASA-CSSA-SSSA, Madison, WI.

Duvick, D. N. 1992. Genetic contributions to advances in yield of US maize. Maydica 37:69–79.

Duvick, D. N. 1999. Heterosis: Feeding people and protecting natural resources. p. 19–29. In: J. G. Coors and S. Pandey (eds.), The genetics and exploitation of heterosis in crops. ASA-CSSA-SSSA, Madison, WI.

Duvick, D. N., and K. G. Cassman. 1999. Post-green revolution trends in yield potential of temperate maize in the north-central United States. Crop Sci. 39:1622–1630.

Evans, L. T., and R. A. Fischer. 1999. Yield potential: Its definition, measurement, and significance. Crop Sci. 39:1544–1551.

Frey, K. J. 1996. National plant breeding survey–1. Special Rep. 98. Iowa State Univer., Iowa Agriculture and Home Economics Experiment Station, Ames.

Goodman, M. M. 1999. Broadening the genetic diversity in maize breeding by use of exotic germplasm. p. 139–148. In: J. G. Coors and S. Pandey (eds.), The genetics and exploitation of heterosis in crops. ASA-CSSA-SSSA, Madison, WI.

Hill, R. R., Jr., J. S. Shenk, and R. F. Barnes. 1988. Breeding for yield and quality. p. 809–875. In: A. A. Hanson et al. (eds.), Alfalfa and alfalfa improvement. Agronomy Monograph 29. Amer. Soc. Agron., Crop Sci. Soc. Amer., Soil Sci. Soc. Am. Madison, WI. p. 809–825.

Holland, J. B., and E. T. Bingham. 1994. Genetic improvement for yield and fertility of alfalfa cultivars representing different eras of breeding. Crop Sci. 34:953–957.

Khalil, I. H., B. F. Carver, E. G. Krenzer, C. T. MacKown, and G. W. Horn. 2002a. Genetic trends in winter wheat yield and test weight under dual purpose and grain-only management systems. Crop Sci. 42:710–715.

Khalil, I. H., B. F. Carver, E. G. Krenzer, C. T. MacKown, G. W. Horn, and P. Rayas-Duarte. 2002b. Genetic trends in winter wheat grain quality with dual purpose and grain-only management systems. Crop Sci. 42:1112–1116.

Marshall, S. W., and W. F. Tracy. 2003. Sweet corn. (in press). In: P. E. Ramstad and P. White (eds.), Corn chemistry and technology, 2nd edition. American Association of Cereal Chemists, Minneapolis, MN.

Meredith, W. R., Jr., and R. R. Bridge. 1984. Genetic contributions to yield changes in upland cotton. p. 75–87. In: W. R. Fehr (ed.), Genetic contributions to yield gains of five major crop plants. CSSA Special Publication 7. ASA-CSSA-SSSA, Madison, WI.

Miller, F. R., and Y. Kebede. 1984. Genetic contributions to yield gains in sorghum, 1950–1980. p. 1–14. In: W. R. Fehr (ed.), Genetic contributions to yield gains of five major crop plants. CSSA Special Publication 7. ASA-CSSA-SSSA, Madison, WI.

Peng, S., K. G. Cassman, S. S. Virmani, J. Sheehy, and G. S. Kush. 1999. Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. Crop Sci. 39:1552–1559.

Riday, H., and E. C. Brummer. 2002. Forage yield and heterosis in alfalfa. Crop Sci. 42: 716–723.

Specht, J. E., D. J. Hume, and S. V. Kumudini. 1999. Soybean yield potential: A genetic and physiological perspective. Crop Sci. 39:1560–1570.

Specht, J. E., and J. H. Williams. 1984. Contribution of genetic technology to soybean productivity—retrospect and prospect. p. 49–74. In: W. R. Fehr (ed.), Genetic contributions to yield gains of five major crop plants. CSSA Special Publication 7. ASA-CSSA-SSSA, Madison, WI.

Undersander, D., N. Martin, D. Cosgrove, K. Kelling, M. Schmitt, J. Wedberg, R. Becker, C. Grau, J. Doll, and M. E. Rice. 1994. Alfalfa Management Guide. NCR547. Am. Soc. Agron., Madison, WI.

USDA Agricultural Statistics Bulletins. 1900–2001. United States Department of Agriculture, Washington, DC.

Ustun, A., F. L. Allen, and B. C. English. 2001 Genetic progress in soybean in the US midsouth. Crop Sci. 41:993–998.

Voldeng H. D., E. R. Cober, D. J. Hume, C. Gillard, and M. J. Morrison. 1997. Fifty-eight years of genetic improvement of short season soybean cultivars in Canada. Crop Sci. 37:428–431.

Warren, G. F. 1998. Spectacular increases in crop yields in the United States in the Twentieth Century. Weed Technol. 12:752–760.

Wych R. D., and D. C. Rasmusson. 1983. Genetic improvement in malting barley cultivars since 1920. Crop Sci. 23:1037–1040.

4

Long-term Selection in a Commercial Hybrid Maize Breeding Program

D. N. Duvick
Department of Agronomy
Iowa State University
Ames, Iowa 50011-1010

*J. S. C. Smith and M. Cooper**
Pioneer Hi-Bred International, A DuPont Company
Johnston, Iowa 50131

*We thank the many Pioneer scientists who have contributed to the collection, analysis, and interpretation of data from the Era hybrid studies. We are indebted to the farmer breeders and professional plant breeders that created this germplasm.

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-46892-4

I. INTRODUCTION

A. Utility of Studying Long-term Commercial Breeding Programs

Commercial plant breeders have the same goal as plant breeders in the public sector—to produce improved cultivars that suit the needs of farmers who plant them. But commercial breeding differs from public sector breeding in at least one respect: retrospective analyses of long-term commercial breeding programs are seldom published in the scientific literature. Job security for a commercial breeder depends on production of successful cultivars, not on production of peer-reviewed publications. However, both private and public plant breeders could benefit from such reports. The reports could show the consequences (intended or unintended) of specific breeding and selection programs, measure rates of gain for important traits such as yield or pest resistance, and identify and quantify useful sources of and changes in genetic diversity. Such information and analysis could help breeders to refine and/or change their breeding programs in desired directions and to make qualified estimates of future progress.

B. A Long-term Selection Program in the Private Sector: 1930s to 2000s

This report will summarize the findings of a series of studies (Duvick 1977, 1984, 1992, 1997; Duvick et al. 2003) of a long-term commercial plant-breeding program—a "long-term selection experiment." The studies compare field performance, parental origins, and genotype changes for a time-series of maize hybrids (see Table 4.1, "Era hybrids") that have been released sequentially in the West-central U.S. Corn Belt during the past seven decades—from the early 1930s through 2001. Each hybrid was successful and widely grown in its time. All of the hybrids were bred, produced, and sold by Pioneer Hi-Bred International. This constantly lengthening series of hybrids has been grown in side-by-side per-

Table 4.1. List of Era hybrids and year of hybrid release. Open pollinated cultivars included in the list arbitrarily are classified as 1930, a year in which they were widely grown and well regarded.

Cultivar	Year	Cultivar	Year	Cultivar	Year
RYD1[z]	1930	329	1954	3378	1983
BYD	1930	354A	1958	3475	1984
KYD	1930	328	1959	3379	1988
RYD2	1930	3618	1961	3362	1989
351[y]	1934	3206	1962	3417	1990
307	1936	3306	1963	3394	1991
322	1936	3376	1965	3563	1991
317	1937	3390	1967	3279	1992
330	1939	3571	1968	3489	1994
336	1940	3334	1969	3335	1995
340	1941	3388	1970	34G81	1997
339	1942	3517	1971	33A14	1997
344	1945	3366	1972	33G26	1998
352	1946	3301A	1974	34B23	1999
350B	1948	3529	1975	33P66	1999
347	1950	3541	1975	33P67	1999
301B	1952	3382	1976	34G13	2000
354	1953	3377	1982	34M95	2001
				33R77	2001

[z]RYD1 = Neal Reid Yellow Dent; BYD = Black Yellow Dent; KYD = Krug Yellow Dent; RYD2 = Reid Yellow Dent. BYD, KYD and RYD obtained from USDA Plant Introduction Station, Ames, IA. RYD1 obtained from Iowa farmer, Neal.

[y]Numerical description of hybrids.

formance trials annually during the past 25 years, and was also subjected to other phenotypic and genotypic measurements. From time to time the accumulated data have been summarized, analyzed, and published. We will review pertinent comments and data from those published reports and also present new data relative to this "long-term selection experiment."

1. Intended Breeding Goals. Higher grain yield has been the Pioneer breeders' avowed primary breeding goal over the decades, but from time to time various biotic and abiotic problems have necessitated the urgent implementation of additional goals. For example:

> Severe drought during the early years of the 1930s pushed selection in the direction of drought tolerance, but the occurrence of ear smut [*Ustilago zeae* (Beckm.) Unger] in some of the

drought-tolerant germplasm then necessitated selection for resistance to smut.

The onset of European corn borer [*Ostrinia nubilalis* (Hübner)] in the 1950s brought new needs for insect tolerance.

Farmer use of higher plant densities and increased rates of nitrogen fertilizer, starting in the 1960s, increased needs for hybrids with stronger roots and stalks, and greater resistance to barrenness.

Plant density continues to increase to this day, even though nitrogen application amounts have leveled off. Constantly increasing plant density constantly increases needs for drought and shading tolerance, as well as for other kinds of stress tolerance.

In recent years no-till and minimum-till planting have increased the need for hybrids with tolerance to new diseases such as gray leaf spot (*Cercospora zeae-maydis* Tehon & E.Y. Daniels).

Thus, one might say that the breeders' constant goal has been to develop hybrids that produce higher yields in spite of a constantly enlarging array of biotic and abiotic stresses. The stresses were brought on in part by changing cultural techniques and in part by the increasingly monoculture nature of maize production in the U.S. Corn Belt.

2. Starting Materials. Open pollinated cultivars (OPCs), such as 'Reid Yellow Dent' and 'Krug', provided germplasm for the first round of inbred development (started in the 1920s) and subsequent hybrid production. The OPCs usually were chosen because of superior performance in yield trials such as those conducted by Iowa State College, or because they were highly regarded by farmers. Crosses among some (but not all) of these first inbreds were the foundation of the next cycle of selfing and inbred production. Those inbreds were supplemented by a few new inbreds that had been developed from additional OPCs. Later cycles of inbred production continued this pattern of recycling currently successful inbreds while also incorporating germplasm of a few new inbreds from other sources. In the early decades, public inbred lines such as WF9 and M14 significantly contributed to pedigrees of the Era hybrids. They supplemented the small number of inbreds that had been developed by the initially small number of Pioneer breeders (one breeder for the entire company, in the beginning). But over the decades the proportion of Pioneer-bred inbred lines increased continually to the point that no public inbreds were used directly in any of the hybrids. During the past

two decades, all inbreds in Era hybrids have been Pioneer-bred. However, some of the important public inbred lines from earlier decades (e.g., Oh43, B37, B73) were progenitors of highly useful inbred lines as developed by Pioneer breeders. Details of this evolutionary history will be shown in later sections of this account.

3. Breeding Methods, Breeders. Pedigree breeding—to develop new inbreds by selfing crosses of proven inbred lines—has been the primary means of developing new inbreds during the seven decades of breeding Era hybrids. Improved populations occasionally have produced successful inbred lines or (more frequently) contributed as a parent when crossed to one or more successful inbred lines. A small number of outstandingly important inbred lines have come from synthetic populations (e.g., the public inbreds B37 and B73 from BSSS, the Iowa Stiff Stalk Synthetic). But the great majority of the inbreds in the Era hybrids were developed by means of pedigree breeding at the hands of Pioneer breeders.

During the past 70 years, many breeders have contributed to development of the Era hybrids, despite the small number of breeders in the beginning. Collectively, they have encompassed a wide range of education and experience and, consequently, they have differed in how they practiced the art and science of plant breeding. The composition of the group has changed continually over the seven decades of this long-term selection program. The first breeders had little or no training in plant breeding and genetics but their replacements had more training in these fields and eventually all breeders had advanced degrees in plant breeding or allied fields of science. Not more than two or three breeders at a time were directly responsible for making and testing inbreds and hybrids for central Iowa (the adaptation region of the Era hybrids). However, Pioneer breeders from other parts of the U.S. Corn Belt have contributed numerous inbred lines or hybrids (especially in the later decades) that ended up in one or more Era hybrids.

The one consistent feature of the plant breeding group was its pragmatism. If a method or source of germplasm worked, it was used whether or not it fit the current styles in breeding theory. All newly proposed theoretical approaches were tried thoroughly, although not at the expense of practical techniques that were producing good results. If the new approaches worked, they were used; if they proved to be unproductive after thorough testing, they were discontinued. The net result (as noted above) has been that over the years the breeders primarily, but not exclusively, have relied on pedigree breeding. It has been the most

dependable method for producing improved inbred lines. Germplasm for the most part came from inbred lines that had proven their utility in commercial hybrids. Widespread on-farm performance of released hybrids was used to identify the top-performing inbreds, to winnow the best from merely average germplasm.

Over the years the pedigree breeding effort has evolved from a structure analogous to a single population improvement program to a two-population improvement program. With the formation of the current Stiff Stalk (SS) and Non Stiff Stalk (NSS) heterotic groups of germplasm (e.g., Casa et al. 2002), this breeding approach can be considered to be an open reciprocal recurrent selection (RRS) strategy, with pedigree breeding being undertaken within both the SS and NSS heterotic groups. These groups can be further divided into sub-groups and heterosis is evident among some of these sub-groups. New inbreds developed within one heterotic group are evaluated on their testcross performance in multi-environment trials, with testers being drawn from one or more pools of elite inbreds drawn from a complementary heterotic group.

II. RESULTS

A. Performance Trials

1. Commercial Hybrids

Grain Yield. Previous studies of the Era hybrids consistently have shown linear increases in grain yield, from oldest to newest hybrids (Duvick 1977, 1984, 1992, 1997; Duvick et al. 2003). Genetic yield gain has been continual and constant in amount (on average) over the years. The studies also consistently show that the oldest hybrids make their maximum yields at lower plant densities typical of maize farming in the early decades, whereas the newer hybrids yield the most at higher plant densities typical of recent decades. Plant densities in the 1930s were ca. 30 thousand plants/ha. Planting rates have gradually increased until today in central Iowa plant densities are ca. 75 thousand plants/ha or higher.

In contrast to annual increases in yield per unit area, the reports show that yield potential per plant (maximum possible grain yield per plant) has not increased appreciably over the years. That is, the oldest hybrids yield nearly as much as the newest hybrids when plants are widely spaced (10 thousand plants/ha), have minimal levels of biotic and abiotic stress, and, consequently, are able to maximize the yield on individual plants. This result shows that increases in yield per unit area of

the newer hybrids are owed not to increased yield potential per plant but, rather, to the hybrids' ability to produce a minimum amount of grain (about 0.15 kg) on every plant when they are grown at higher plant densities. At today's higher plant densities, the older hybrids typically have a high percentage of barren plants, or ears with very few kernels, whereas the new hybrids have an ear on every plant.

The yield data from recent years agree with the previous results. Trials of Era hybrids (listed in Table 4.1) conducted during the past 10 years (1991 to 2001) show that the annual genetic yield gain is still linear, averaging 77 kg/ha (Fig. 4.1). Fig. 4.1 shows the yield of each hybrid at the plant density in which it made the highest yield. (The hybrids were compared in split-plot trials at 30, 54, and 79 thousand plants/ha.) Use of the "optimum plant density" yield gives a more realistic measurement of genetic yield gain, as compared with measurements at any single plant

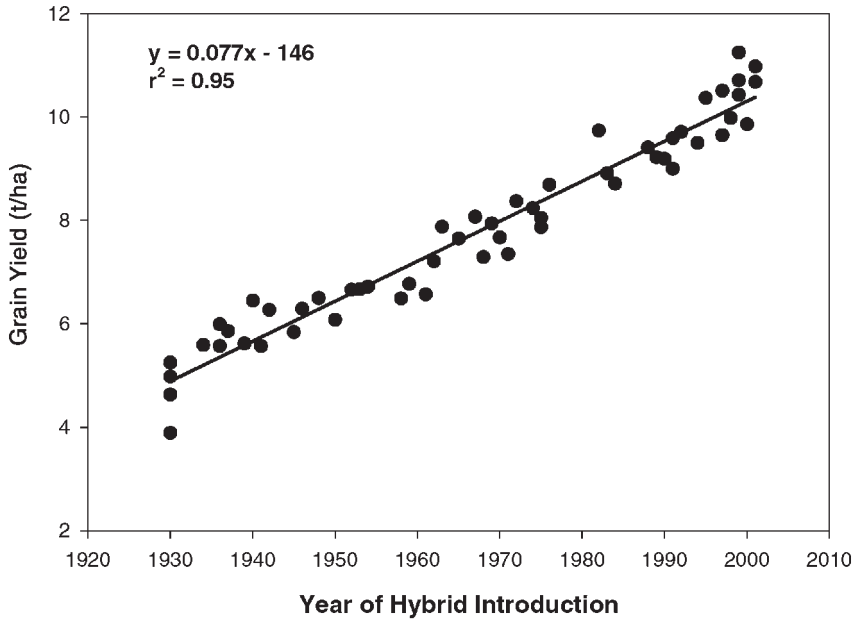

Fig. 4.1. Grain yield per hybrid regressed on year of hybrid introduction. Best Linear Unbiased Predictors (BLUPs) of hybrid grain yield based on trials grown in the years 1991 to 2001, at three locations per year in central Iowa, at three densities (30, 54, and 79 thousand plants/ha), one replication per density. Yield per hybrid is for the density giving the highest average yield.

density, because each hybrid is evaluated at a reasonably close approximation of the plant density at which it was bred and tested. As noted earlier, usually the older hybrids yield most at lower plant densities and the newer hybrids yield most at higher plant densities.

Recent yield data also agree with those from earlier years (and thus shorter time-series) in showing a strong interaction between hybrid age and plant density in regard to their effects on grain yield (Fig. 4.2). Fig. 4.2 summarizes the 1991 to 2001 data for Era hybrids when grown at three widely different plant densities: ca. 10, 30, and 79 thousand plants/ha. (The 10 thousand plants/ha density was planted alongside the 3-density split-plot trial, essentially giving a split-split-plot trial. It was intended to measure potential yield per plant at minimal levels of stress.) Fig. 4.2 demonstrates clearly that although genetic yield gain over the years is linear and positive at all three plant densities, the gain is minimal or nil at 10 thousand plants/ha and maximal at 79 thousand plants/ha.

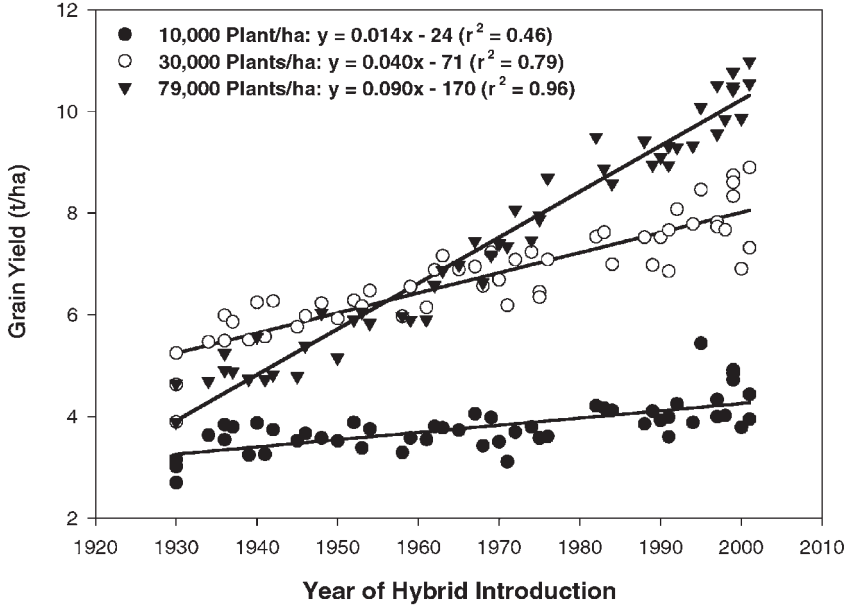

Fig. 4.2. Grain yield per hybrid regressed on year of hybrid introduction at each of three plant densities (10, 30, and 79 thousand plants/ha). Best Linear Unbiased Predictors (BLUPs) for hybrid grain yield based on trials grown in the years 1991 to 2001, three locations per year, one replication per density.

Yield gains for the time-series were nearly as great in low-yield (i.e., high stress) seasons as in high-yield (i.e., low stress) seasons. For example, trends were linear and similar in amount in three widely different seasons (Fig. 4.3). The seasons are described as follows:

1992: Exceptionally high yields because of a "nearly perfect" growing season. Statewide yields in 1992 were at record highs.

1993: Low yields because of waterlogged soil and cool temperatures during most of the growing season. ("The Year of the Floods," was one of the coolest and wettest growing seasons on record for Iowa.)

2001: Low yields because of a season-long local drought, especially severe at the sensitive anthesis-silking period.

The newer hybrids outyielded the older hybrids in low-yield as well as in high-yield environments, not only between seasons but also within

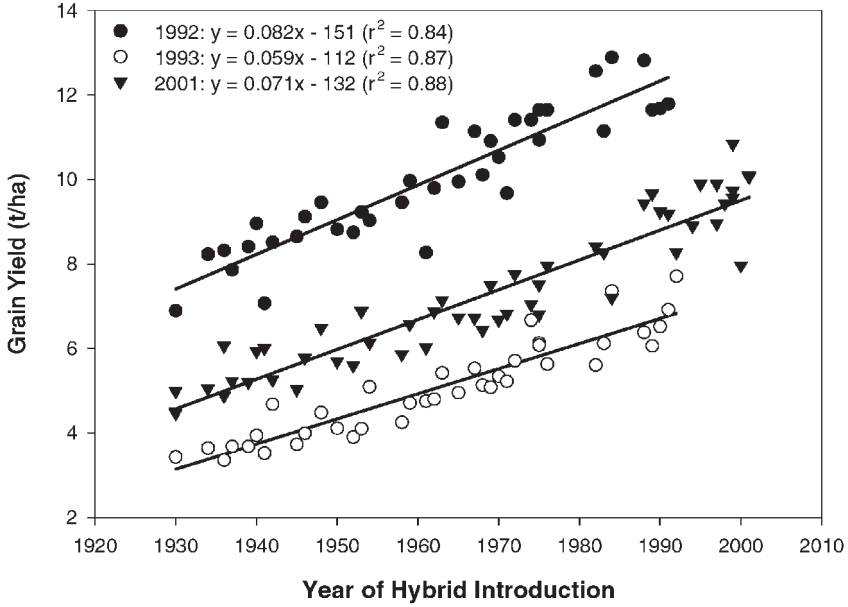

Fig. 4.3. Grain yield per hybrid regressed on year of hybrid introduction for trials grown in 1992, 1993, and 2001. Yield per hybrid is for the density giving the highest average yield.

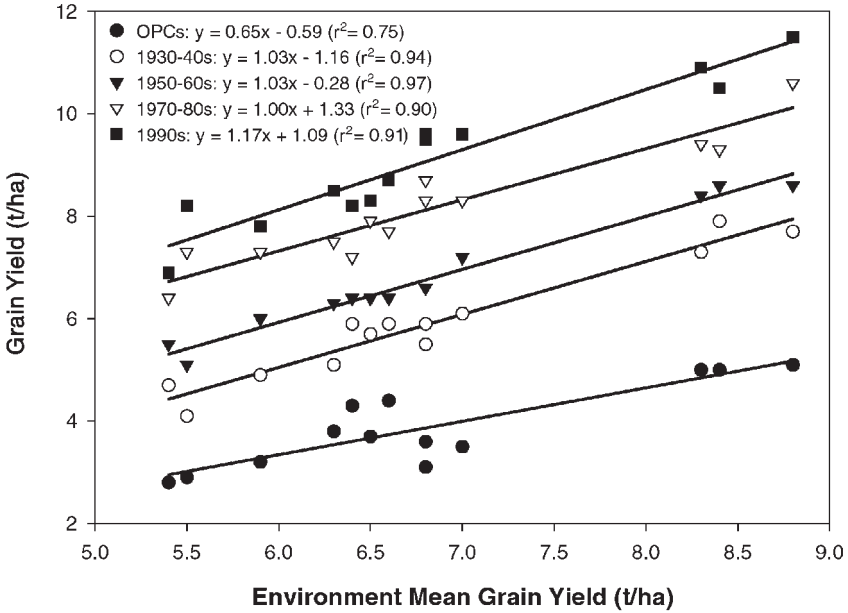

Fig. 4.4. Mean grain yield of hybrids released within two-decade spans, and of three OPCs, regressed on mean yield of all hybrids per environment. Trials grown in a total of 13 environments during the years 1996 through 2000. Means of three plant densities per environment (30, 54, and 79 thousand plants/ha).

seasons. When hybrids are aggregated by double-decade of release (1930s plus 1940s, etc., with OPCs in a class by themselves), the newer the class of hybrids the higher the yield, in low-yield as well as high-yield trial locations (Fig. 4.4). Data are from 13 locations, accumulated over the years 1996 to 2000.

Other Traits. Table 4.2 summarizes various observations that have been made from time to time on additional traits of the Era hybrids. Several of these traits have changed over the years. The changes can be sorted arbitrarily into two groups: (1) changes that add efficiency to the maize plant's grain production process, and (2) changes that improve or give evidence for improvement in stress tolerance and thus improve chances for good yields in stressful growing conditions. (We emphasize that this is an arbitrary classification and note that to list a trait in one category does not imply that it cannot also have some effect in the alternate category; for example, efficiencies from reduced tassel size could well increase stress tolerance in regard to grain production.)

Table 4.2. Linear regressions (b) of trait on year of introduction of Era hybrids introduced from 1934 to 2001. Regressions include some or all of the hybrids in Table 4.1, depending on the year in which measurements were taken. The data for all of the 1991 to 2001 summaries (based on Best Linear Unbiased Predictors), and for leaves per plant, fodder weight, harvest index, and mean value per trait are reported herein for the first time. All other data are from Table 2 of Duvick 1997.

Trait change/stability	Trial years	Density (000 ha^{-1})	b (10 yr^{-1})	R^2	Trait mean
Change, Efficiency					
Tassel weight	1992	30,54,79	−0.5 g	0.70	3.5
Tassel branch number	1992	30,54,79	−2.5 branch	0.66	15.5
Tassel size score[z]	1991–2001	30,54,79	−0.9 score	0.91	5.4
Grain protein (%)	1992	30,54,79	−0.3%	0.68	9.8
Grain starch (%)	1992	30,54,79	+0.3%	0.56	70.4
Leaf angle score	1991–2001	30,54,79	+1.0 score	0.81	4.7
Non-tillered plants (NT) (%)	1992, 1994	30,54,79	+4% NT	0.48	86.4
Change, Stress tolerance					
ASI	1991–2001	30,54,79	−13 GDU	0.81	48
Ears per 100 plants	1991–2001	30,54,79	+3.6 ears	0.78	92.9
Staygreen score	1991–2001	30,54,79	+0.6 score	0.75	5.1
Not stalk-lodged (%)	1991–2001	30,54,79	+1.6%	0.69	91.3
Not root-lodged (%)	1991–2001	30,54,79	+4.1%	0.67	81.5
ECB2 tunnel length	1992	30,54,79	−1.5 cm	0.46	9.9
ECB2 damage score	1992, 1994	30,54,79	+0.5 score	0.58	2.9
NLB tolerance score	1994	30,54,79	+0.7 score	0.34	4.3
Stability, Intentional					
Plant height	1991–2001	30,54,79	−1 cm	0.11	270
Ear height	1991–2001	30,54,79	−3 cm	0.40	116
50% anthesis, GDU	1991–2001	30,54,79	+4 GDU	0.07	1412
Grain moisture (%)	1991–2001	30,54,79	+0.1%	0.05	21.88
Stability, Unintentional					
Leaves per plant	1992	30,54,79	+0.1	0.05	13.3
Fodder weight per plant	1985	30,54,69	+3 g	0.01	220
Harvest Index (%)	1985	30,54,69	+1.0	0.45	40
Rows per ear	1992	30,54,79	−0.5 rows	0.36	16.7
Kernels per row	1992	30,54,79	+0.4 k/row	0.06	40.2
Kernels per ear	1992	30,54,79	−11 kernels	0.11	670
100 kernel weight	1992	30,54,79	+0.7 g	0.31	31.7
Test wt. since 1955	1991–2001	30,54,79	+8.7 kg m^{-3}	0.65	704
Grain oil (%)	1992	30,54,79	−0.0%	0.07	4.5
ECB1 leaf feed score	1994	30,54,79	+0.3 score	0.19	2.6
Heterosis (SX-MP)					
Plant height	1992–1993	30,54,79	−9 cm	0.26	+193
50% Anthesis	1992–1993	30,54,79	−0.3 GDU	0.00	−117
Grain Yield (t/ha)	1992–1993	30,54,79	+0.1 t/ha	0.01	+4.3

[z]Tassel size score: 1 to 9, 9 = largest size; Leaf angle score: 1 to 9, 9 = most upright; ASI: anthesis-silking interval; GDU: growing degree units, °C; Staygreen score: 1–9, 9 = least premature senescence; ECB2: European corn borer, 2nd generation; ECB2 damage score: 1–9, 9 = least damage; Harvest index: grain to fodder ratio; ECB1: European corn borer, 1st generation; ECB1 leaf feeding score: 1–9, 9 = least leaf feeding; NLB: Northern leaf blight; NLB Score: 1–9, 9 = most tolerant; SX-MP = single cross minus mid-parent mean.

Some traits have not changed, or the amount of change has been nonsignificant—the traits have been relatively stable over the years. These "stable" traits also can be sorted into two groups: (a) stability enforced by way of breeders' selection (such as a ceiling on grain moisture at harvest time) and (b) stability in absence of breeder attention (or in some cases, stability despite breeders' hope for change).

Changes that increase efficiency of grain production are: smaller tassels (more energy available for grain production, and less shading of the leaves); reduced grain protein percentage and increased grain starch percentage (less energy is required to make starch than to make protein); more upright leaves (better light interception in dense plantings); and fewer tillers (assuming that tillers without ears use water and nutrients that otherwise would be available for grain production).

Changes that demonstrate or provide greater stress tolerance are: shorter anthesis-silking interval (an indication that ear development is not hindered); more ears per 100 plants (fewer barren plants at higher plant densities or in other stressful environments, and more two-eared plants at lower plant densities and in more favorable environments); higher staygreen score (plants are less prone to premature death); less stalk lodging (stronger stalks with better resistance to stalk rot organisms); less root lodging (better ability to maintain erect plants when strong winds follow heavy rainfall); less tunneling and less visible damage by second generation European corn borer (increased genetic resistance to the insect); and slight trend to improvement in tolerance to northern corn leaf blight [*Exserohilum turcicum* (Pass.) K. J. Leonard & E.G. Suggs].

As noted earlier, breeders deliberately selected for no change in certain traits. Such intentionally stable traits were: plant height and ear height (essentially no change in plant height over the years to satisfy farmers' antipathy to overly-tall plants, and only minimal reductions in ear height); and hybrid maturity (time of anthesis and grain moisture at harvest were unchanged during 70 years of breeding and selection, to ensure ripening of the grain before average date of first frost).

Other traits also did not change materially, even though breeders did not directly select for stability. Hybrids showed little or no change in: plant size as measured by leaves per plant, leaf area, and fodder weight (no change over the years); harvest index (very little improvement as averaged over plant densities, although there is a trend to higher harvest index as plant densities are increased); rows per ear, kernels per row, kernels per ear, and 100-kernel weight (little or no change, although there is a slight trend to fewer and larger kernels); test weight (increased consistently since 1955 but the average annual increase has been small—

about 0.1% per year); grain oil percentage (unchanged over the years); tolerance to leaf feeding of first generation European corn borer (no improvement); and heterosis (no substantial increase on average during the period 1930s through 1980s, as measured for plant height, flowering date, and grain yield. Heterosis is calculated as value of a single-cross mean minus that of its mid-parent mean. See section IIA2, for further discussion of heterosis effects.)

Genotypic and Genotype-by-Environment Interactions. When examining genetic gain for traits in a target population of environments, it is important to consider the relative influences of different sources of genotype-by-environment interactions. Here we were interested in the relative sizes of the variance components for genotypic variance, genotype interactions with the important management variable plant density, and genotype interactions with the range of environments that were sampled at different year-location combinations in the Era experiments.

Analyses of variance were conducted for grain yield and agronomic traits using the data from the Era experiments conducted from 1991 to 2001. To accommodate the inherent imbalance that is introduced into the data set when new hybrids are added sequentially over years, the results of the experiments were analyzed as a mixed-model. Environments (year-location combinations) and hybrids were treated as random effects and plant density as a fixed effect. Since the Era experiments are conducted as single replicates for each year-location combination, the residual term in the analysis is a combination of the hybrid-by-environment-by-plant density interaction and experimental error. The trait performance of the hybrids was computed as a Best Linear Unbiased Predictor (BLUP). All analyses were conducted using the ASREML software (Gilmour et al. 1998). The mixed model enabled estimation of components of variance for hybrids, hybrid-by-environment (H×E) interaction and hybrid-by-plant density (H×D) interaction. The variance components were used to obtain estimates of heritability for the traits to provide a measure of degree of genotypic determination for the phenotypic observations in the Era experiments. Analyses were conducted for the complete set of hybrids and also for sets of hybrids released in three time-periods (see Table 4.1):

1930 to 1959, representing the period when double-cross hybrids dominated

1960 to 1989, representing the period when the Stiff Stalk and Non Stiff Stalk pools of germplasm were formalized and single-cross hybrids appeared and dominated

1990 to 2001, representing the most modern germplasm consisting of single-cross hybrids combining inbreds from the Stiff Stalk and Non Stiff Stalk pools

Examined over the complete set, the genotypic ("Hybrid") variation for grain yield and most agronomic traits was significantly larger than H×E and H×D sources of genotype-by-environment interaction (Table 4.3). Heritability estimates on a single environment or on multiple environment bases were moderate to large. As expected, for those traits where selection has resulted in direct or indirect changes, the genotypic variance component within the three time periods was smaller than when considered across the complete set of hybrids. The H×E and H×D interaction components of variance changed with time period and the pattern of change between the time periods differed among the traits. For grain yield the hybrid, H×E, and H×D variance components increased from the 1930 to 1959 time period to the 1960 to 1989 time period. The increases in these components of variance are indicative of the broadening of the genetic variation among the successful hybrids created by the Pioneer breeding program over these time periods. They also indicate the important influences of H×D and H×E interactions on the grain yield performance of the hybrids in the target population of environments. In the 1990 to 2001 time period, compared with the 1960 to 1989 time period, there was a slight reduction in the hybrid variance component (0.33 ± 0.14 *cf.* 0.43 ± 0.17) and a more noticeable reduction in the H×E interaction (0.07 ± 0.04 *cf.* 0.16 ± 0.03) and H×D interaction (0.01 ± 0.02 *cf.* 0.18 ± 0.05) components of variance. These trends suggest that the modern hybrids (1990 to 2001, Table 4.1) are all similarly adapted to the range of plant densities considered in the Era experiments and are more stable in their higher yield performance across environments than were the hybrids from the 1960 to 1989 time period.

The heritability estimates within the three time periods were usually lower than for the complete data set. These lower estimates of heritability (within the three time periods) are indicative of the more limited viewpoint that a corn breeder would have of the extent of genotypic variation among these hybrids and potential for further progress while working within a time period. This can be contrasted with the more obvious changes in the traits that can be observed over the whole period encompassed by the sequence of hybrids included in the Era study (Fig. 4.5).

Hybrid Yield at Time of Release versus Yield in Era Trials. Today's agronomic practices, pest problems, and perhaps climatic conditions are different from those of earlier decades (e.g., in the 1930s), and therefore

Table 4.3. Estimates of Hybrid (genotypic), Hybrid-by-Environment (H×E), Hybrid-by-Density (H×D) and residual variance components (± Standard Errors) and heritability ($h^2_{(1\ env)}$ = on a single location, single density, single year basis; $h^2_{(9E,3D)}$ = on a line-mean across 9 environments and 3 density basis) for grain yield and agronomic traits based on available data from Era experiments conducted from 1991 to 2001. #Number, indicates the number of hybrids that were used to estimate the components of variance. (This number can differ among traits for a time period because some hybrids were not measured, particularly for the more recent hybrids.)

Trait	#Number	Set	Hybrid	H×E	H×D	Residual	$h^2_{(1\ env)}$	$h^2_{(9E,3D)}$
Yield (t/ha)	**55**	**All**	**2.65 ± 0.55**	**0.19 ± 0.02**	**0.48 ± 0.07**	**0.69 ± 0.02**	**0.66**	**0.93**
	17	1930–1959	0.17 ± 0.07	0.09 ± 0.02	0.03 ± 0.01	0.60 ± 0.03	0.19	0.81
	19	1960–1989	0.43 ± 0.17	0.16 ± 0.03	0.18 ± 0.05	0.68 ± 0.03	0.29	0.81
	15	1990–2001	0.33 ± 0.14	0.07 ± 0.04	0.01 ± 0.02	0.79 ± 0.06	0.28	0.89
ASI (GDU)	55	**All**	**11.7 ± 2.4**	**1.7 ± 0.2**	**0.8 ± 0.2**	**7.2 ± 0.3**	**0.55**	**0.94**
	17	1930–1959	2.4 ± 0.9	0.9 ± 0.4	0.1 ± 0.1	9.0 ± 0.5	0.19	0.84
	19	1960–1989	4.7 ± 1.7	1.3 ± 0.3	0.5 ± 0.2	5.9 ± 0.3	0.38	0.90
	15	1990–2001	0.7 ± 0.4	0.6 ± 0.3	0.0 ± 0.1	3.5 ± 0.3	0.15	0.78
Staygreen	49	**All**	**2.74 ± 0.59**	**0.49 ± 0.06**	**0.06 ± 0.02**	**1.31 ± 0.05**	**0.60**	**0.96**
(1–9 Score)	17	1930–1959	0.71 ± 0.27	0.37 ± 0.08	0	1.23 ± 0.08	0.31	0.89
	19	1960–1989	1.08 ± 0.39	0.37 ± 0.07	0.08 ± 0.04	1.27 ± 0.08	0.39	0.90
	9	1990–2001	0.57 ± 0.40	0.26 ± 0.15	0.11 ± 0.11	1.15 ± 0.17	0.27	0.84
Ear Number	55	**All**	**101.7 ± 22.4**	**10.9 ± 3.7**	**23.9 ± 4.9**	**135.6 ± 5.4**	**0.37**	**0.88**
(ears/100 plants)	17	1930–1959	0.5 ± 4.0	17.8 ± 5.3	16.9 ± 5.9	97.6 ± 6.6	0.00	0.04
	19	1960–1989	39.0 ± 17.4	1.4 ± 5.3	27.9 ± 8.9	143.3 ± 9.0	0.18	0.73
	15	1990–2001	10.7 ± 11.2	5.0 ± 9.2	23.8 ± 12.1	142.6 ± 14.8	0.06	0.44
Leaf Angle	55	**All**	**6.17 ± 1.20**	**0.19 ± 0.03**	**0**	**1.09 ± 0.04**	**0.83**	**0.99**
(1–9 Score)	17	1930–1959	0.32 ± 0.12	0.12 ± 0.05	0	1.31 ± 0.07	0.18	0.84
	19	1960–1989	2.64 ± 0.89	0.23 ± 0.05	0	1.16 ± 0.06	0.66	0.97
	15	1990–2001	0.33 ± 0.14	0.08 ± 0.03	0	0.31 ± 0.03	0.46	0.94

(*continued*)

Table 4.3. (*Continued*)

Trait	#Number	Set	Hybrid	H×E	H×D	Residual	$h^2_{(1\ env)}$	$h^2_{(9E,3D)}$
Tassel Size	55	**All**	**4.89 ± 0.95**	**0.19 ± 0.03**	**0.02 ± 0.01**	**0.79 ± 0.03**	**0.83**	**0.99**
(1–9 Score)	17	1930–1959	0.93 ± 0.34	0.09 ± 0.04	0.03 ± 0.02	0.95 ± 0.06	0.46	0.94
	19	1960–1989	1.43 ± 0.49	0.22 ± 0.04	0.01 ± 0.01	0.85 ± 0.05	0.57	0.96
	15	1990–2001	0.28 ± 0.12	0.12 ± 0.04	0.01 ± 0.01	0.41 ± 0.04	0.35	0.90
Moisture (%)	55	**All**	**2.42 ± 0.49**	**1.01 ± 0.06**	**0.11 ± 0.03**	**1.68 ± 0.05**	**0.46**	**0.92**
	17	1930–1959	1.27 ± 0.47	0.49 ± 0.07	0.09 ± 0.03	1.42 ± 0.07	0.39	0.90
	19	1960–1989	3.02 ± 1.03	1.10 ± 0.09	0.10 ± 0.03	1.23 ± 0.05	0.55	0.94
	15	1990–2001	2.60 ± 1.06	0.80 ± 0.14	0.18 ± 0.09	1.36 ± 0.11	0.53	0.93
Ear Height (cm)	53	**All**	**66.2 ± 14.0**	**8.6 ± 2.8**	**2.3 ± 1.3**	**118.6 ± 4.2**	**0.34**	**0.92**
	17	1930–1959	23.7 ± 10.1	10.8 ± 5.1	5.5 ± 3.1	129.5 ± 7.6	0.14	0.75
	19	1960–1989	51.4 ± 17.7	4.1 ± 3.3	0	98.1 ± 5.2	0.33	0.93
	13	1990–2001	53.3 ± 24.8	5.8 ± 6.3	2.4 ± 3.5	89.5 ± 9.4	0.35	0.92
Plant Height (cm)	53	**All**	**57.8 ± 12.8**	**9.6 ± 4.0**	**3.3 ± 2.0**	**179.4 ± 6.3**	**0.23**	**0.87**
	17	1930–1959	11.3 ± 6.1	0	4.0 ± 4.2	247.4 ± 11.8	0.04	0.52
	19	1960–1989	66.9 ± 23.3	13.6 ± 4.40	1.2 ± 1.7	112.4 ± 6.1	0.34	0.92
	13	1990–2001	68.1 ± 35.5	27.3 ± 13.5	2.4 ± 5.1	156.9 ± 16.5	0.27	0.88
% Not Root Lodged	53	**All**	**149.5 ± 33.8**	**84.9 ± 11.3**	**4.8 ± 4.0**	**200.6 ± 10.1**	**0.34**	**0.89**
	17	1930–1959	30.5 ± 16.4	58.0 ± 16.5	6.3 ± 7.2	213.6 ± 18.0	0.10	0.65
	19	1960–1989	7.9 ± 6.1	41.1 ± 12.0	0	178.8 ± 13.3	0.03	0.42
	13	1990–2001	0.3 ± 1.0	0	0	35.1 ± 3.7	0.01	0.17
% Not Stalk Lodged	55	**All**	**23.4 ± 5.1**	**12.3 ± 1.9**	**0**	**60.0 ± 2.2**	**0.24**	**0.87**
	17	1930–1959	12.1 ± 5.1	10.9 ± 3.8	0	85.5 ± 5.1	0.11	0.73
	19	1960–1989	3.9 ± 1.9	10.8 ± 2.2	0.7 ± 0.7	43.6 ± 2.5	0.07	0.56
	15	1990–2001	0.9 ± 0.8	1.6 ± 1.4	0	18.7 ± 2.0	0.04	0.52

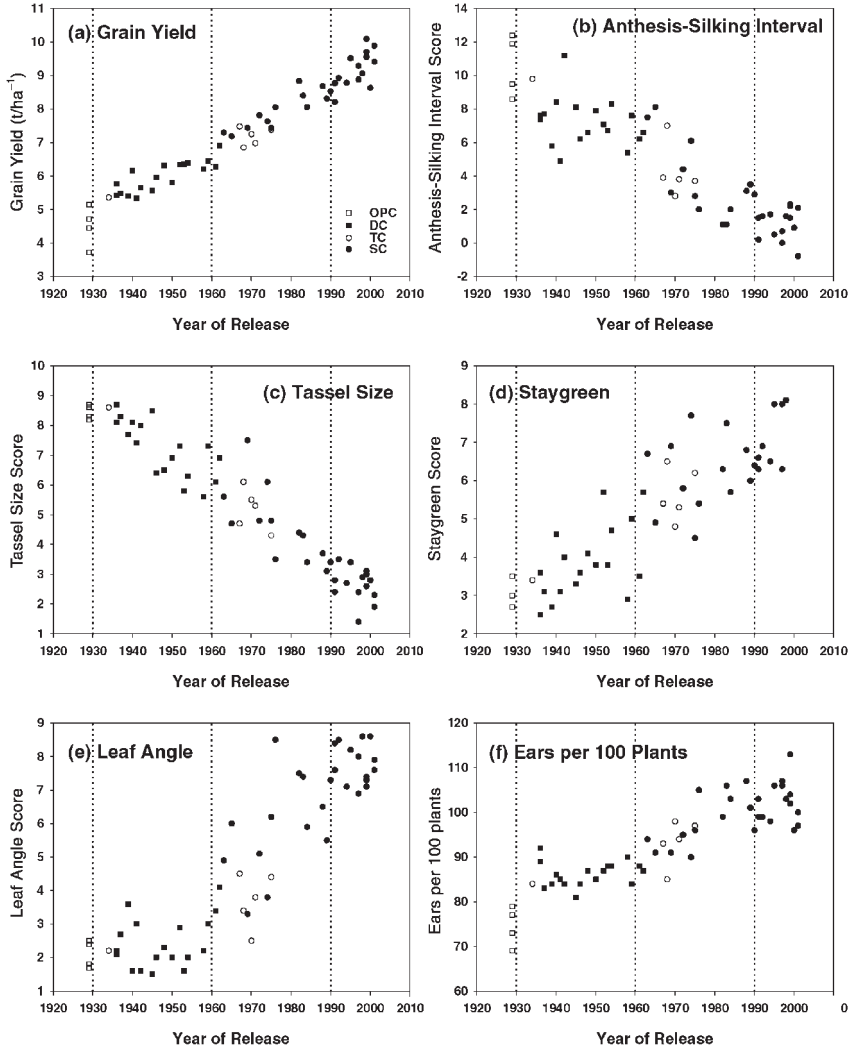

Fig. 4.5. Best Linear Unbiased Predictions (BLUPs) for hybrid grain yield and agronomic traits computed from results of Era experiments conducted from 1991 to 2001. Dotted vertical lines indicate the distinction between three time periods: 1930 to 1959, representing the period dominated by double-cross hybrids; 1960 to 1989, representing the period when the Stiff Stalk and Non Stiff Stalk heterotic groups were developed and single-cross hybrids were introduced; and 1990 to 2001, representing the modern period dominated by single-cross hybrids. All data are BLUPs based on three plant densities: 30, 54, and 79 thousand plants/ha. Key to symbols: OPC, open pollinated cultivars; DC, double cross hybrids; TC, three-way cross hybrids; SC, single cross hybrids.

performance of older hybrids under today's growing conditions may be different than it was when they were at their peak of use and popularity. One could speculate that the older hybrids might not yield as much now as they did at the time of their widespread use, or that they may yield more. For example, in a different crop in another part of the world, an IRRI (International Rice Research Institute) rice variety (IR8) yields less today than it did when first released 30 years ago. A time-series of IRRI rice cultivars, grown today, shows linear increases in yield but the highest yielding variety of recent vintage yields no more than the old variety did at the time of its release (Peng et al. 1999).

To answer the question, How did the hybrids yield in their "era" compared with the present time?, we compared yields of Era hybrids during their "peak years" (years of widespread sales) with yields of the same hybrids in the Era trials of 1996 to 2000. Data for the peak years were taken from concurrent reports of standard small-plot performance trials grown in central Iowa. Data from the present-day Era trials were for yield at the optimum plant density (the plant density with the highest yield) of each hybrid. Fig. 4.6 shows that yields of hybrids in their

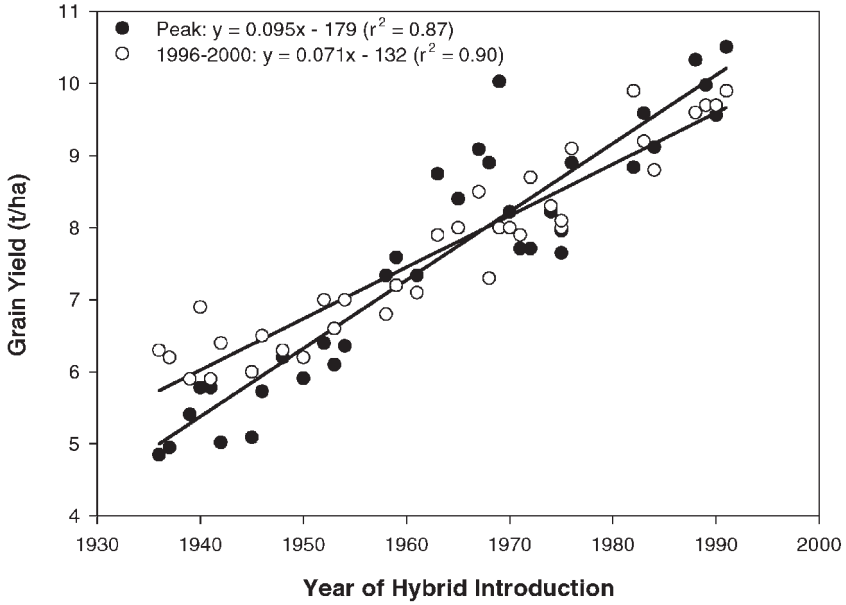

Fig. 4.6. Yields of Era hybrids in 1996 to 2000 Era trials and in their "peak" years (years of widespread planting). Yield data for "peak" years are from contemporary reports of Pioneer hybrid performance trials in central Iowa. Data for Era trials are for optimum plant density for each hybrid.

peak years corresponded closely with yields in the 1996 to 2000 trials. (Comparisons were for 36 hybrids introduced from 1936 through 1991.) Yields increased linearly in both sets ("peak" and "1996 to 2000") and the correlation between the two data sets was 0.90 (data not shown). However, the oldest hybrids tended to yield less in their peak years than in the 1996 to 2000 Era trials and conversely the newest hybrids yielded slightly more in their peak years than in the Era trials. One can speculate that the old hybrids' yields were higher in 1996 to 2000 than they were in the 1930s and 1940s because the current trials had better weed control and/or higher levels of available nitrogen, but this cannot be proven. And one could speculate that the weather was more conducive to high yields in the late 1980s and early 1990s (when the new hybrids were at their peak years) than it was in the late 1990s. But even if these speculations were true, undoubtedly there were several other differences, abiotic and biotic, that cannot be determined today with any degree of certainty. And at any rate the loss/gain in yield between peak year and Era yields (as indicated by regression lines) is small. The main conclusion that can be drawn from this comparison is that yields of the older hybrids have not been underestimated and yields of the newer hybrids have not been overestimated in the recent Era trials.

2. Heterosis: Single-crosses vs. Parental Inbreds. The genetic gains in yield of the Era hybrids conceivably can be caused (1) by increases in heterosis, and/or (2) by improvements in non-heterotic components of the genome, such as in those traits governed primarily by additive gene action. To test the relative importance of heterosis, comparisons were made between pairs of inbreds representing the most widely used male and female inbreds of each decade and the single-crosses of those inbreds. Within each decade (1930s through 1980s), seven pair-wise comparisons were set up as follows: an inbred from the female side of an Era hybrid was crossed with an inbred from the male side of the same hybrid, to maximize potential heterosis. Inbreds and their single-crosses (a total of 42 single-crosses) were compared in split-plot yield trials. Heterosis was calculated as SX – MP, the difference between the yield of the single-cross (SX) and the mean yield of its inbred parents (MP, "midparent mean").

The results of this experiment (as well as of an earlier experiment of similar design) indicate that on the average, hybrid yield gains over the decades (1930s to 1980s) owe very little to increases in heterosis (Duvick 1984, 1999). The linear increase in single-cross yield over the decades is closely paralleled by linear increases in yield of the inbred parents of those single-crosses and consequently SX – MP is constant over the decades on average (Fig. 4.7). And if heterosis (SX – MP) is calculated

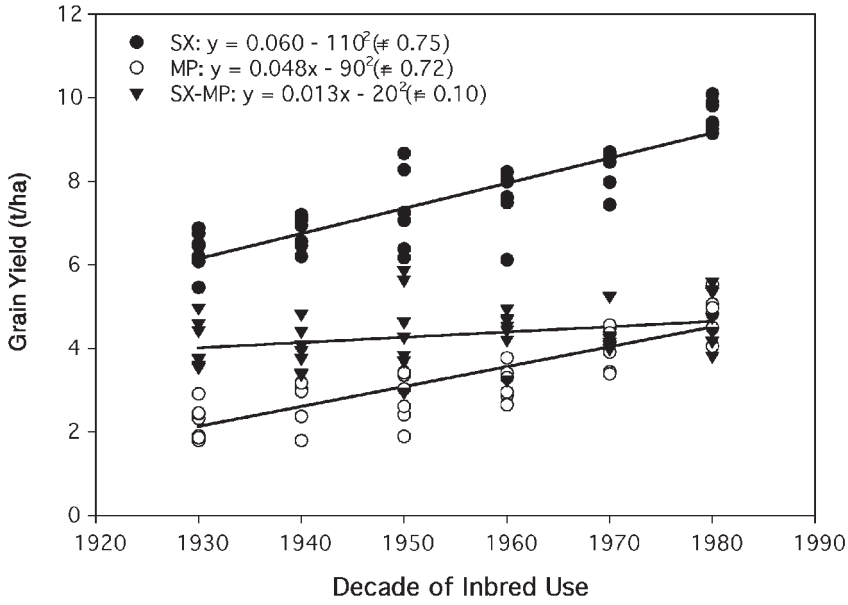

Fig. 4.7. Yields of single crosses (SX) and their inbred parent means (MP), and heterosis as SX – MP. Single-cross pedigrees are based on heterotic inbred combinations in the Era hybrids during the six decades, 1930s through 1980s, 12 inbreds and six single crosses per decade. Means of trials grown in three locations in 1992 and two locations in 1993 at three densities (30, 54, and 79 thousand plants/ha) with one replication per density.

as percent of mid-parent yield, the value for heterosis actually declines in linear fashion across the decades (b = –1.7% yr^{-1}, R^2 = 0.33 for means of 1992–93 at 30, 54, and 79 thousand plants/ha). The reason, of course, is that a constant value for heterosis is divided by increasingly large values for mid-parent yield.

However, the data on hand do show that over the decades heterosis has increased to a greater extent in trials subjected to severe stress than in trials grown in high-yield (lower stress) environments. Examples of stressful environments would be dense planting (e.g., 79 thousand plants/ha) or the abnormally cold wet season of 1993 (Fig. 4.8). This interaction of heterosis with environmental stress simply restates the fact that during the past 70 years of breeding and selection, yielding ability under stress has been improved to a greater extent in hybrids than in their inbred parents.

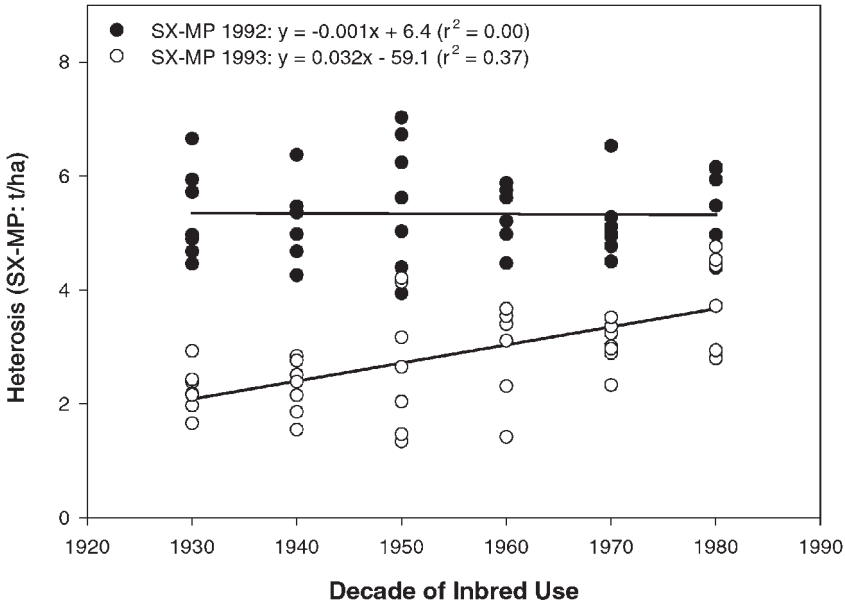

Fig. 4.8. Heterosis (as SX – MP) in two seasons, 1992 and 1993. Single-cross pedigrees based on heterotic inbred combinations in the Era hybrids during the six decades, 1930s through 1980s, 12 inbreds and six single crosses per decade. Trials were grown in three locations in 1992 and two locations in 1993. Means of three densities (30, 54, and 79 thousand plants/ha) with one replication per density.

Heterosis can be calculated on the basis of yield at optimum plant density of single-crosses and of each of their inbred parents. As stated earlier, yield at optimum plant density seems best for comparing hybrids of different eras—each hybrid is evaluated at an approximation of the plant density at which it had been bred and tested. The same argument could be used for comparing inbred lines and their single-crosses. Additionally, one could hypothesize that inbreds on average will require higher plant densities than single-crosses for maximum yield because the inbreds are smaller and will require more plants per acre to produce an equivalent leaf area index (LAI).

Analysis of the 1992–93 data for yield did not bear out this hypothesis. The inbreds interacted with plant densities in about the same manner as the single-crosses. The only obvious difference was that although the oldest single-crosses (of the 1930s and 1940s) lost yield (on average)

at 79 thousand plants/ha as compared with their performance at 30 and 54 thousand plants/ha, the inbreds from those early decades did not show a yield reduction at 79 thousand plants/ha. They maintained (but did not gain) yield at the 79 thousand plants/ha density. Both categories (inbreds and single-crosses) showed an increasing adaptation to high plant densities, progressing from earliest to most recent decades; that is, their yield at the higher plant densities was increasingly greater than that at the lower plant densities.

When heterosis calculations are based on yield at optimum plant density, the net result is that trends and values for heterosis are very similar to those determined on the basis of 3-density means. Inbred yields and single-cross yields at optimum plant density increase in linear fashion, and in equivalent amounts, over the decades. Heterosis calculated as SX – MP shows no significant change over the decades. And when heterosis (as SX – MP) is calculated as percent of mid-parent yield, the value for heterosis declines in linear fashion across the decades (b = –1.8% yr^{-1}, R^2 = 0.38 for 1992–93 means). These values for optimum plant density are almost identical to results of similar calculations for the 3-density means.

B. Pedigree Examination

Study of the Era hybrid pedigrees can identify the germplasm sources (founder sources) of hybrids in the time-series. It also can reveal the continuity or extinction of breeding families that trace back to those founders. And finally it can show the breadth (or narrowness) of the germplasm base that has supported Era hybrids during any particular time, such as the decade of the 1950s or the decade of the 1990s.

1. Founder Sources. The time-series of Era hybrids through the year 2000 traces back to 53 founder sources (Duvick et al. 2003) (Table 4.4). Landraces predominate in this list, and they come primarily, but not entirely, from the U.S. Corn Belt—they are "Corn Belt Dents." However, founders also came from the southeastern and eastern United States, and (rarely) from Latin American countries such as Argentina. Synthetic populations and first generation inbred lines also are identified as founder sources; all of these are essentially U.S. Corn Belt Dent in breeding.

2. Proportionate Contribution of Founders. The above count of the number of founder genotypes gives an indication of the potential amount of genetic diversity that could have contributed to genomes of the Era hybrids. But it is also important to know which family lineages have

Table 4.4. Founder inbred lines and open pollinated cultivars (OPC) of hybrids in Table 4.1. Source: Duvick et al. 2003.

Founder germplasm[z]	Background	OPC Background	Origin	Country
A237	Minnesota 13 × Reid Yellow Dent Population	Minnesota 13 & Reid	Minnesota	USA
ABCOMP	Pioneer Composite A × Pioneer Composite B		Iowa	USA
AFLF	Argentinean Flint/Lady-finger Popcorn	Argentinean Flint & Ladyfinger popcorn		Argentina/ USA
ALBRTFLINT	Alberta Flint Population	Alberta Flint	Alberta	Canada
ARGMAIZARM	Argentinean Maiz Amargo	Argentinean Maiz Amargo		Argentina
BH940				USA
BLACKSDCO	Black's County Reid Dent; landrace selection from Reid	Blacks (Reid)		USA
BLF				USA
BOONECOWH	Boone County White	Boone County White		USA
Luxe's Boone County White		Luxe's Boone County		USA
White Mastodon		White Mastodon		USA
BR2USDA				USA
BROOKINGS86	Developed from Minnesota 13	Minnesota 13		USA
BSSS	Iowa Stiff Stalk Synthetic			USA
I 461-73 Troyer Reid		Reid		USA
AH 83 Funk 176A Reid		Reid		USA
TR9-1-1-6 Troyer Reid		Reid		USA
F1B1-7-1 Troyer Reid		Reid		USA
CI 540 Illinois Two Ear		Leaming		USA
Hy Illinois High Yield		Hy		USA
CI 187-2 Krug		Krug		USA

(*continued*)

Table 4.4. (*Continued*)

Founder germplasm[z]	Background	OPC Background	Origin	Country
BSSS (*cont.*)				
LE23 Illinois Long Ear (Leaming)		Leaming		USA
Ill 2E Illinois Two Ear (Leaming)		Leaming		USA
CI 617 Funk 176A Reid		Reid		USA
A3G-3-1-3 I159 (Iodent Reid) × BL345 (Black's Reid Yellow Dent)		Iodent (Reid) & Blacks (Reid)		USA
Iodent Reid		Iodent (Reid)		USA
I 224 Iodent Reid		Iodent (Reid)		USA
OS 420 Osterland (Selection From Reid)		Osterland (Reid)		USA
WD 456 Walden Dent (Selection From Reid)		Walden (Reid)		USA
OH 3167B Clarage		Clarage		USA
COKER616		Coker Synthetic	Southern US	USA
DOCKDRF101	Dockendorf 101 Synthetic		Iowa	USA
FCOMP2	Pioneer Female Composite			USA
FSOUTH[y]	Pioneer Far South Open Pollinated	Southern US OPCs		USA
FUNKS176A	Funks 176A; population selected from Reid	Reid	Illinois	USA
FUNKYDENT	Funks 176A; population selected from Reid	Reid	Illinois	USA
G16Y	Inbred from Lancaster Sure Crop landrace	Lancaster Sure Crop	Iowa	USA
GE440	Inbred line HT resistance source		Georgia	USA

GOLDENGATE		Goldengate		USA
ILLHY	Illinois High Yield-inbred from Hy population		Illinois	USA
ILLOEAR	Illinois Low Ear population		Illinois	USA
ILLONG	Illinois Long Ear; Leaming open-pollinated cultivar	Leaming		USA
ILLTWOEAR	Illinois Two Ear; Leaming open-pollinated cultivar	Leaming		USA
IODENT	Iodent Selection from Reid	Iodent (Reid)	Iowa	USA
K140	Inbred line		Iowa	USA
KRUG	Krug-Reid and Goldmine	Krug	Iowa/Illinois	USA
LADYFINGER	Ladyfinger Popcorn	Ladyfinger popcorn		USA
LAGUNAOP	Laguna open-pollinated-developed by USDA from Laguna Population	Laguna population		Mexico
LANCCOMP	Lancaster Composite-from Lancaster Sure Crop landrace	Lancaster Sure Crop		USA
LANCLOBRK	Lancaster Low Breakage-population from Lancaster Sure Crop landrace	Lancaster Sure Crop		USA
LANCSURCROP	Lancaster Sure Crop-Open-pollinated landrace cultivar	Lancaster Sure Crop		USA
LE	Illinois Long Ear developed by Pioneer	Leaming	Iowa/Illinois	USA
LLE	Lindstrom Long Ear	Lindstrom		USA
LONGFELFLT	Longfellow open-pollinated landrace cultivar	Longfellow	Kansas	USA
M3204	Mississippi 3204 hybrid		Mississippi	USA
MA111				USA

(*continued*)

Table 4.4. (*Continued*)

Founder germplasm[z]	Background	OPC Background	Origin	Country
MARYLDYDENT	Maryland Yellow Dent	Maryland Yellow Dent		USA
MHW				USA
MIDLAND	Midland Yellow Dent	Midland	Kansas	USA
MINN13	Minnesota 13 open-pollinated cultivar	Minnesota 13	Minnesota	USA
NWDENT	Northwestern Dent; Indian cultivar or Red Flint × White Dent	Northwestern Dent		USA
OSTERYDNT	Osterland open-pollinated cultivar (selection from Reid)	Osterland (Reid)	Iowa	USA
PROLC	Prolific Composite	Reid		USA
REID	Reid Yellow Dent open-pollinated cultivar	Reid	Minnesota	USA
SPROL	Pioneer Prolific Composite		Minnesota	USA
SYNWF9	Wilson Farm Reid Synthetic	Reid		USA
TROYERREID	Troyer Reid, selection from Reid Yellow Dent	Reid		USA
U886				USA
UHTSOURCE	University source of resistance to *Helminthosporium turcicum*			
US558W	USDA hybrid			USA
WFRYD	Wilson Farm Reid Yellow Dent; landrace selection from Reid	Reid		USA

[z]Founder sub-sets are in lowercase.

[y]Synthetic of southern U.S. open-pollinated cultivars.

persisted over the decades, and in what proportions. Forty-two founder genotypes contributed more than 1% in any single decade (Duvick et al. 2003) (Table 4.5). Of these 42, five OPCs contributed more than 5% on average across the decades (1930s through 2000s). They were 'Reid Iodent' (15%), 'Krug' (8%), 'Lancaster Sure Crop' (6%), 'Leaming' (6%), and 'Reid Yellow Dent' (33%). An improved population, 'Iowa Stiff Stalk Synthetic' (BSSS), also contributed significantly (mean per decade of 22%). Five of the six major contributors were U.S. Corn Belt Dent in origin; the sixth contributor, 'Lancaster Sure Crop', originated in the eastern United States but it also was a dent.

3. Variability in Proportionate Contribution. The relative importance of different founder lineages has varied over time in the list of Era hybrids. Some founders have risen and then fallen in importance. For example, 'Krug' reached a peak use of 23% in the 1940s, but then rapidly declined to a steady level of about 3%. 'Lancaster Sure Crop' peaked twice, at 11% in the 1940s and 11% in the 1970s, and is currently in decline at about 3%. 'Reid Yellow Dent' was a prominent contributor in the early decades, ranging from 40 to 50% through the 1950s. It has since declined in importance but is still a significant contributor, at levels of 20 to 30% for the past five decades. 'Reid Iodent' is the only founder that was present in the first decade and has not only persisted but has increased in importance during recent decades, starting from a level of about 5% in the 1950s and moving up to its present contribution (in the 2000s) of 26%.

Some founders made initial contributions but have since disappeared. For example, 'Illinois Low Ear' was at 25% concentration in the 1930s but abruptly disappeared in the 1940s, 'Maryland Yellow Dent' contributed 5% in the 1960s and then disappeared, and 'Boone County White' appeared in the 1960s and was gone by the 1980s.

Other founders appeared late in time and have persisted since then. Argentinean Maiz Amargo appeared in the 1980s and has contributed 4% to 5% in each of the past two decades. 'Pioneer Female Composite' and 'Pioneer Prolific Composite' appeared in the 1980s and 1990s, respectively. They have contributed between 3% and 8% per decade since their first appearance.

Some founders have persisted throughout many or all of the decades but always at a low level. For example, 'BSSS-Clarage' has never exceeded 3%, 'Lindstrom Long Ear' has varied between levels of 1% to 4%, and the inbred Hy has never exceeded 5%.

Table 4.5. Percentage contribution per decade (by pedigree) for each of 42 founder genotypes that contributed more than 1 percent in any single decade. Source: Duvick et al. 2003.

Founder genotypes	Pedigree background	1930s	1940s	1950s	1960s	1970s	1980s	1990s	2000s	Decade mean
LANDRACES										
Argentinean Maiz Amargo	**ARGMAIZARM**	**0**	**0**	**0**	**0**	**0**	**2.5**	**5.38**	**4.36**	**1.53**
Boone County White	**BOONECOWH**	**0**	**0**	**2.08**	**1.01**	**0.88**	**0**	**0**	**0**	**0.5**
Clarage	**BSSS-CLARAGE**	**0**	**0**	**0**	**1.33**	**2.48**	**2.38**	**2.46**	**2.21**	**1.36**
Krug	BSSS-KRUG	0	0	0	1.33	2.48	2.38	2.46	2.21	1.36
Krug	KRUG	10	22.92	12.5	3.13	1.34	0.63	0.98	0.5	6.5
Krug Total		**10**	**22.92**	**12.5**	**4.46**	**3.82**	**3.01**	**3.44**	**2.71**	**7.86**
Lancaster Sure Crop	LANCCOMP	0	0	0	1.79	7.81	0.94	1.97	1.89	1.8
Lancaster Sure Crop	LANCLOBRK	0	0	0	0	2.68	2.51	2.34	1.56	1.14
Lancaster Sure Crop	LANCSURCROP	5	11.46	7.81	0.34	0.29	0	0	0	3.11
Lancaster Total		**5**	**11.46**	**7.81**	**2.13**	**10.78**	**3.45**	**4.31**	**3.45**	**6.05**
Leaming	BSSS-LEAMING	0	0	0	3.98	7.45	7.15	7.38	6.63	4.13
Leaming	ILLONG	0	0	0	0	0.11	1.88	1.46	0.99	0.55
Leaming	ILLTWOEAR	5	0	0	0	0.11	1.88	1.46	0.99	1.18
Leaming Total		**5**	**0**	**0**	**3.98**	**7.67**	**10.91**	**10.3**	**8.61**	**5.81**
Lindstrom Long Ear	**LLE**	**0**	**0**	**1.04**	**5.14**	**4.09**	**2.3**	**1.94**	**3.61**	**2.27**
Maryland Yellow Dent	**MARYLDYDENT**	**0**	**0**	**0**	**3.57**	**0**	**0**	**0**	**0**	**0.45**
Midland	**MIDLAND**	**0**	**0.26**	**12.5**	**15.18**	**0.78**	**5.01**	**3.24**	**2.55**	**4.94**
Minnesota 13	BROOKINGS86	0	0	0	0	0	1.26	0.68	0.34	0.29
Minnesota 13	MINN13	0	0	3.25	5.36	3.89	5.53	3.63	6.2	3.48
Minnesota 13 Total		**0**	**0**	**3.25**	**5.36**	**3.89**	**6.79**	**4.31**	**6.54**	**3.77**
Reid Yellow Dent	BSSS-Blacks	0	0	0	0.64	1.19	1.14	1.18	1.06	0.65
Reid Yellow Dent	BSSS-Butler	0	0	0	0.64	1.19	1.14	1.18	1.06	0.65
Reid Yellow Dent	BSSS-Funks	0	0	0	2.65	4.97	4.77	4.92	4.42	2.72
Reid Yellow Dent	BSSS-Osterland	0	0	0	1.33	2.48	2.38	2.46	2.21	1.36

(*continued*)

Reid Yellow Dent	BSSS-Reid	0	0	0	0.64	1.19	1.14	1.18	1.06	0.65
Reid Yellow Dent	BSSS-Troyer	0	0	0	2.65	4.97	4.77	4.92	4.42	2.72
Reid Yellow Dent	BSSS-Walden	0	0	0	1.33	2.48	2.38	2.46	2.21	1.36
Reid Yellow Dent	FUNKS176A	0	8.33	0	0	0	0	0	0	1.04
Reid Yellow Dent	FUNKYDENT	0	0	0	0.89	3.89	0.47	0.79	0.67	0.84
Reid Yellow Dent	OSTERYDNT	0	10.68	16.67	3.57	0	2.68	2.6	2.29	4.81
Reid Yellow Dent	REID	30	8.33	2.08	0	0	0.59	0.91	0	5.24
Reid Yellow Dent	TROYERREID	10	3.65	2.22	3.97	1.02	1.68	0.65	1.61	3.1
Reid Yellow Dent	WFRYD	0	20.83	25	7.14	7.8	0.63	1.36	1.35	8.01
Reid Yellow Dent Total		**40**	**51.82**	**45.97**	**25.45**	**31.18**	**23.77**	**24.61**	**22.36**	**33.15**
Reid-Iodent	BSSS-IODENT	0	0	0	3.31	6.21	5.96	6.15	5.52	3.39
Reid-Iodent	IODENT	10	5.21	4.95	14.13	11.2	17.14	11.73	20.48	11.86
Reid Iodent Total		**10**	**5.21**	**4.95**	**17.44**	**17.41**	**23.1**	**17.88**	**26**	**15.25**
Landrace Total		**70**	**91.67**	**90.1**	**85.05**	**82.21**	**83.22**	**77.87**	**76.79**	**82.94**
OTHER SOURCES										
Dockendorf 101 Synthetic	**DOCKDORF101**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**1.34**	**0.79**
Hy	BSSS-HY	0	0	0	1.33	2.48	2.38	2.46	2.21	1.3
Hy	ILLHY	5	0	2.08	4.02	2.46	0	0	0	1.7
Hy Total		**5**	**0**	**2.08**	**5.35**	**4.94**	**2.38**	**2.46**	**2.21**	**3**
Illinois Low Ear Population	**ILLOEAR**	**25**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3.13**
Pioneer Composites A × B	**ABCOMP**	**0**	**0**	**0**	**3.57**	**3.57**	**0**	**0**	**0**	**0.9**
Pioneer Female Composite	**FCOMP2**	**0**	**0**	**0**	**0**	**0**	**7.5**	**8.8**	**4.54**	**2.61**
Pioneer Prolific Composite	**SPROL**	**0**	**0**	**0**	**0**	**0**	**0**	**5.47**	**3.07**	**1.03**
Inbred line	**K140**	**0**	**2.08**	**2.08**	**0**	**0**	**0**	**0**	**0**	**0.52**
Inbred line	**MA111**	**0**	**0**	**0**	**3.57**	**3.57**	**0**	**0**	**0**	**0.9**
Mississippi hybrid	**M3204**	**0**	**0**	**0**	**0**	**1.79**	**0**	**0**	**0**	**0.22**
BSSS[z]		0	0	0	21.2	39.72	38.13	39.35	35.34	21.72

[z]Already broken out to pedigree sources.

C. Molecular Marker Changes

Although informative, pedigree data have limitations because they do not positively identify genetic materials in the pedigree lineages, either qualitatively or quantitatively. Molecular marker data can give positive identification of genetic material. For example, one can trace a given DNA fragment from one generation to the next, and thus enable quantification of the amount of founder germplasm that persists in successive generations.

1. Number of Alleles. In a previous report, we used SSRs to enable such quantification and identification of genetic materials, scoring 969 alleles for the 1930 to 2000 array of Era hybrids and OPCs (Duvick et al. 2003). These alleles were identified from a study of 100 SSR loci distributed across all 10 chromosomes. Table 4.6 shows numbers, frequencies, and percent change from the previous decade for these SSR alleles. It shows that the number of alleles fluctuated from decade to decade, that about 40% to 50% of the 968 alleles were present in any one decade, and that there is a weak trend toward fewer numbers per decade, starting in the 1980s.

2. "New" Alleles. "New" SSR alleles in the context of this report are defined as those that have not been identified in any previous decade. Table 4.7 enumerates new SSR alleles, as found in the array presented in Table 4.6. The figures for hybrids of the 1930s may be inflated because of the small sample of OPCs to which they were compared. The large increase in "new" alleles from the 1930s to the 1940s agrees with

Table 4.6. Number of SSR alleles in each decade, percent change from previous decade, and percent of total identified in the hybrid series through 2000. Source: Duvick et al. 2003.

Decade	No. of alleles	Change from previous decade	% of total found in study (968)
1920s	387		40%
1930s	480	+24%	50%
1940s	590	+23%	61%
1950s	484	–18%	50%
1960s	599	+24%	62%
1970s	543	–9%	56%
1980s	430	–21%	44%
1990s	476	+11%	49%
2000s	369	–22%	38%

Table 4.7. Number of "new" alleles in hybrids released during each decade. Source: Duvick et al. 2003.

		No. "new alleles"							
Analyses		1930s	1940s	1950s	1960s	1970s	1980s	1990s	2000s
No. "new" alleles		241	105	44	48	30	29	19	26
No. loci with "new" alleles		84	61	36	39	24	24	15	20
No. chromosomes with "new" alleles		10	10	10	10	10	9	8	8
"New" alleles per inbred	min	17	0	0	0	0	0	0	0
	max	43	19	21	13	11	9	6	6
	mean	26.7	9.6	3.6	2.9	3.2	4.6	2.2	1.9
"New" alleles per hybrid	min	82	15	1	4	0	7	1	2
	max	121	53	30	22	16	13	7	10
	mean	97.6	38.2	15.3	9	7	9	3.6	3.9

the pedigree data, which showed that several founder families were dropped, and others were introduced, in the 1940s.

The several categories of examination (loci with "new" alleles, "new" alleles per inbred, etc.) generally agree in showing a reduction in "new" alleles starting in about the 1960s or 1970s. This corresponds with the time during which Era hybrids shifted from double-cross to single-cross status (with an intermediate stage in which some hybrids were 3-way crosses or "modified" single-crosses, meaning one parent was a cross of closely related inbreds).

3. Formation of Stiff Stalk and Non Stiff Stalk Heterotic Groups. Recent work has expanded the number of SSR loci, from 100 to 298, measured on the parents of the Era hybrids. The number of OPCs was increased from 3 to 8. Germplasm from 'Lancaster Sure Crop', 'Leaming', 'Minnesota 13', and 'Midland Yellow Dent' is now represented, in addition to that from 'Reid Yellow Dent' and 'Krug'. The average number of alleles per locus was estimated for the OPCs and the hybrids and their female and male parents by decade (Fig. 4.9). The average number of alleles per locus decreased over time. There was a decrease from the OPCs to the double-cross hybrids (1930s to 1960s) and a further decrease with the move from double-cross hybrids to single-cross hybrids (1970s to 2000s). However, several of the OPCs that feature in the pedigree backgrounds of the Era hybrids are exotic and were never grown as such in the central U.S. Corn Belt. Therefore, our measurement of the decline in

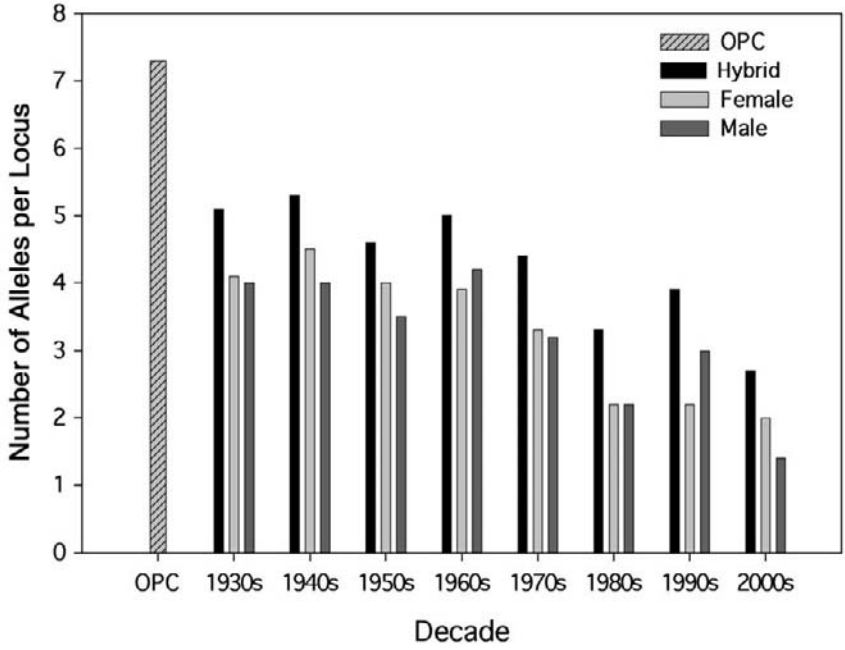

Fig. 4.9. Average number of SSR alleles per locus for 298 SSR loci for eight open pollinated cultivars (OPC) and for the Era hybrids and female and male parents of the hybrids by decade of release.

number of alleles during the transition from OPCs to hybrids may overestimate the actual decline in allelic diversity that occurred when U.S. Corn Belt OPCs were replaced by hybrids. On the other hand, our representation of OPC diversity in the central U.S. Corn Belt is more likely an underestimate of the allelic diversity that was extant in the many variations of Reid Yellow Dent and other OPCs in the central U.S. Corn Belt. More thorough surveys of OPC diversity in the 1920s are necessary to give an accurate estimate of the change in genetic diversity as OPCs were replaced by hybrids. Our intent here is to provide simply a first appraisal of OPC diversity in the 1920s and 1930s.

The number of alleles per locus was similar for the female and male parents of the hybrids in all decades. Further analysis of the allele polymorphism among the inbred parents of the Era hybrids by multidimensional scaling (Kruskal and Wish 1978) separates the older inbred parents of the double-cross hybrids from the modern inbred lines that comprise the Stiff Stalk and Non Stiff Stalk heterotic groups (Fig. 4.10). The SSR polymorphism data indicate a trend toward reduction in the

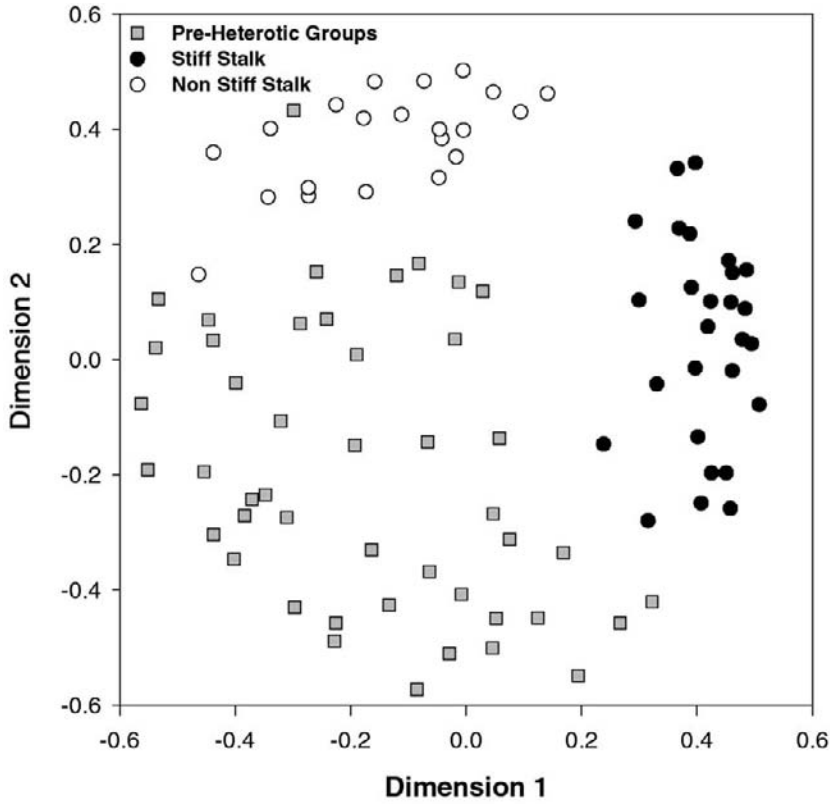

Fig. 4.10. Scores for 94 inbreds contributing to the Era hybrids on the first two dimensions of the multi-dimensional scaling analysis of the SSR polymorphism data for 298 SSR loci ($R^2 = 0.45$ for the two dimension model).

average number of alleles per locus and a clear divergence between the allele profiles of the inbreds created by pedigree breeding in the Stiff Stalk and Non Stiff Stalk heterotic groups.

III. POSTULATED CAUSES AND CONSEQUENCES OF SEQUENTIAL CHANGES IN HYBRID PERFORMANCE

A. Genetic Yield Gain

The data summarized in this review suggest that genetic yield gain primarily is caused by increase in genetic tolerance of the biotic and abiotic stresses that normally occur in a hybrid's region of adaptation.

"Yield genes" (when yield is defined as "yield per unit area") often may simply be "stress tolerance genes," genes that give service when needed in some seasons or some localities but not in others, depending on such factors as the weather, soil fertility, and disease pressure. This inference is based on the fact that increased yield of the Era hybrids consistently has been accompanied by increased tolerance to heat and drought, to cool and wet growing conditions, and to low- as well as high-yielding sites. This trend has lasted over a 70-year time-span. Further evidence of improved stress tolerance is the continuing improvement in standability (better root and stalk strength) and improvements in tolerance to the common disease and insect pests of the region where the hybrids are adapted.

A second way to increase yield per unit area is to improve the maize plant's efficiency in transformation of sunlight, soil nutrients, and carbon dioxide into grain. The newer Era hybrids in this study have more upright leaves, smaller tassels, make less grain protein and more starch, and make fewer tillers. Upright leaves improve the plant's ability to capture sunlight for photosynthesis at higher plant densities. Smaller tassels, fewer tillers and a grain protein/starch ratio changed in favor of starch presumably reduce the plant's energy requirements for general maintenance and so make more energy available for grain production. "Efficiency genes" may be a second category of "yield genes," differing from stress tolerance genes in that they operate at all times and all places, rather than only in particular stress conditions. But in a sense they also may be stress tolerance genes if the increased efficiency that they impart provides more energy for stressed (and presumably energy-hungry) plants.

Of course, in regard to both stress tolerance and efficiency, it may well be that the critical genetic changes primarily are in the regulatory parts of the gene, rather than in the expressed portion. Timing of gene action or threshold levels of stimulus may be more important than the gene product per se. We consider this to be an important area of research if we are to understand the basis of the genetic improvements in adaptation of maize hybrids over the course of a breeding program.

Increases in stress tolerance and in production efficiency increase the ability of the maize hybrids to tolerate ever-higher plant densities, which invariably increase the burdens of biotic and abiotic stress. Farmers have increased their planting rates over the years (since at least the 1950s), and they therefore have increased the amounts of abiotic and biotic stress on their maize plantings, exposing the weaknesses of

hybrids that may have been satisfactory at lower plant densities. Breeders, reacting to rejection of those hybrids, have developed newer hybrids with tolerance or resistance to the biotic or abiotic stresses that were the source of trouble and have planted their new-hybrid yield trials at constantly higher plant densities. Thus, the breeders continually have increased the selection pressure for tolerance to the most troublesome stresses of previous seasons, in addition to constant selection pressure for higher grain yield. Consequently, they have produced a stream of hybrids with steadily increasing amounts of stress tolerance and continually increasing efficiency in grain production, especially in high-density plantings. They have produced hybrids with the ability to make respectable amounts of grain on every plant at ever higher plant densities and under a variety of growing conditions, both favorable and unfavorable. The net product has been a stream of hybrids that continuously outyield their predecessors year in and year out, in the region of their adaptation.

Conversely, the Era data show that the time-series' increase in yield per unit area is not due to increased yield potential per plant. The Era time-series showed little or no gain in yield at 10 thousand plants/ha (i.e., one plant per square meter). At 10 thousand plants/ha, especially in favorable seasons, individual plants presumably are nearly stress-free and can express their full yield potential. Although grain yields of individual plants of the newest hybrids averaged about 0.4 kg/plant at 10 thousand plants/ha, the newest hybrids produced their highest yields at 79 thousand plants/ha with averages of 0.13 kg/plant. Maximum grain yield per plant is not necessarily a predictor of high yield per unit area.

This study is not the only one to show that maize yield gains over the years owe much to (or at the least are associated with) increases in stress tolerance (Bänziger et al. 1999; Castleberry et al. 1984; Edmeades et al. 1997, 1999; Tollenaar and Wu 1999; Tollenaar et al. 1994, 2000). And other investigations find, as well, that changes in phenotype and physiology similar to those described herein for the Era hybrids often are associated with (and may be consequences of) improvements in yield and stress tolerance (Chapman and Edmeades 1999). The Era hybrid time-series results probably are not unique, but rather they may epitomize any selection program that has the goal of increasing varietal yield and dependability in a defined region of adaptation.

A final comment, a negative one, in regard to linear increases in grain yield: when stated as percent gain, the increases in yield will be smaller each year on average, because a constant value for yield gain is being

divided by an ever larger value for hybrid yield. Thus, the percent gain in grain yield (the rate of gain) is declining and has declined from the beginning of this time-series. Unfortunately, the cost per unit of yield gain has not declined, at least as reported in 1984 (Duvick 1984) and as can be inferred also from a recent report on human and financial resources devoted to plant breeding research and development in the United States (Frey 1996). The cost per unit of yield gain has risen continually in past years and it probably will continue to increase unless new efficiencies in breeding are introduced.

B. Correlated Responses or Lack Thereof

The maize plant has made several decisions "on its own" when subjected to intense selection for higher and more stable yield. Without express breeder intention and/or selection, successive hybrids have (for example) smaller tassels, more upright leaves, less grain protein, and shorter anthesis-silking interval. As noted earlier, these changes probably increase efficiency and stability in grain production, and are a consequence of selection for high and stable yield. It may be important that tassels cannot become much smaller or leaves much more upright, and, therefore, these changes will not be of much help in the future as ways to increase efficiency in grain production. Also, one would suppose that except for specialty hybrids, breeders would not wish to lower grain protein percentage any further.

Harvest index increased, but was minimal, approximately one percentage unit per decade, according to measurements made in 1985. The increase was much less than that shown for Green Revolution wheat and rice cultivars in the early years of those crops, but it was relatively constant and was still increasing in 1985. Fodder weight showed no increase in the 1985 measurements. One can infer, therefore, that yield gain of the newer hybrids depends on increased efficiency in production of grain per plant (increase in harvest index at the plant density for which the hybrids were bred) added to the hybrids' generally increased tolerance to the stresses of higher density planting. Tolerance to higher density planting probably is more important than increased harvest index per se, but the probability of complex interactions between the two traits precludes any definitive statement about which is more important. Also, the harvest index data at hand are for one year only and do not include Era hybrids of recent years, so one should not attempt to infer or predict too much from them. At most, one can say that improved harvest index has played a much smaller role in yield gain of the Era maize

hybrids than it did for the early generations of the Green Revolution wheat and rice cultivars.

If protein percentage was reduced (and starch percentage increased) because more energy is needed to make protein than to make starch, one might expect oil percentage to have been reduced as well and to a greater extent, because even more energy is required for synthesis of oil. Since oil percentage was not reduced, it seems likely that a minimum grain oil percentage is required for some function, such as germination. One can hypothesize that the kernel cannot go below that limit and still function properly as (for example) a germinating seed.

Similarly, one might logically expect that if ECB2 (European corn borer, 2nd generation) tolerance/resistance is increased, ECB1 (European corn borer, 1st generation) tolerance should be increased simultaneously. The fact that it was not would seem to indicate that the factor that imparted increased tolerance/resistance to ECB2 is not present at the time of ECB1 establishment, or it is not present in tissues that ECB1 borers attack. Denser stalks or stalks with more lignin (and more resistance to stalk lodging) may be a reason for increased resistance to damage from ECB2. Such structures are not yet present during the time that ECB1 borers are active.

C. Heterosis

The minor role for heterosis in yield increase of the Era hybrids seems to be at variance with reports that show a major increase of heterosis over the years in inbred/hybrid comparisons (e.g., Meghi et al. 1984) and in population improvement programs (e.g., Carena and Hallauer 2001). This result also is at variance with the generally accepted belief that because heterosis is the basis for superiority of hybrids over their parents, further increase in yield will depend primarily on further increases in heterosis.

It is true that heterosis (as SX – MP) has made some increase over the decades when measured in stressful environments (Fig. 4.8), but nevertheless the data on hand indicate that heterosis for grain yield was less important than non-heterotic factors for improving yields over the years. Indeed, when heterosis (as SX – MP) is stated as percent of midparent yield (a frequent procedure) rather than as an absolute amount, the value for heterosis actually declines in low-stress conditions and at best is merely constant in high-stress growing conditions.

On sum, it seems likely that the primary reason for increase in yields of the Era hybrids is as summarized by Hallauer (1999, p. 486) for results

of recurrent selection. He says, "the additive effects of alleles with partial to complete dominance were of greater importance but dominant and epistatic effects could not be discounted." A predominant but not exclusive role for additive gene action would explain the concomitant increase in yields of inbreds and their hybrids but with slightly higher average increase for hybrids than for inbreds, at least in stressful growing conditions. Again, results in this data series are not unique; other researchers have found that heterosis for grain yield and for accompanying traits such as biomass is increased in stressed environments (e.g., Giauffret et al. 1997; Meghi et al. 1984).

Finally, heterosis values for plant height or for flowering date (anthesis) have not increased at all over the years (Table 4.2). Heterosis for plant height actually has diminished, although not very consistently or to any large degree.

D. Pedigree Dynamics

The pedigree data in this study show that it is a fallacy to believe that most U.S. maize hybrids are founded upon a genetic base of only a handful of widely used public inbred lines. (The inbred lines B14, B37, B73, Mo17, and Oh43 occasionally are cited as encompassing most of U.S. maize diversity.) Indeed, one could argue from the history of migration of maize into the North American continent and its subsequent usage that this region represents a center of diversity for maize. The relative breadth of current diversity in U.S. elite maize is shown by the large number of founder lines and landraces that underpin the germplasm developed by one breeding organization for one region of the United States. The germplasm in this study includes contributions from ancestral lines originally bred in regions exotic to the central U.S. Corn Belt, (e.g., Georgia, Pennsylvania, and Tennessee). But this array does not represent the full range of diversity that currently exists in regions of the United States outside of the central U.S. Corn Belt.

The history of maize in North America does not fit the model for small grain cereals [wheat (*Triticum* spp. L.), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), rice, millet (*Pennisetum glaucum* R. Br.), or sorghum (*Sorghum* bicolor (L.) Moench)] where relatively few inbred cultivars were brought to the United States during the past two to three hundred years. A broader diversity of maize has been extant in North America during the past two to three hundred years. Two races that are genetically very distinct (the Northern Flints and Southern Dents) were hybridized during the mid-nineteenth century to create the Corn Belt

Dents. Further crossing and selection for adaptation then was practiced by generations of farmer breeders to create the founder germplasm from which inbred lines and hybrids were initially developed in the early decades of the twentieth century. Formal breeding programs have allowed new diversity to be created by bringing together germplasms that otherwise would have been isolated in different regions of the country. Breeders therefore have continued to create and to test new combinations of diversity, a reality that differs from another misconception whereby breeders are said to have taken an initial small sample of corn inbreds and essentially then boiled away most of the diversity.

This study shows that the creation, testing, and incorporation of new diversity has accompanied the development of robust new hybrids that increasingly are tolerant of pests and stressful climatic conditions and that, consequently, can yield more per unit area of land. These increasingly robust hybrids make more effective use of available soil, water, sunlight, and nutrient resources. It seems likely that infusions of new germplasm have played an essential role in development of the improved hybrids. If this is true, we can assume that additional, fresh genetic diversity will be required to provide the potential for further adapting corn hybrids to future changes in husbandry, different climates, new forms of pests and diseases, and to new demands from consumers. An important challenge therefore will be to further increase breeders' abilities to identify and incorporate potentially useful new exotic diversity, including that from germplasm that currently resides outside the United States. Maize productivity thereby will become yet more efficient and will contribute increasingly to food, health, and environmental wellbeing.

Pedigree breeding, augmented with population improvement to create some important publicly bred Stiff Stalk lines, has been the major breeding strategy underpinning the hybrids reported upon in this study. The program has evolved into a large reciprocal recurrent selection program, with pedigree breeding operating within each of the two main heterotic groups. There has been a reduction in the average number of alleles per locus, as measured by SSRs distributed across the genome. While there has been a reduction in the number of alleles per locus within the heterotic groups, there has been a concomitant increase in diversity of alleles between the heterotic groups. The resulting pattern of change in allele diversity over time at a locus varies across positions in the genome. These changes in allele numbers per locus and in allelic diversity at individual loci may be, in part, a consequence of the combined effects of selection for favorable additive alleles within the

heterotic groups and selection for complementary alleles between the heterotic groups.

IV. SUMMARY, COMMENTS, AND PREDICTIONS

An array of commercial hybrids representing seven decades of breeding for the West-central U.S. Corn Belt shows linear increases in grain yield and in tolerance to biotic and abiotic stress, in particular to stresses typical of the West-central U.S. Corn Belt. Phenotypic changes (or lack thereof) in the hybrids partly are the result of breeders' planned selection practices and partly appear to be correlated responses to overt selection for other traits.

Increases in yield per unit area primarily are dependent on the ability of new hybrids to tolerate increased plant densities and the stresses they induce, rather than to increases in yield potential per plant, or to improvements in harvest index (when hybrids are planted at their appropriate plant density). As plant densities have increased across the years, newer generations of hybrids bred for those higher plant densities continue to make about the same amount of grain per plant at the higher plant densities, thus increasing yield per unit area.

Increases in heterosis have made a minimal contribution to the yield gains, although the contribution of heterosis in absolute amounts (calculated as SX – MP) increases when hybrids are subjected to increased levels of abiotic stress.

Pedigree and molecular marker analyses have shown the dynamic nature of germplasm shifts over the decades. Pedigree breeding, albeit with a few important inputs from population improvement, has been the primary breeding method during the 70 years of this long-term selection program.

Founder sources are numerous but are almost entirely of U.S. origin, especially from the U.S. Corn Belt. Over the decades, founders have appeared, disappeared, or persisted in hybrid pedigrees, as multigenerational inbred families arose, died out, or persisted. Persistent founders have varied considerably in proportionate contribution over the decades, increasing, decreasing, or sometimes maintaining a fairly constant contribution.

Molecular marker analysis of the Era hybrids and their inbred parents has shown that over the years the average number of alleles per locus has declined, and that, in recent decades, the alleles have been sorted into two contrasting groups corresponding to Stiff Stalk and Non Stiff

Stalk breeding pools. One can hypothesize that the contrasting marker alleles are associated with specific additive and/or partially dominant genes that allow inbreds in the two pools to complement each other's weaknesses. As well, they could be associated with genes affecting traits needed to make a good seed parent (Stiff Stalk) or a good pollinator parent (Non Stiff Stalk).

There is as yet no indication that yield gains are leveling out in this long-term breeding program. However, it would appear that contributions from some of the yield-enhancing trait changes (such as smaller tassels, more upright leaves, or reduced grain protein percent) have gone as far as they can go. Parenthetically, we note (and emphasize) that many of the trends that have been described in this report can be discerned as significant only because of the long-term nature of the breeding and selection program. Heritability estimates are much more robust for the 70-year period than for intervals within that period.

Past genetic gains in yielding ability have been closely associated with and dependent upon interactions with changes in agronomic practices (such as earlier planting and higher plant densities). One can expect that new and presently unforeseen changes in agronomic practices (and probably in climate) will give tomorrow's breeders new challenges and new opportunities to breed and select for enhanced yielding ability under new growing conditions and (probably) with new infusions of germplasm from around the world. Regardless of the nature of the future genetic changes, one can be confident that they will improve hybrid yields and dependability (an important aspect of yield) only if they improve the hybrids' tolerance to the prevalent biotic and abiotic stresses of future eras.

LITERATURE CITED

Bänziger, M., G. O. Edmeades, and H. R. Lafitte. 1999. Selection for drought tolerance increases maize yields across a range of nitrogen levels. Crop Sci. 39:1035–1040.

Carena, M. J., and A. R. Hallauer. 2001. Expression of heterosis in Leaming and Midland Corn Belt Dent populations. J. Iowa Acad. Sci. 108:73–78.

Casa, A. M., S. E. Mitchell, O. S. Smith, J. C. Register III, S. R. Wessler, and S. Kresovich. 2002. Evaluation of Hbr (MITE) markers for assessment of genetic relationships among maize (*Zea mays* L.) inbred lines. Theor. Appl. Gen. 104:104–110.

Castleberry, R. M., C. W. Crum, and C. F. Krull. 1984. Genetic yield improvement of U.S. maize cultivars under varying fertility and climatic environments. Crop Sci. 24:33–36.

Chapman, S. C., and G. O. Edmeades. 1999. Selection improves drought tolerance in tropical maize populations: II. Direct and correlated responses among secondary traits. Crop Sci. 39:1315–1324.

Duvick, D. N. 1977. Genetic rates of gain in hybrid maize yields during the past 40 years. Maydica XXII:187–196.

Duvick, D. N. 1984. Genetic contributions to yield gains of U.S. hybrid maize, 1930 to 1980. p. 15–47. In: W. R. Fehr (ed.), Genetic contributions to yield gains of five major crop plants, CSSA Special Publ. No. 7. Crop Sci. Soc. Amer., Amer. Soc. Agron., Madison, WI.

Duvick, D. N. 1992. Genetic contributions to advances in yield of U.S. maize. Maydica 37:69–79.

Duvick, D. N. 1997. What is yield? p. 332–335. In: G. O. Edmeades et al. (eds.), Developing drought- and low N-tolerant maize. Proc. Symp., March 25–29, 1996, CIMMYT, El Batàn, México. CIMMYT, México, D.F.

Duvick, D. N. 1999. Heterosis: feeding people and protecting natural resources. p. 19–29. In: J. G. Coors and S. Pandey (eds.), The genetics and exploitation of heterosis in crops. Amer. Soc. Agron., Inc., Crop Sci. Soc. Amer., Inc., Soil Sci. Soc. Amer., Inc., Madison, WI.

Duvick, D. N., J. S. C. Smith, and M. Cooper. 2003. Changes in performance, parentage, and genetic diversity of successful corn hybrids, from 1930 to 2000. In: C. W. Smith et al. (eds.), Corn: Origin, history, technology and production. John Wiley, Hoboken, NJ. In press.

Edmeades, G. O., M. Bänziger, H. R. Mickelson, and C. B. Peña-Valdivia (eds.). 1997. Developing drought- and low N-tolerant maize: Proc. Symp., March 25–29, 1996, CIMMYT, El Batán, Mexico, p. 1–566. CIMMYT, El Batán, Mexico, D.F.

Edmeades, G. O., J. Bolaños, S. C. Chapman, H. R. Lafitte, and M. Bänziger. 1999. Selection improves drought tolerance in tropical maize populations: I. Gains in biomass, grain yield, and harvest index. Crop Sci. 39:1306–1315.

Frey, K. J. 1996. National plant breeding study—I: Human and financial resources devoted to plant breeding research and development in the United States in 1994. Iowa Agr. and Home Econ. Expt. Sta., and Cooperative State Res., Educ. & Ext. Serv./USDA Cooperating, Ames.

Giauffret, C., R. Bonhomme, and M. Derieux. 1997. Heterosis in maize for biomass production, leaf area establishment, and radiation use efficiency under cool spring conditions. Maydica 42:13–19.

Gilmour, A. R., B. R. Cullis, S. J. Welham, and R. Thompson. 1998. ASREML program user manual. NSW Agr. Biometrics Bul., Australia.

Hallauer, A. R. 1999. Heterosis: What have we learned? What have we done? Where are we headed? p. 483–492. In: J. G. Coors and S. Pandey (eds.), Genetics and exploitation of heterosis in crops. Amer. Soc. Agron., Inc., Crop Sci. Soc. Amer., Inc., Soil Sci. Soc. Amer., Inc., Madison, WI.

Kruskal, J. B., and M. Wish. 1978. Multidimensional scaling. Sage University Paper Series on Quantitative Applications in the Social Sciences, series no. 07-011. Sage Publ., Beverly Hills and London.

Meghi, M. R., J. W. Dudley, R. J. Lambert, and G. F. Sprague. 1984. Inbreeding depression, inbred and hybrid grain yields, and other traits of maize genotypes representing three eras. Crop Sci. 24:545–549.

Peng, S., K. G. Cassman, S. S. Virmani, J. Sheehy, and G. S. Khush. 1999. Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. Crop Sci. 39:1552–1559.

Tollenaar, M., and J. Wu. 1999. Yield improvement in temperate maize is attributable to greater stress tolerance. Crop Sci. 39:1597–1604.

Tollenaar, M., J. Ying, and D. N. Duvick. 2000. Genetic gain in corn hybrids from the Northern and Central Corn Belt. p. 53–62. 55th Annual Corn and Sorghum Research Conference, Vol. 55. Amer. Seed Trade Assoc., Chicago.

Tollenaar, M., S. P. Nisssanka, A. Aguilera, S. F. Weise, and C. J. Swanton. 1994. Effect of weed interference and soil nitrogen on four maize hybrids. Agron. J. 86:596–601.

5

Long-term Divergent Selection for Ear Length in Maize

Arnel R. Hallauer, Andrew J. Ross, and Michael Lee
Department of Agronomy
Iowa State University
Ames, Iowa 50011-1010

I. INTRODUCTION

Selection is a key element of all plant improvement programs. Selection can take different forms, and its effectiveness is dependent on factors that either are under direct control by the plant breeder or are affected by forces that are under limited control by the plant breeder. The effectiveness of artificial selection is determined by how the plant breeder can either control or manipulate the known and unknown factors. Factors that can be controlled in selection include types of progenies evaluated, extent of test information, parentage control, effective population size, methods of intermating, duration of each cycle of selection, and, in some instances, environmental effects (Eberhart 1970). One of the more important unknown factors that can cause differences in trait expression is the combination of climatic factors that can occur during different cycles of selection. Other unknown factors include relative amount and types of genetic effects affecting trait expression, decreases in parental

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-46892-4

control because of possible contamination, errors in measurement, field husbandry practices (e.g., seedbed preparation, pest controls, and cultivation), and correlated changes that can occur in other traits when selection emphasizes one trait. In all instances, the main concern is to attain the greatest heritability to increase effectiveness of selection.

Selection methods can have different objectives in applied plant breeding. In all instances, the main goal of selection is to increase the frequency of favorable alleles for the target trait(s). For germplasm enhancement, selection emphasizes the improvement of a limited number of traits of genetically broad-based populations and the maintenance of genetic variation for continued selection. In programs that emphasize cultivar development, intense selection is practiced in appropriate choice of parental materials and during inbreeding and testing to identify elite genotypes that have good, consistent performance across environments. Although the goals for the two aspects of plant improvement are different, factors that affect effectiveness of selection are similar. Usually, but not always, either one or a few traits are emphasized during improvement of genetically broad-based populations, whereas 10 or more traits may be considered when developing elite cultivars for commercial use.

Mass selection, or phenotypic selection, was used in development of the original landrace cultivars of maize (*Zea mays* L.). Selection was based on the phenotype of individual plants, and its effectiveness depended on the heritabilities of the traits considered during selection. Mass selection was effective for some traits (e.g., maturity, plant stature, and grain color and types), but it was not effective for other traits (e.g., productivity). Modifications (increased parental control and reduced environmental effects) were made to increase effectiveness of mass selection (Gardner 1961), but the heritability on an individual plant basis remained less than on a progeny mean basis.

Specific selection studies are conducted to gain information on the heritability of traits, responses to selection, and correlated changes of traits that are not under direct selection. Divergent selection studies are designed to obtain these types of information (Falconer 1960). Selection is practiced in two directions for the same trait in divergent selection studies: one phase of selection is applied to increase allele frequency to enhance a specific trait in one direction, and the second phase of selection is to increase allele frequency in the opposite direction. Responses observed from long-term divergent selection studies permit interpretations relative to the genetic structure of the original population under selection; such interpretations are based on rates of response to selection and factors that affected the different rates of response over cycles

of selection. Collorary information also can be obtained from the correlated changes that occur for other traits (i.e., correlated responses) not considered in divergent selection for specific traits. The Illinois maize selection studies that emphasized divergent selection for protein and oil are good examples (e.g., Dudley 1977).

Divergent mass selection in maize was initiated in 1963 in the synthetic cultivar designated as 'Iowa Long Ear Synthetic'. This selection study was conducted to determine the effectiveness of mass selection for ear length and its effects on grain yield. Evidence from previous quantitative genetic studies in maize populations suggested that ear length was positively correlated with grain yield and that the heritability of ear length was greater than for grain yield (Robinson et al. 1951). This information suggested that grain yield would increase with an increase in ear length.

II. MATERIALS

The 'Iowa Long Ear Synthetic' (BSLE) cultivar was developed by intermating 12 inbred lines (Russell et al. 1971). Observations were made in breeding nurseries of lines that had above average ear length. Ear length data were not taken from replicated trials to determine the choice of 12 inbred lines intermated to form BSLE (Table 5.1). The 12 inbred lines had different levels of inbreeding and testing, some lines had not been released, and the use of the lines in hybrid breeding programs varied (i.e., limited to extensive). None of the lines themselves was included in the summaries of lines that had extensive use as parental lines to produce commercial hybrids, but C103 and 187-2, for example, were the parents of Mo17, an extensively used line (Zuber 1975; Zuber and Darrah 1980; Troyer 1999, 2001; Hallauer 2002). The 12 inbred lines did not represent any specific heterotic group. Germplasm from different strains of 'Reid Yellow Dent', different strains of 'Lancaster Surecrop', 'Midland', and other sources were represented in these inbred lines. Four lines, B56, C103, Lancaster Composite-34, and (L317 × 187-2)-1-1-9 ('Alph' cultivar probably includes 'Lancaster Surecrop' germplasm) included varying percentages of 'Lancaster Surecrop'; four lines, B217 (waxy), N22A, N25, and W-17R-B, included varying percentages of 'Reid Yellow Dent', one line (B15 × B18)-16 included a 'Midland' line as one parent, and the remaining three lines (B50, B55, and Oh29) were derived via pedigree selection from crosses that included lines of different origins. The BSLE population was formed by intermating the 12 lines in the following manner: (1) the 12 lines were crossed to produce six single

Table 5.1. Pedigree of 12 maize lines intermated to form Iowa Long Ear Synthetic (Russell et al. 1971).

Line	Source[z]
B50	[(M14 × A206) × Oh4c]-26
B55	(Oh45 × W92)-1-1-2
B56	(Alph4 × 38-11)-432
B217 (waxy)	(High Oil × B10$^{/3}$)-1-1-2-1-1; B10 is BSSS-507-193-4-1
C103	Lancaster Surecrop (from Noah Hershey)
N22A	N22 outcross (N22 from Krug)
N25	Reid Yellow Dent
Oh29	Oh28 × Ia.159 L1
W-17R-B	KROsf (Osterland Yellow Dent-Os420)
(B15 × B18)-16	(WF9 × D17)-561 × M4-345 (from Midland)
Lancaster Composite-34	P.I. 213697
L317 × 187-2)-1-1-9	Lancaster Surecrop (Richey strain) for L317 and Krug for 187-2

[z]Gerdes, J. T., et al. (1993).

crosses; (2) the six single crosses were then used to produce three double crosses; and (3) three possible double-double crosses were produced using the three double crosses. Equal quantities of the three double-double crosses were bulked and then intermated for three generations by open-pollination in isolation fields.

III. METHODS

Mass selection for divergent ear length was initiated in an isolation field during the third generation of intermating for a seed increase of BSLE cycle 0 (BSLEC0) in 1963. Plant density was about 36,000 plants ha^{-1}. The grid system of mass selection suggested by Gardner (1961) was superimposed on the field to form 100 grids (or plots) before selection was initiated. Each of the 100 plots included 40 competitive plants. Within each plot of 40 plants, ears were harvested from plants with the 5 to 8 shortest ears and from plants with the 5 to 8 longest ears. The two groups of ears were kept separately for future measurements. After harvest, the 100 shortest and 100 longest selected groups of ears were dried at approximately 35°C to a uniform moisture level of 130 to 140 g kg^{-1}.

After drying, each ear within the 200 groups (100 groups of short ears and 100 groups of long ears) of ears selected phenotypically within the isolation field was measured to determine the three shortest and three longest ears within each of the 100 plots. A total of 300 ears were selected that had either the shortest or longest ears. Selection intensity was 7.5% (k = 1.8882) for the shortest and longest ear length. Three bulks were formed from each of the selected shortest and longest ears to form the C1 cycle of selection: (1) 50 seeds were taken from each selected ear and bulked (15,000 seeds) for planting the following year; (2) 50 seeds were taken from each selected ear, bulked and put in cold storage as remnant seed if the current planting was lost; and (3) remaining seeds on each of the shortest and longest ears were shelled in bulk to form short-ear and long-ear bulks and put in cold storage for future use.

Subsequent cycles of mass selection were conducted in two separate isolation fields that were themselves a minimum of 200 m from other fields of maize. The short-ear bulk from 1963 was planted in one isolation field and the long-ear bulk from 1963 was planted in the second isolation field. Plant density within each field was approximately 39,000 plants ha^{-1}. Mass selection methods used in subsequent cycles of selection were similar to those used in the first cycle except selection was conducted in two isolation fields: selection for shorter ear length in one isolation field and selection for longer ear length in the second isolation field. Modifications were made in subsequent cycles of selection for methods of planting, weed control, fertilizer applications, and an increase in plant density (about 48,000 plants ha^{-1}). Methods of selection and selection intensities were the same in all cycles of selection. Because of damage from invading livestock and wild animals, a few of the isolation fields did not produce adequate seed for the next cycle of selection. Thirty cycles of mass selection for divergent ear length were completed.

Response to divergent mass selection was monitored at the 10th (Cortez-Mendoza and Hallauer 1979), 15th (Salazar and Hallauer 1986), and 27th (Lopez-Reynoso and Hallauer 1998) cycles of selection. Response to mass selection was determined in replicated trials that included entries of the BSLEC0 population and different cycles of selection. In all instances, remnant seed of the BSLEC0 population and selection cycles was used to reproduce seed of the BSLEC0 and selection cycles in the same season. Seed reproduction was done to reduce possible seasonal and age effects on seed quality for BSLEC0 and selection cycles. Approximately 100 to 150 plants were planted in the breeding nurseries either to reproduce BSLEC0 and each selection cycle, to produce crosses between BSLEC0 and testers, and crosses between the

long- and short-ear strains of different selection cycles. Hand pollinations were used for reproducing populations and for producing population crosses. Attempts were made to include every plant during pollination. All pollinated ears were harvested, dried at approximately 35°C to uniform moisture level of 130 to 140 g kg^{-1}, and an equal number of seeds was harvested from each ear to form a balanced bulk for the evaluation trials. Replicated evaluation trials were conducted at locations in central and southern Iowa. Details for the specific trials were given by Cortez-Mendoza and Hallauer (1979), Salazar and Hallauer (1986), and Lopez-Reynoso and Hallauer (1998).

Evaluation trials included both hand and machine harvesting to determine response to mass selection for divergent ear length. In some instances, all ears were hand harvested and in other instances 10 ears were harvested from 10 competitive plants within each plot, and the remaining plants were machine harvested to determine grain yield. Ear measurements were made on the 10 hand-harvested ears, and shelled grain from the 10 ears was included with the machine-harvested ears to determine total grain yield. Data also were taken on dates of flowering, plant and ear height, grain yield, ear diameter, kernel depth, and kernel-row number. Data collected on traits, other than ear length, were used to determine the correlated effects of divergent mass selection for ear length on other plant and ear traits.

Trait means from the combined analyses of variance were used to determine the direct and indirect effects of divergent mass selection for ear length. Regression analyses were used to measure rate of direct response to selection for shorter and longer ear length. Direct responses were expressed as cm $cycle^{-1}$ of selection. Standard errors were calculated to determine if direct response to selection was significant, and if the direct responses for shorter and for longer ears were significantly different. Regression and correlation analyses were used to determine the correlated, or indirect, changes that occurred with mass selection for shorter and for longer ears.

IV. RESULTS AND DISCUSSION

Evaluation trials at the 10th, 15th, and 27th cycles of divergent mass selection for ear length indicate selection was effective for longer and for shorter ear length (Table 5.2). Selection for greater ear length increased at about the same rate throughout all selection cycles, whereas selection for decreased ear length tended to decrease with continued selection. The estimates of the coefficients of regression were significantly ($P \leq$

Table 5.2. Response to divergent mass selection for ear length in Iowa Long Ear Synthetic (BSLE) of maize after 10, 15, and 27 cycles of selection.

Source	Response to selection (cm)		No. cycles of selection	No. replications	No. locations
	Shorter ears	Longer ears			
Cortez-Mendoza and Hallauer (1979)	–0.64 ± 0.05	0.32 ± 0.05	10	4	5
Salazar and Hallauer (1986)	–0.46 ± 0.03	0.38 ± 0.03	15	3	7
Lopez-Reynosa and Hallauer (1998)	–0.37 ± 0.03	0.27 ± 0.03	27	3	5

0.01) different from zero with selection for longer and for shorter ears, and the differences between the two regression coefficients also were significantly ($P \leq 0.01$) different in all instances. The rate of change with selection for shorter ears was greater than selection for longer ears when evaluated after 10, 15, and 27 cycles of selection (Fig. 5.1). The rate of

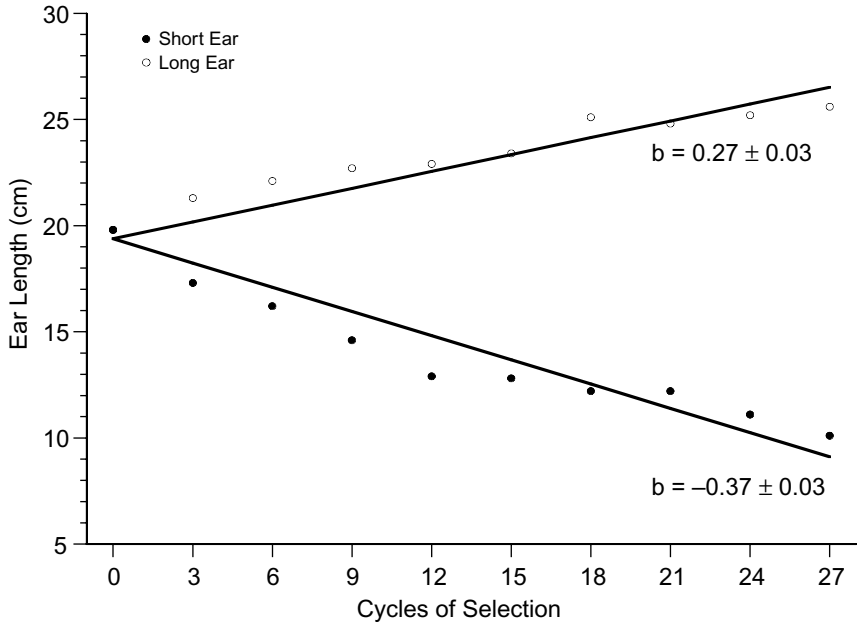

Fig. 5.1. Direct response for ear length with 27 cycles of divergent mass selection for ear length in Iowa Long Ear Synthetic cultivar of maize.

response to selection for shorter ear length after 15 and 27 cycles of selection was less than for the first 12 cycles of selection; the rate of change for shorter ear length has decreased with continued mass selection. Selection for shorter ears decreased mean ear length from 19.8 cm for BSLEC0 to 12.9 cm for the 12th cycle of selection, a decrease of 6.8 cm. Ear length of the 27th cycle was 10.1 cm, a decrease of 2.8 cm for the last 15 cycles of selection.

Correlated responses to divergent selection for ear length were detected but not considered in selection (Table 5.3). Selection for longer and for shorter ears affected grain yield; there was no significant change with selection for longer ears and a significant decrease with selection for shorter ears (Fig. 5.2). There was greater variation in the response of grain yield to cycles of selection for longer ears compared with consistent decreases in grain yield with selection for shorter ears (Fig. 5.2). Changes for other plant and ear traits were consistent throughout the 27 cycles of selection. Selection for longer ears resulted in decreases in ear diameter, kernel-row number, and kernel depth, increases in plant and ear height, and later flowering dates (Table 5.3). Later selection cycles

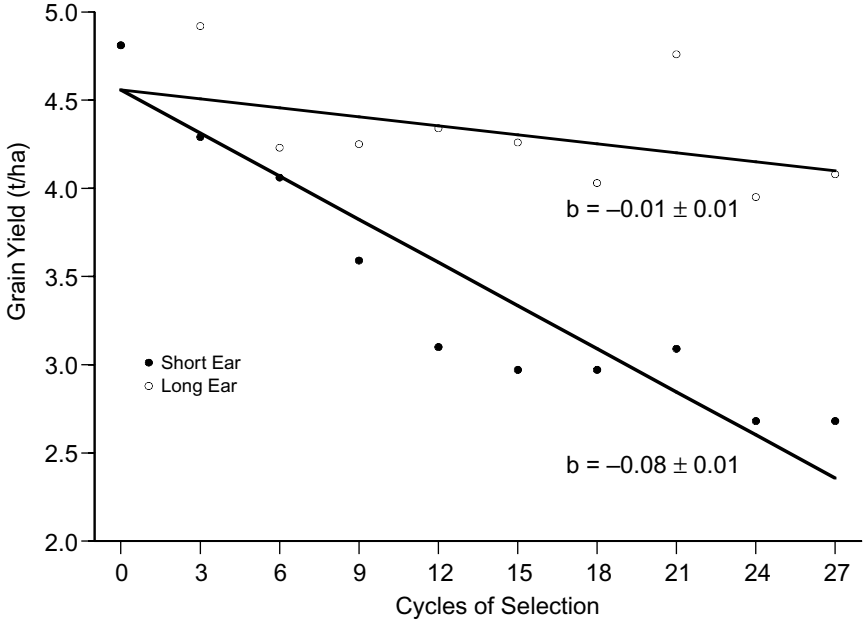

Fig. 5.2. Correlated response for grain yield with 27 cycles of divergent mass selection for ear length in Iowa Long Ear Synthetic cultivar of maize.

Table 5.3. Correlated responses for plant and ear traits with divergent mass for ear length in Iowa Long Ear Synthetic (BSLE) synthetic of maize after 10, 15, and 27 cycles of selection.

	Cortez-Mendoza and Hallauer (1979)		Salazar and Hallauer (1986)		Lopez-Reynosa and Hallauer (1998)	
Traits	Long ear	Short ear	Long ear	Short ear	Long ear	Short ear
Yield (t ha^{-1})	−0.01 ± 0.03	−0.23 ± 0.03	−0.04 ± 0.01	−0.10 ± 0.01	−0.01 ± 0.01	−0.08 ± 0.01
Ear diameter (cm)	−0.02 ± 0.01	0.01 ± 0.01	−0.02 ± 0.01	0.02 ± 0.01	−0.02 ± 0.01	0.01 ± 0.01
Row number	−0.06 ± 0.04	0.07 ± 0.04	−0.08 ± 0.02	0.15 ± 0.02	−0.06 ± 0.01	0.11 ± 0.01
Kernel depth (×10) (cm)	−0.10 ± 0.03	0.03 ± 0.03	−0.09 ± 0.02	0.07 ± 0.02	—[z]	—[z]
Ear height (cm)	1.07 ± 0.39	−2.35 ± 0.39	1.67 ± 0.21	−2.14 ± 0.21	0.99 ± 0.25	−0.83 ± 0.25
Plant height (cm)	—[z]	—[z]	2.39 ± 0.35	−3.23 ± 0.35	1.54 ± 0.37	−1.59 ± 0.37
Days to flower (no.)	0.49 ± 0.14	0.05 ± 0.14	0.33 ± 0.06	−0.26 ± 0.06	0.21 ± 0.04	−0.21 ± 0.04

[z]Data not recorded.

for increased ear length also had greater root and stalk lodging and increased prolificacy (data are not shown). Compared with selection for longer ear length, selection for shorter ear length caused significantly lower grain yield, and caused trends of increasing ear diameter, kernel-row number, and kernel depth, accompanied by reduced plant and ear height and earlier flowering dates (Table 5.3). Not all of the regression coefficients for correlated plant and ear traits were significant, but the trends were consistent for both phases of selection.

A biparental mating design suggested by Comstock and Robinson (1948) was used to estimate the relative amounts of additive genetic variance and variance due to dominance deviations and correlations between plant and ear traits in BSLEC0 (Hallauer 1968). This study included 84 males each mated to four females (336 full-sib families) that were evaluated at three Iowa locations. Data were collected for ear length, grain yield, and six other plant and ear traits on an individual plant basis. Estimates from the combined analyses of variance indicated that the greatest portion of the total genetic variance for ear length and grain yield was due to additive genetic effects. Genetic (0.38) and phenotypic (0.45) correlations between ear length and yield were greater than for ear diameter, kernel-row number, and weight of 300 kernels. Heritability estimates based on full-sib progeny means were 0.45 for ear length and 0.29 for grain yield. But the heritability estimates expressed on the basis of individual plants with grid system of mass selection were 0.07 for ear length and 0.21 for grain yield, indicating that indirect selection, based on length, would not be effective to increase grain yield.

Lopez-Reynoso and Hallauer (1998) determined the genetic variation within the original BSLEC0 population and in the populations after 24 cycles of selection for shorter [BSLE(M-S)C24] and longer [BSLE(M-L)C24] ears. Genetic variability was estimated from the use of 100 unselected S_1 lines developed from the BSLEC0, BSLE(M-L)C24, and BSLE(M-S)C24 populations that were evaluated at two locations for two years with two replications for each location. Based on the variation among the 100 S_1 progenies, there was no evidence that genetic variation (σ^2_G) for ear length in BSLE(M-S)C24 ($\sigma^2_G = 2.61$) and BSLE(M-L)C24 ($\sigma^2_G = 3.78$) was reduced after 24 cycles of selection; estimate of genetic variation within BSLEC0 was 2.12 with all estimates significantly ($P \leq 0.01$) different from zero. Correlated responses among the 100 S_1 lines for other plant and ear traits were similar to those listed in Table 5.3. Estimates of heritability for ear length on an individual plant basis were 0.05 for BSLEC0 and 0.02 for BSLE(M-S)C24 and BSLE(M-L)C24. The estimate of heritability for ear length (0.05) for BSLEC0 was similar to

the estimate (0.07) previously reported by Hallauer (1968) using a biparental mating design. The estimate of heritability for grain yield on an individual plant basis for grain yield (0.02) reported by Lopez-Reynoso and Hallauer (1998) was 10 times less than the estimate (0.21) reported by Hallauer (1968). The genetic correlation between yield and ear length for the S_1 progenies was –0.07. Estimates of heritability from the use of different types of progenies (full-sib families and S_1 progenies) could have been a factor for the differences in the estimates between the two studies.

The use of S_1 progenies rather than individual plants suggests greater progress could be realized using S_1 progenies on a per cycle basis (i.e., 1 year for mass selection vs. 2 to 3 years for S_1 progeny selection). Heritability estimates for ear length based on S_1 progeny means were 0.78 for BSLEC0, 0.59 for BSLE(M-S)C24, and 0.53 for BSLE(M-L)C24. Similarly, the estimates for grain yield were 0.59, 0.70, and 0.66 for BSLEC0, BSLE(M-S)C24, and BSLE(M-L)C24, respectively. The choice of selection method depends on the resources available and the goal(s) of selection. Although the estimates of heritabilities for S_1 progeny means were significantly greater than those based on individual plants, selection responses for ear length and other plant traits were similar, and it is not known if S_1 progeny selection would have changed the final results.

If indirect selection for grain yield is to be more effective than direct selection, the heritability for the primary trait (e.g., ear length) under selection must be greater than the secondary trait and the secondary trait (e.g., grain yield) must be correlated with the primary trait under selection. Predicted genetic gain (Δ_G) for one trait can be expressed as $kh^2\sigma_P$, where k is standardized selection differential, h^2 is the heritability estimate, and σ_P is the square root of the phenotypic variance. If selection is practiced on ear length (trait 1) and it is desired to predict genetic gain for grain yield (trait 2), predicted genetic gain in grain yield ($\Delta_{G_{2.1}}$) based on selection for ear length becomes $\Delta_{G_{2.1}} = r_{G_{12}}h_1h_2\,\sigma_{P_2}\,k_1$, where $r_{G_{12}}$ is the genetic correlation between grain yield and ear length, h_1 and h_2 are square roots of the heritabilities for ear length and grain yield, respectively, σ_{P_2} is the square root of phenotypic variance for grain yield, and k_1 is the standardized selection differential for ear length. Relative efficiency (RE) of selection can be expressed $\Delta_{G_{2.1}}/\Delta_{G_2}$, or gain by indirect selection divided by gain by direct selection. Predicted direct responses for decreased and increased ear length were reported by Lopez-Reynoso and Hallauer (1998) as 0.30 cm cycle^{-1}. Observed responses for longer (b = 0.27 cm) and shorter (b = –0.37 cm) were similar to the predicted direct response (Fig. 5.1). Predicted direct response for grain yield was 1.61 g

plant^{-1}, but the predicted correlated response for grain yield with selection for ear length was –0.31 g plant^{-1}. The observed correlated response of yield for shorter ears (–0.31 g plant^{-1}) was equal to the predicted correlated response, whereas the observed correlated response for longer ears (–2.25 g plant^{-1}) was greater than predicted correlated response. Lopez-Reynoso and Hallauer (1998) reported that response for grain yield based on selection for ear length was 19.2% relative to direct response for grain yield. Response to direct selection for grain yield itself was expected to be 80.8% greater than to indirect selection for ear length, which is very similar to the 79.2% previously reported by Hallauer (1968). Cortez-Mendoza and Hallauer (1979) and Salazar and Hallauer (1986) reported that response to direct selection for grain yield was 44 and 68%, respectively, greater than to selection based on ear length. Indirect selection for greater yield decreased with selection for increased ear length. The failure of indirect selection to be more effective than direct selection was because the genetic correlation ($r_{G_{12}}$) between grain yield and ear length and heritability of ear length (h_1^2) were not sufficiently large enough for indirect selection to be more effective than direct selection.

Mass selection for divergent ear length was not symmetrical. Response to selection for shorter ear length was significantly ($P \leq 0.01$) greater than selection for longer ear length throughout the 27 cycles of selection. Falconer (1960) discussed possible causes of asymmetrical response resulting from divergent selection. Population sizes of the present study were at a minimum of 4,000 plants for the plots within each isolation. Additional plants surrounded the plots used for selection to provide adequate competition to reduce possible biases in selection for ear length. It is estimated that each isolation field included a minimum of 8,000 to 10,000 plants. If we use Falconer's formula for determining effective population size with 300 females and 8,000 males, effective population size would be more than 2,300 for each cycle of selection. It seems that effective population sizes were adequate to minimize the effects of random drift and inbreeding. Selection for ear length was based only on individual plants within each of the 100 plots, but pollen to fertilize the ears could come from any of the plants within the isolation field. Pollen sources of the plants of the selected ears would originate from tassels of both the selected and unselected plants. Parental control (c) would have been greater if pollen had been provided only by tassels of the plants with the selected ears, and theoretically this would have increased response to selection, provided other factors remained constant. Predicted response in ear length (b = 0.30 cm), however, was similar to observed response to both shorter (b = 0.37) and longer (b = 0.27) ears (Fig. 5.1). Lopez-

Reynoso and Hallauer (1998) discussed possible reasons for the asymmetrical response to selection for ear length and concluded that the initial gene frequencies of BSLEC0 may have been an important factor. BSLEC0 was developed by intermating lines with above average ear length. Allele frequencies for greater ear length would be assumed to be greater than 0.5, which would contribute to asymmetrical response of selection for shorter and longer ear length. The asymmetry to response was greater initially than in later cycles of selection (Table 5.2). Response from BSLEC0 to BSLE(M-S)C12 was 6.9 cm vs. 2.8 cm from BSLE(M-S)C12 to BSLE(M-S)C27. Response during the first 12 cycles of selection was 2.5 times greater than the response during the last 15 cycles of selection, suggesting that alleles with major effects were important for shorter ear length and were driven to fixation in the earlier cycles of selection. Selection intensities were the same for short-ear and long-ear selection during all selection cycles. Selection differentials, however, may have differed because of the unequal effects of natural selection, pollen fertilization, scale effects, and changes in husbandry practices during the 27 cycles of selection. Evidence from Lopez-Reynoso and Hallauer's (1998) study suggested that genetic variances within BSLE(M-S)C24 and BSLE(M-L)C24 were similar to the estimate of genetic variance within BSLEC0 for ear length, grain yield, and most of the other plant and ear traits.

Limits to future response to selection for shorter and for longer ears may occur. In all instances, ears included for selection were restricted to those with complete seed sets. Within the long-ear selection phase, poorer pollination of the basal ovaries was sometimes observed. It seems that the growth and penetration of the pollen tubes to the basal ovaries may have reduced selection response for greater ear length because of the failure to fertilize ovaries with longer silks. Within the short-ear selection phase, ears with viable kernels are required to ensure reproduction of the plant and there would be an ultimate limit for shorter ears. If size of pollen grains is increased to permit pollen tube growth in longer silks, rate of response to selection for longer ears may become greater than rate of response to selection for shorter ears. Based on the data for the first 27 cycles of divergent selection for ear length, it seems the initial allele frequency in BSLEC0 was the main causal factor for the observed asymmetrical response to selection.

Grain yield of maize is a complex trait that includes several components, such as ear length, ear and cob diameter, kernel size, depth, and weight, number of kernel rows, number of kernels, and number of ears per plant. There is genetic variation among cultivars for the different

components (e.g., greater ear length and fewer kernel rows for 'Lancaster Surecrop' vs. greater number of kernel rows on shorter ears for 'Reid Yellow Dent'), but environmental effects from time of planting to time of harvest also can cause significant changes in the expression for each of the components of yield. Positive and significant genetic correlations between grain yield and ears per plant (0.43), ear length (0.38), ear diameter (0.41), cob diameter (0.10), kernel depth (0.51), kernels per row (0.45), and kernel weight (0.25) suggest that each of the components contribute to greater grain yield (Table 5.16, in Hallauer and Miranda 1988). Correlations of several yield components with grain yield are similar, suggesting that each component has similar relative importance for their contribution to total grain yield. Divergent mass selection studies for two other components of yield have been reported in maize. Torregroza (1973) selected for greater ear number (prolificacy) and single-ear plants in the highland open-pollinated maize cultivar, 'Harinoso Mosquera', to produce prolific and nonprolific subpopulations. Selection for multiple ears plant^{-1} increased grain yield (35%) and prolificacy (48%) compared with decreases for yield (7%) and prolificacy (16%) in the single-ear strain of 'Harinoso Mosquera'. Odhiambo and Compton (1987) summarized 20 cycles of divergent mass selection for seed size in a strain of 'Krug Yellow Dent'; their results were similar to those for ear length. Grain yield did not increase with selection for greater seed size, but grain yield decreased significantly ($P \leq 0.01$) with selection for smaller seed size. They also reported correlated responses in seed weight, number of seeds, kernel-row numbers, and ear length with divergent mass selection for seed size.

Grain yield of maize is affected by many different plant and ear components. Later plants, which also tend to be taller, within a given maturity zone tend to have a greater yield than earlier maturity plants. Most ear components (ear length, ear diameter, kernel depth, number of kernels, number of ears, and kernel weight) have positive correlations ($r = 0.4$ to 0.6) with grain yield (Hallauer and Miranda 1988). But the correlations between the different components of the ear can be either near zero or negative. Selection for greater ear length resulted in fewer kernel rows and reduced kernel depth because of greater cob diameter. Compensations among the different components of the ear are made when selection emphasizes only one of the components. Grafius (1963), for small grains, and Rinke (1960), for maize, suggested that manipulation of the components of yield was a feasible method to enhance total grain yield. Geadelmann and Peterson (1976, 1978) reported data from the study proposed by Rinke (1960) for maize. The proposal by Rinke (1960) included backcross breeding for the parallel convergence of com-

ponents of yield to increase yield of hybrids. Geadelmann and Peterson (1976, 1978) concluded that no yield increases were derived from yield component modification of inbred parents of superior hybrids, and that backcross-selection for yield components does not seem to be a useful method for improving high-yielding maize hybrids grown at the plant densities common in the U.S. Corn Belt. Similar to Torregroza (1973), they found selection for greater ears per plant was more effective than for ear length or kernel depth for increasing yield of hybrids. If an increase in grain yield is desired, direct selection for grain yield will be more effective. An optimal balance among the ear components occurs with direct selection for increased grain yield, and, if adequate genetic information is available for the populations under selection, appropriate selection indices would increase effectiveness of selection (Baker 1986).

LITERATURE CITED

Baker, R. J. 1986. Selection indices in plant breeding. CRC Press, Boca Raton, FL.

Comstock, R. E., and H. F. Robinson. 1948. The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. Biometrics 4:254–266.

Cortez-Mendoza, H., and A. R. Hallauer. 1979. Divergent mass selection for ear length in maize. Crop Sci. 19:175–178.

Dudley, J. W. 1977. 76 generations of selection for oil and protein percentage in maize. p. 459–473. In: E. Pollak et al. (ed.), Proc. Int. Conf. Quant. Genet. Iowa State Univ. Press, Ames.

Eberhart, S. A. 1970. Factors affecting efficiencies of breeding methods. African Soils 15:669–680.

Falconer, D. S. 1960. Introduction to quantitative genetics. The Ronald Press Co., New York.

Gardner, C. O. 1961. An evaluation of effects of mass selection and seed irradiation with thermal neutrons on yields of corn. Crop Sci. 1:241–245.

Geadelmann, J. L., and R. H. Peterson. 1976. Effects of yield component selection on the general combining ability of maize inbred lines. Crop Sci. 16:698–701.

Geadelmann, J. L., and R. H. Peterson. 1978. Effects of two yield component selection procedures on maize. Crop Sci. 18:387–390.

Gerdes, J. T., C. F. Behr, J. G. Coors, and W. F. Tracy. 1993. Compilation of North American maize breeding germplasm. CSSA, Madison, WI.

Graffius, J. E. 1963. Vector analysis applied to crop eugenics and genotype-environment interaction. p. 197–217. In: W. D. Hanson and H. F. Robinson (eds.), Statistical genetics and plant breeding. Publ. 982, NAS-NRC, Washington, DC.

Hallauer, A. R. 1968. Estimates of genetic variance in Iowa Long Ear Synthetic (*Zea mays* L.). Adv. Frontiers Plant Sci. 22:147–162.

Hallauer, A. R. 2002. Integration of germplasm improvement with corn breeding. p. 11–41. In: 38th Annu. Illinois Corn Breeders School. 4–5 March. University of Illinois, Urbana, IL.

Hallauer, A. R., and J. B. Miranda, Fo. 1988. Quantitative genetics in maize breeding. 2nd ed. Iowa State Univ. Press, Ames.

Lopez-Reynoso, J. J., and A. R. Hallauer. 1998. Twenty-seven cycles of divergent mass selection for ear length in maize. Crop Sci. 38:1099–1107.

Odhiambo, M. O., and W. A. Compton. 1987. Twenty cycles of divergent mass selection for seed size in corn. Crop Sci. 27:1113–1116.

Rinke, E. H. 1960. Reaching for yield. p. 61–67. In: Proc. 15th Annu. Hybrid Corn Ind. Res. Conf. Chicago, IL. Am. Seed Trade Assoc., Washington, DC.

Robinson, H. F., R. E. Comstock, and P. H. Harvey. 1951. Genotypic and phenotypic correlations in corn and their implications in selection. Agron. J. 43:282–287.

Russell, W. A., L. H. Penny, A. R. Hallauer, S. A. Eberhart, G. E. Scott, W. D. Guthrie, and F. F. Dicke. 1971. Registration of maize germplasm synthetics. Crop Sci. 11:140–141.

Salazar, M. A., and A. R. Hallauer. 1986. Divergent mass selection for ear length in maize. Brazil. J. Genet. 9:281–294.

Torregroza, M. 1973. Response of a highland maize synthetic to eleven cycles of divergent mass selection for ears per plant. p. 16. In Agronomy Abstracts. ASA, Madison, WI.

Troyer, A. F. 1999. Background of U.S. hybrid corn. Crop Sci. 39:601–626.

Troyer, A. F. 2001. Temperate corn—background, behavior, and breeding. p. 393–466. In: A. R. Hallauer (ed.), Specialty corns. 2nd ed. CRC Press, Boca Raton, FL.

Zuber, M. S. 1975. Corn germplasm base in the U.S.: Is it narrowing, widening or static? p. 277–286. In: Proc. 30th Annu. Corn and Sorghum Industry Res. Conf. Chicago, IL. Am. Seed Trade Assoc., Washington, DC.

Zuber, M. S., and L. L. Darrah. 1980. 1979 U.S. corn germplasm base. p. 234–249. In: Proc. 35th Annu. Corn and Sorghum Res. Conf., Chicago, IL. Am. Seed Trade Assoc., Washington, DC.

6

Inferences on the Genetics of Quantitative Traits from Long-term Selection in Laboratory and Domestic Animals

*William G. Hill**
Institute of Cell, Animal and Population Biology
School of Biological Sciences, University of Edinburgh
West Mains Road, Edinburgh, EH9 3JT, UK

*Lutz Bünger**
Animal Biology Division, Scottish Agricultural College
West Mains Road, Edinburgh, EH9 3JG, UK

*We are grateful to many colleagues for kindly providing unpublished information and for permission to reproduce published results, especially Ulla Renne and Paul Siegel for new data, to Peter Keightley and an anonymous referee for helpful comments on the manuscript, and to the Biotechnology and Biological Sciences Research Council for financial support.

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-46892-4

I. INTRODUCTION

A large number of selection experiments have been conducted on various species in the laboratory, and a lesser number on commercial species under field conditions, notably of course the Illinois corn experiment, the centenary of which we celebrate (Dudley and Lambert 2004). Whereas response to selection in early generations can be expressed solely in terms of initial variances and is, generally, robust to the underlying distribution of gene effects and frequencies, the influences on long-term response are more complex. Long-term selection experiments, here taken as experiments of over 30 generations of selection, should, in principle, provide information on the architecture of the trait in the species under selection, and perhaps beyond. Experiments in microorganisms, invertebrate animals, and plants are being reviewed by others, and the analysis here will be restricted to selection experiments conducted in laboratory mammals and birds and to breeding programs in commercial livestock. The latter will be focused predominately on poultry, for which there has been continued intense selection, and on horses and dogs for racing performance.

The aim is to review these breeding programs and experiments and consider what can be concluded about the genetic architecture of quantitative traits from them, focusing particularly on the variance maintained within populations. There are many open questions in quantitative genetics on what factors maintain the levels of variability that we see. For example, why do traits have quite consistent values of variance or coefficient of variation (CV) across populations? For example, body weight typically has a CV of about 10% in many species at different ages, in both selected and unselected lines, while litter size has a higher CV. Why does heritability take quite consistent values in different species and populations, even though we know it is a function of the actual gene frequencies? Thus heritability of growth traits is typically about one-

quarter to one-third in mammals and birds, even, as we shall see, in populations after long-term selection. The CV of a trait of reproduction, such as litter size, is typically much higher than of weight, but the heritability is lower. It is not surprising that the heritability of litter size is lower, because environmental (simply chance) variance for the trait may be high, and because natural selection erodes additive genetic variability. Nevertheless, it is not clear why, typically, the genetic CV (evolvability) is higher for litter size than for weight or growth. Mutation generates variability, but why is the value so consistently about 0.1% of the environmental variance for most traits and species studied?

To address some of these questions, we have to consider both the relevant theory and other experimental observations, including those on the base populations (or, often, equivalently on early generations of selection) from which the selection lines were derived, and those from QTL mapping. Even so, there are many lacunae in our understanding, and we answer only a fraction of the questions that we would wish to. Initially we review some of the relevant theoretical background.

II. FACTORS AFFECTING RESPONSE IN SELECTION EXPERIMENTS

In the first few generations, response is simply a function of the composite variances and is rather insensitive to the genetic architecture of the trait under selection. This no longer holds as selection continues, because gene frequency changes at individual loci have to be taken into account in order to predict the changes in variance. There has been extensive discussion in the literature of factors influencing maintenance of variability under selection and selection response (see, for example, Robertson 1960; Falconer and Mackay 1996; Bürger 2000; Hill 2000; Walsh 2004) and we merely summarize some of these in two categories: those that depend on the genetic architecture of the trait itself and those that the experimentalist determines and may modify to provide information about the trait from the responses to selection.

A. Influences Intrinsic to the Trait

1. The Distribution of the Effects of Individual Genes. Genes of large effect are expected to change most rapidly in frequency and thus in the variance they contribute. With few loci responsible for variability in the trait, plateaus would be anticipated earlier and at a lower level in relation to the initial rate of response, that is, a lower ‘half-life’ of response.

This applies whether in an infinitely large population, when in theory all favorable alleles are ultimately fixed, and in a finite population where favorable alleles may be lost by chance (Robertson 1960). If an exponential or similar shaped distribution of effects is expected, then the response will continue longer, but the pattern of response is some function of that distribution (Hill and Rasbash 1986a).

2. The Distribution of Gene Frequencies. If all alleles are at intermediate frequencies, it can be expected that variance will decline monotonically with time (assuming additivity), whereas if some are at low frequency, a rise in variance might be anticipated. If the distribution of frequencies is U shaped, then the increase in variance of those at low frequency might be expected to outweigh the decrease from those at high frequency. Analyses undertaken by Hill and Rasbash (1986a) for finite populations indicate that the pattern of response is somewhat robust to the gene frequency distribution.

3. The Degree of Non-additivity of Gene Action. For genes showing dominance, the response is also influenced by the inbreeding depression associated with the increase in homozygosity in a finite population. With dominant genes of small effect, inbreeding depression becomes a simple linear function of the inbreeding coefficient. More generally, the effects are confounded with those due to gene frequency change by selection. Whilst it is obvious that patterns of long-term response are influenced by epistatic interactions among loci, there is little theory on which to base predictions. Basically, there is an infinite range of epistatic models, and little evidence on which to base them. Relaxation of selection is, however, expected to produce some regression of response in the case of additive $\times$ additive interaction (Griffing 1960). With overdominance, variation can remain in the population, even though no response is being obtained.

4. Scale Effects. Although scale effects can be regarded as non-additivity, a logarithmic transformation turns multiplicative into additive effects, for example, so it may be more useful to view the transformation as a separate process if, for example, environmental effects are also multiplicative. Scale effects have to be considered both when comparing response to high and low selection and when analyzing long-term response: log transformed means may then give a different picture of the late vs. early response rates than do the untransformed means if, for example, the coefficient of variation rather than the standard deviation remains relatively constant.

5. The Relation of the Trait to Fitness. If individuals of extreme phenotype are less fit, then response may be attenuated. This can arise in two formally different ways. One is where fitness is solely a function of the mean: individuals may be less fit de facto, as is obviously the case in a selection experiment for low litter size, or because there is an intermediate optimum and thus stabilizing selection, for example at high and low birth weight in humans. The second is where genes that influence the trait and whose frequencies have been changed by selection have pleiotropic effects on fitness, with the relation between effect on the trait and on fitness varying among loci. In either case, a difference between intended and realized selection differentials might be seen unless all fitness differences are expressed solely within litters. Also, in these cases, relaxation of selection would be anticipated to lead to a reversal of response, and reverse selection to more rapid response downwards than was previously observed up. Distinguishing between the two models of effects on fitness is harder. One possible experiment is to compare crosses among high selected, among low selected, and between high and low (symmetric) selected lines, where stabilizing selection effects but not pleiotropic effects should be eliminated in the high $\times$ low cross.

6. The Direct Effect of Selection on Variability. With intense selection, individuals with genotypes that have higher intrinsic variability (if there are such) are more likely to be among the extreme, selected, animals. Selection therefore acts directly to increase the phenotypic variability (Hill 2002).

7. The Rate of Mutation and Distribution of Mutant Effects. The asymptotic rate of response, due to mutation, increases in proportion to the rate at which new variability is generated by mutation. Further, the greater the effects of individual mutants, the more likely are these to contribute significantly early on but also to give a more variable pattern of response.

8. Physiological Limits. There are real biological limits to response, for example percent oil in the Illinois corn selection lines cannot fall below 0%, but arguably there are others such as the tight regulation of egg production in poultry to the diurnal cycle. An example of another may be body size in vertebrates, where energy expenditure requirements may set lower limits. Those such as the last are more debatable, in the sense that processes are themselves under genetic control, and it may be argued that these so-called "physiological limits" are merely expressions of current genotypes. For example, in principle mammals could become smaller by eating more, or finding a warmer environment (as in the

laboratory), but ultimately gut volume or surface may indeed set limits; no mammals have mature weights under 1g (Bünger et al. 2001b).

B. Factors under the Control of the Experimentalist or Breeder

1. Choice of the Base Population. If, for example, the base is a two-way cross of highly inbred lines, then initial frequencies are close to 0.5 for all traits. If the base population is an outbred and selection is on a trait with presumably a negligible or weak linear relationship with fitness, then a symmetric distribution of initial frequencies can be assumed. This would, however, be a less reasonable assumption if selection is on a trait of reproduction, or the base population has already been under some artificial selection for the trait, such as a growth trait in a farmed livestock species.

2. Selection Intensity. The selection intensity affects the distribution of selective values of genes affecting the trait, and influences the relative strengths of directional and natural selection.

3. Population Size. The impact of population size depends on the other factors listed above, notably the distribution of gene effects and frequency. The basic theory was provided by Robertson (1960). With an infinitesimal model in a random mating selection line, the genetic variance V_{Gt} at generation t declines as $V_{Gt} = (1\text{-}F)V_{G0}$, where F is the inbreeding coefficient; and hence the cumulative response is given by $i(1\text{-}F)2N_eV_{G0}/\sqrt{V_{P0}}$, with a half-life at $1.4N_e$ generations, where i, N_e and V_{P0} are respectively the selection intensity, effective population size, and initial phenotypic variance. This formally requires some modification to take account of the change in phenotypic variance due to the Bulmer effect (Bulmer 1971) and finite population size (Wei et al. 1996). If gene effects are not all infinitesimally small, then the pattern of response changes, the half-life getting shorter. Similarly, the selection limit is also reduced, if selection is continued that far and mutations can be ignored. The population size also determines the number of mutations and the steady state variation maintained (Hill 1982).

4. Selection Criterion. Selection based fully or partly on family mean or relatives' performance may lead to a higher rate of initial response, but a lower long-term response and limit because effective population size is reduced (Robertson 1960). Selection within families, however, may

reduce short-term but increase long-term response since N_e is maximized.

5. Divergent Selection. Selection high and low from the same base population should give some information on the mean initial frequency of genes influencing the trait, and perhaps on the relation between the trait and fitness when these are not symmetric. A particular problem in interpretation of such experiments is that of scale (as indeed it can be in the interpretation of experiments conducted for only one generation).

6. Relaxed and Reversed Selection. Relaxation of selection or reversal of its direction potentially provides information on the relation between the trait and fitness, but does not directly distinguish between stabilizing and pleiotropic effects. Resumption of selection in the same direction as previously can be practiced, which if it occurred with increased speed might imply either that deleterious genes contributing to variance but not response had been eliminated, or perhaps that unfavorable linkages had been broken up. Such experiments may also reveal whether the distribution of effects of mutation genes on the trait is symmetric.

7. Inbreeding of Selected Lines. Inbreeding depression indicates the presence of dominance, as in an unselected population. In principle, inbreeding depression of populations at a selection plateau is indicative of overdominance, albeit even here confounded with the effects of rare deleterious recessives.

8. Environmental Change. To some extent this may be under the control of the breeder, or can be eliminated by use of an unselected control population. There may also be confounding of genotype × environment, such that the selected populations respond differently.

C. Factors Affecting the Variance of Selection Response

The above factors are largely concerned with the expected response to selection. The variance of response among replicates and between generations is also affected by the architecture of the trait and the influences on it. That applies to most of the factors listed above, but there are important other factors.

1. Distribution of Gene Effects. Under the infinitesimal model, even with selection, the variance among replicates is simply proportional to t/N_e initially, to $2FV_{G0}$ more generally, and thus continues to increase

with generations (ignoring mutations). If the distribution of gene effects is leptokurtic, then the variance may be larger initially, as the line mean is sensitive to the frequency of the genes of large effect, especially if they are at extreme frequency. (See Hill and Rasbash 1986a,b, for an analysis.) However, such genes have a higher probability of fixation, and hence, in theory the variance can drop as they move towards fixation.

2. Mutation. Similarly, the variance among replicates depends on mutation: basically the greater the variance and leptokurtosis of the distribution of mutation effects, the greater the variance among replicates expected.

3. Natural Selection. Natural selection would lead to a reduction in variance if based on stabilizing selection, since the limit is a function of the actual position of the mean. If, however, the effect of selection on fitness is due to pleiotropic effects, both of genes initially present and of de novo mutations, then the variance may continue to increase, dependent on the actual joint effects of the gene on the trait and on fitness.

4. Effective Population Size. The variability among replicates is inversely proportional to N_e under the infinitesimal model, and is likely to be approximately so in early generations more generally (Hill 1971).

5. Environmental Differences among Generations. Differences in the quality of the environment among generations cause variability in response. This can be eliminated by using an unselected control population unless there are genotype × environment interactions between the selected and the control lines or if the control population is maintained at small effective size.

III. LONG-TERM SELECTION IN COMMERCIAL LIVESTOCK

Our domesticated animals have long been under artificial selection, but with selection targeted on many traits, not least the ability to perform under domestication. The most obvious achievement for a single trait is the range of body size among breeds of dogs, which range some 100-fold in body weight; but many of their other characteristics, such as behavior, have also been greatly changed. Modern breeding programs and laboratory experiments have not produced such spectacular changes, but population sizes (at least in the laboratory animal studies) are likely to have been much smaller and time scales of directed selection in livestock and laboratory animals have been substantially shorter, at least in

terms of the total selection (generations × intensity) that could be accumulated. As we do not have appropriate historical data, however, our discussion will be restricted to genetic progress in current breeding programs and in laboratory experiments.

A. Poultry for Meat Production

Intensive genetic improvement in recent times has been carried out most intensively in poultry, particularly in broilers, in which growth rate can be recorded on juveniles of both sexes, and intense selection pressure can be applied. Genetic change has been spectacular, as Havenstein et al. (1994a,b) and McKay et al. (2000) have shown by contemporary comparisons between modern and old commercial populations, the latter maintained without selection. Havenstein et al. undertook comparisons of 1957 and 1991 broilers each reared on two diets, corresponding to those typical for those years. Juvenile body weight of the 1991 strain grown on the 1991 diet was at least three-fold higher than that of the 1957 strain when grown on the 1957 diets. Comparisons of each strain on the other diets showed that diet accounted for only 15% of the difference. McKay et al. (2000) estimated annual improvement rates currently of 60g increase in live weight to 42 days (2.4% of the mean/year), a 0.02 reduction in feed eaten/weight gain (feed conversion ratio, FCR) (1.2%/year) and a 0.25% increase in yield of breast meat in the carcase (1.4%/year). Their unselected lines were established in 1972 (equivalent to 1976 commercials). Since then live weight at 42 days has increased from 1050g to 2600g, age at reaching 2 kg fallen from 63 to 36 days, yield of breast meat at 2 kg increased from 250g to 340g, and feed required for 1 kg of breast meat fallen from 20 kg to under 10 kg. Havenstein et al. (1994a) observed substantially higher incidences of mortality to market age and leg abnormality in the (then) modern versus control populations they compared. There is indication from the more recent analysis of McKay et al. (2000), however, that the problems of leg abnormality, at least, have been very substantially reduced by selection on bone conformation.

Similarly, there is rapid progress in turkeys and in ducks for meat production (McKay et al. 2000). In turkeys, the male of 2000 grew to about 19.6 kg at 21 weeks, with FCR 2.63, a total meat yield of approximately 50% and breast meat yield of 28%. Annual rates of improvement in daily live weight gain are approximately 3% in male and 1% in female parent lines, that is, about 2% in commercial birds. Then current commercial ducks grew to 3.4 kg at 45 days, at an FCR of 2.2. Annual rates of improvement in ducks are approximately 1% in live weight gain, 2% in FCR, and 1.3% in breast meat yield.

Intensive selection for growth and related traits has been practiced for over 50 years in broiler chickens, with approximately annual generations. Information on effective population sizes is not available; but it is likely that the broiler breeders' nucleus populations had quite large effective size when selection was entirely on juvenile growth. As emphasis on traits such as feed conversion efficiency and leg conformation and so on has increased, however, more selection has presumably been based on family information and effective population sizes must have fallen through co-selection of relatives. Furthermore, the breeders have surely not been able to utilize any native populations in recent years because they are so far behind as to be non-competitive. It is possible that there has been some migration of stock among competitive breeders, but even so the overall relevant pool is still not enormous. Hence, most of the response has been within populations (either those of the individual breeder but perhaps also of competitors). The continued response indicates that variation has been retained within populations, but there is also direct evidence. Estimates of heritability for growth rate have typically remained at about 25% since estimates were first made many years ago. For example, Koerhuis and Thompson (1997) obtained estimates of 32% and 27% from current data on two intensely selected nucleus populations of a commercial breeder. Breeders have exploited variation in the various founder breeds and populations, and it is likely that they are now mainly utilizing genetic variation that has come from mutations subsequent to the start of intense selection for growth rate.

B. Poultry for Egg Production

There has long been concern that selection limits were being reached in poultry for egg production. This is based both on the light-cycle dependent limit of one egg produced per day and perceived lack of success of breeding programs (Dickerson 1955), but some of this pessimism seems ill founded. Evidence for substantial and continued genetic improvement was given by McMillan et al. (1990) for the period 1950–1980, and genetic variance is being maintained and genetic progress is seemingly still being made some half a century later.

Anderson and colleagues compared contemporary birds from control strains established in 1950, 1958, and 1972 with a 1993 commercial strain (Tharrington et al. 1999; Jones et al. 2001). Body weight progressively decreased and egg weight progressively increased, with percentage yolk in the egg declining but shell quality at least maintained. The modern strain had the highest egg production and, for example, egg mass and income over feed costs. Preisinger and Flock (2000) gave estimates of the heritability of percent egg production (egg number per day

in lay, %) in a modern population: phenotypic variation and heritability were both low at peak production when most birds lay daily, but earlier and later in the laying year, heritability values were typically about 20%. They also obtained estimates of genetic trends from phenotypic trends in random sample tests, which have been maintained in a consistent fashion, although inter-year changes in environment, such as disease exposure, cannot be ruled out. For a series of three-year averages, starting in 1980/82 and ending in 1995/97, the mean feed conversion ratio (kg feed/kg eggs) had improved, that is, reduced as follows: 2.46, 2.39, 2.35, 2.32, 2.25, 2.10 (Preisinger and Flock 2000). These results indicate that progress in improvement of these production traits has been steady. Further, egg production has increased not because birds lay more than an egg every day, but because they lay eggs daily or almost daily for a much longer part of the laying year; and other traits have also been improved.

C. Other Livestock for Food Production

There is extensive evidence for genetic change in other domesticated species, and no indications of selection limits of which we are aware. Long-term changes in pigs have recently been reviewed by Merks (2000), and these show substantial continuing genetic changes in growth rate and leanness. In dairy cattle, estimates of trend come from comparisons of predicted breeding values by best linear unbiased prediction (BLUP) or by comparisons utilizing control populations. The U.S. Holstein population is probably the best indicator of within-population change, as there has been much migration in recent decades from North America to European and other populations. Over the 30-year period 1967–97, mean breeding value for milk yield appears to have increased by about 6000 kg (McGuirk 2000). Indeed, the heritability of milk yield in dairy cattle populations appears to have risen over recent decades (from about 25% to 35%), despite the selection practiced; presumably part of the change is due to better methods of management, recording, and data analysis, however. In view of the lower intensities and often less consistent objectives over long periods than in poultry (for example, emphasis on reducing fat in pigs has altered over the years), these mammalian species do not provide much additional information about long-term change and will not be reviewed in further detail.

Whilst commercial breeding programs are aimed at improving or at least retaining performance in multiple traits, deterioration in other traits may occur, for example in fitness-associated traits such as fertility in dairy cattle or leg quality in meat animals. Rauw et al. (1998) reviewed these for a range of livestock species. It is, however, the case

that when selection effort has been put onto secondary traits, deterioration can be attenuated or reversed. Thus, growth plate quality in leg bones has been improved, enabling the amount of culling for leg defects in broilers to be reduced (McKay et al. 2000).

D. Selection for Racing Performance: Horses and Dogs

One notable exception to the general observation that breeding can change any trait is speed in Thoroughbred racehorses. There are no data on average performance of the population, but an indicator of change in mean performance comes from extreme values, expressed as the time of the winner in the classic races, in which the best horses are entered and are not handicapped. These times have increased little in the last half century, as shown by Gaffney and Cunningham (1988) for English classic races, and Fig. 6.1 gives a similar picture for the Kentucky Derby. For example, the winner's time in the 1950 Kentucky Derby was 2 min 0.1 sec, the same time as the winner's in 2000, and the record (1 min 59.04 sec) has been held by Secretariat since 1973. Speed for this race over 1¼ miles seems to have reached a plateau at just under 60 km/hr.

Nevertheless there is evidence of strong selection being practiced, for example as shown by the stud fees of classic winners and the attention paid to pedigree by breeders and punters. Further, analyses of pedigree

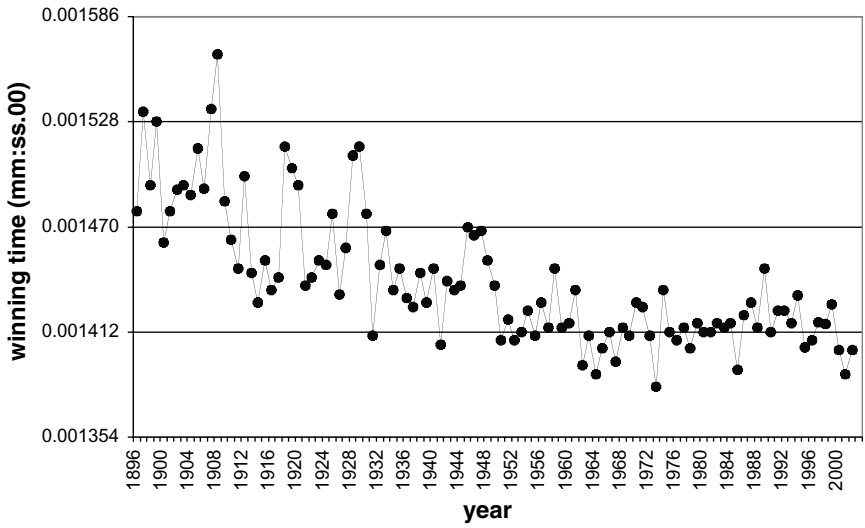

Fig. 6.1. Progress in Thoroughbred racehorses: winning times in the Kentucky Derby. (Data source: *http://www.drf.com/home/crown2001/kd/derby_stats.html*)

records give estimates of heritability well above zero for traits such as prize money won or ratings of comparative performance used for handicapping, for example 'TIMEFORM' in the UK, for which Gaffney and Cunningham (1988) estimated h^2 to be ca. 0.5. There has been some inconclusive debate on the explanations for the lack of phenotypic change. One argument is that genotypic values for speed are indeed increasing, as judged by BLUP analysis of TIMEFORM data (Gaffney and Cunningham 1988), but that times are not improving because the environment is changing, for example courses are being watered more. A further argument put forward is that a race is a competition among horses, mainly involving other behavioral traits in addition to speed; but these traits should have a genetic basis as well. The most plausible explanation of the data seems to be that indeed there is no real genetic change, that the heritability estimates are biased upwards by environmental correlations through special treatment of progeny of the best horses, and the BLUP estimates are consequently biased by overestimation of heritability (Hill 1988).

For Standardbred trotters in Sweden, Arnason (2001) found that speeds, estimated as the best racing time (sec/km in a race of at least 1640 m) for horses that had completed at least five races as 3–5 year olds, were still improving during the period 1976 to 1994 (Fig. 6.2 gives results for males; those for females are very similar but almost 1 sec/km slower). The distribution of these times is skewed upwards (coefficient ca. 0.4, i.e. with the long tail for slow speed). (As the CV is only about 2%, this could not be explained simply by its inverse, speed (km/sec), being normally distributed.) Further, the CV appears to be falling; this could represent a real trend or, perhaps, a change in culling rates of animals before completing five races. Thus, while mean times ($t \pm$ SD) for males were 80 ± 2.1 sec/km in 1976–9, they were 77.7 ± 1.6 sec/km (i.e., 46 km/h) in 1991–4. Arnason found that a log transformation, $\ln(t - 68.2)$, best normalized the data, and that this function was declining linearly, implying a plateau at 68.2 sec/km, approached in mid-century.

Data on winning times of Greyhound dogs also suggest that progress is currently slow or almost non-existent (Fig. 6.3). The English Derby was run over 480 m until 1974 (apart from a war break), then over 500 m till 1984 at White City (London), and again at 480 m, but at Wimbledon (near London), from 1985 onwards. Although there was clearly improvement in times from the mid-1920s until the 1950s, there is little evidence of much progress since then, when times have ranged between 28.5 and 29 sec (approximately 60km/hr) for the 480 m race.

In contrast, records are still being broken in races among humans. Although genetic selection between generations can be almost totally ruled out, there has been a wider screening of the population and environmental changes including nutrition, equipment, and training methods

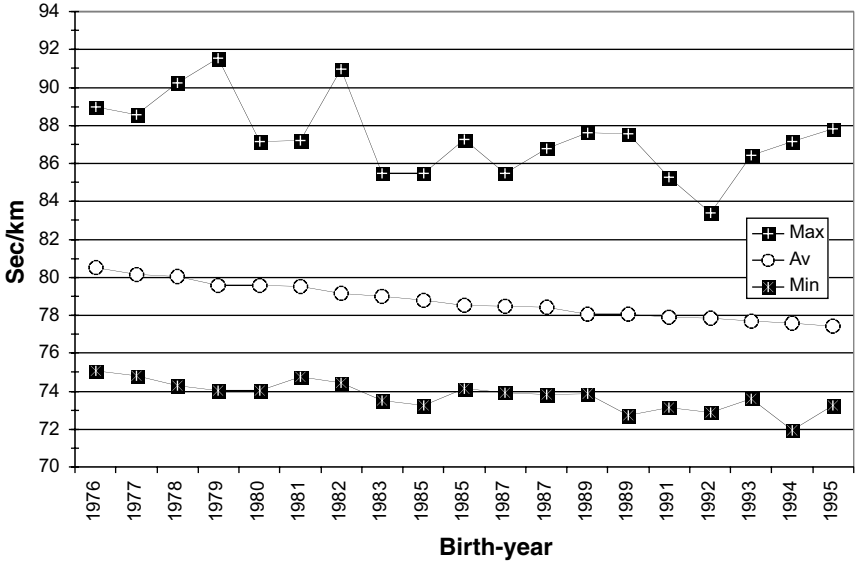

Fig. 6.2. Progress in trotting horses: racing times (average, minimum, and maximum) of Swedish Standardbred males. (Source: Arnason 2001)

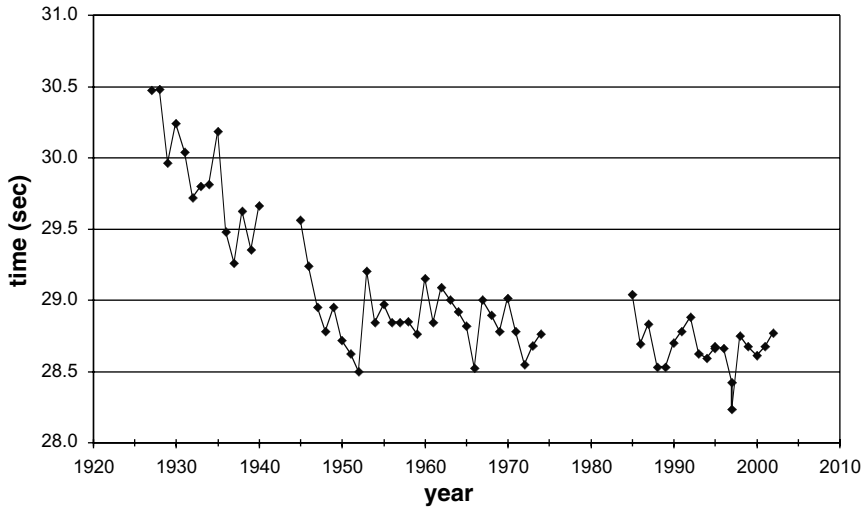

Fig. 6.3. Progress in Greyhound dogs: winning times for the English Greyhound Derby (480 m except 500 m 1975–84, run at White City to 1984, subsequently Wimbledon). (Data source: *http://www.greyhound-data.com*)

(and drugs?). Most of these factors might also be of importance in dogs and horses for racing. As an illustration, winning times for the men's 400 m at the Olympic Games are given in Fig. 6.4. There was a substantial improvement till about 1970, when the winning time fell below 44 sec (ca. 33 km/hr), and rather little change since. There is a similar tendency for the rate of improvement to slow in other sprints; but times for longer races, especially for women, continue to fall.

E. Comment

There is a striking difference between the progress being made in commercial populations of livestock, illustrated most fully for broiler chickens, and that of winning times in Thoroughbred horses and Greyhound dogs. The absence of phenotypic and, presumably, genetic progress in the winning times and therefore speeds is quite puzzling. Although there may have been strong natural selection for speed before domestication, to enable them to, respectively, escape and catch their prey, other animals run faster (cheetah, almost 100 km/hr). The genetic bases of the current Thoroughbred and Greyhound breeds may have been small, but for all traits analyzed in other species new variation from mutation contributes

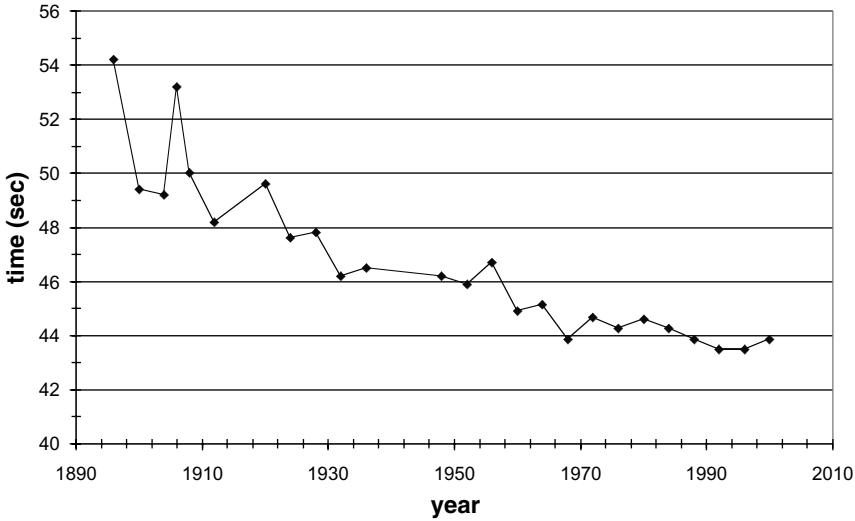

Fig. 6.4. Progress in middle distance track events: winning times in the Olympic Games for the men's 400 m. (Data source: *http://62.232.35.140/athletics-heroes/stats_athletics/olympics_trackandfield/400_m.htm*)

substantially to variation (Hill 1982; Falconer and Mackay 1996; Keightley 2004). Furthermore, there is evidence of genetic variation for speed-related performance in the horse populations (we do not know of relevant data on dogs). The results used are for extremes of performance (classic winners), and perhaps the population mean is changing, but not the extremes. There is some evidence of this for speed in the Standardbred horses (Arnason 2001) and for weight in mice (see later), but a very skewed distribution would be needed. Possible explanations include a strongly skewed distribution of mutation effects, most reducing speed, or very strong unfavorable correlations with other aspects of fitness; but neither is proven. We return to some of these issues later.

IV. LONG-TERM LABORATORY SELECTION EXPERIMENTS: POULTRY

A number of long-term selection experiments for traits of growth and reproduction that are closely associated with commercial performance have been conducted in the chicken, the turkey, and the Japanese quail, which has a shorter generation interval. Experiments of short duration or for other traits will not be considered; nor is the coverage comprehensive. We consider, in particular, the magnitude of the responses, the evidence for opposing natural selection, changes in phenotypic and genetic variability, and, where information is available, changes in the distribution of the selected trait.

A. Growth

1. Chickens. P. B. Siegel and colleagues at VPI&SU, Blacksburg, VA commenced selection with annual generations about 1960 from an inbred line cross base population. Selection was practiced high and low in both sexes on individual 8-week body weight (BW8wk). Numbers of sires and dams selected were 8 or 12 and 48 to generation 25, and 14 and 56 subsequently (Liu et al. 1994). Contemporary samples of an unselected control population were also reared in most generations. There has been substantial response in both high (HWS) and low (LWS) selected lines, such that birds from the high line birds are now about eight-fold heavier than those from the low at eight weeks (Fig. 6.5). Response per generation has declined during the experiment and is erratic, but there is no clear evidence that a plateau has been reached (Dunnington and Siegel 1996).

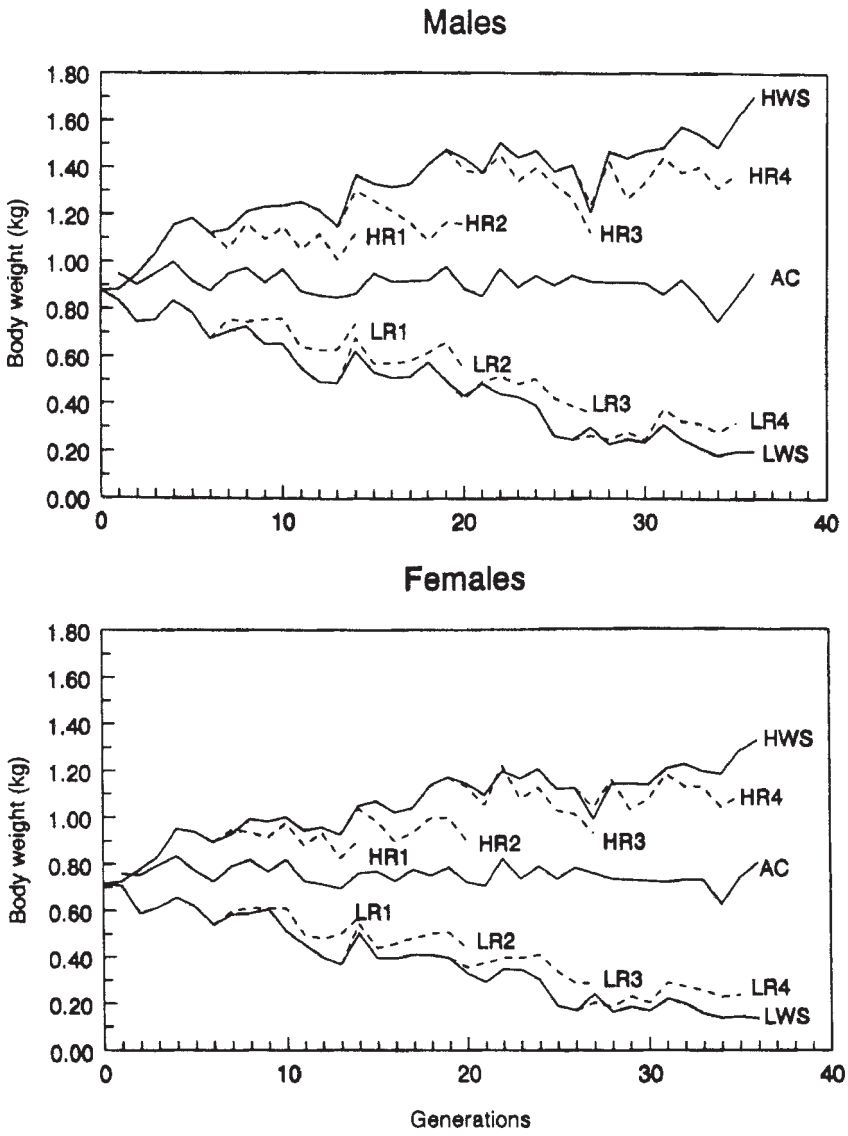

Fig. 6.5. Response to long-term selection for body weight in chickens: mean body weight at 8 weeks (BW8w) of chickens selected high (HWS) and low (LWS) for BW8wk, and of relaxed lines (HR1, HR2, . . .) recurrently drawn from them. (Source: Dunnington and Siegel 1996)

Relaxed selection lines were drawn from the selected lines at intervals of 6–7 generations and each maintained for 8 or so generations. Over each of these periods, the selected line diverged from the most recent relaxed line (Fig. 6.5). The pattern of absolute changes varied somewhat, presumably due to environmental or disease level changes; for example, the high relaxed line from generation 19 showed a substantial decline in mean, but that from generation 26 did not. Birds from the high line were fed a restricted diet after 8 weeks of age after generation 18 to avoid over fatness and reproductive problems, but realized selection differentials were as expected. In the low line, as some birds showed anorexia from generation 26 on (but responded to forced feeding), realized selection differentials became increasingly lower than expected (Liu et al. 1994).

Estimates of realized heritability of BW8wk for generations 0–18 and 19–36 appeared to fall in the high line (0.26 and 0.14, respectively), and, in contrast to the selection differentials, rise in the low (0.20 and 0.37) (although estimates were sensitive to the analysis used) (Liu et al. 1994). The total response is of the order of $5\sigma_P$ (phenotypic SD) in each direction; or, assuming $h^2 = 0.25$, some 10 σ_G (genetic SD). As σ_P changed little during the course of the experiment in the high line, its CV fell. In the low line, however, there was a decline in σ_P, but the CV rose markedly; and the increase in realized heritability indicated that σ_G changed less than σ_P. The 8-fold or greater difference in line means is associated with a less than 3-fold difference in σ_P (Table 6.1). P. B. Siegel (pers. commun.) commented that the distributions of weights in "the high lines are reasonably normal, while the low line has some very small individuals due to some being anorexic."

2. Turkeys. K. E. Nestor and colleagues at the experiment station in Wooster, Ohio used a cross of two commercial large-bodied lines in 1966 as the base for a randombred control population and a line (F) selected

Table 6.1. Means and standard deviations of 8-week body weight (BW8wk, g) in selected lines of broiler chickens (data kindly provided by Dr. Paul B. Siegel, pers. commun.)

	High line		Low line	
Generation	Male	Female	Male	Female
01	944 ± 120	776 ± 100	868 ± 112	705 ± 93
10	1238 ± 126	1000 ± 118	655 ± 91	511 ± 88
20	1439 ± 111	1144 ± 118	432 ± 63	330 ± 69
30	1472 ± 122	1135 ± 146	238 ± 62	169 ± 45
40	1559 ± 139	1264 ± 110	201 ± 54	138 ± 40

for high 16-week body weight (BW16wk). The selected line was maintained with 36 males each generation, each mated to one female to generation 21 and subsequently with two. After 30 generations of selection, substantial response was made (Fig. 6.6, showing deviations from the control) and BW16wk in the selected line was almost double that in the control (Nestor et al. 2000; although actual means not given). Over this period, the responses do not appear to depart far from linearity, and there is no impression of a plateau being achieved (Nestor et al. 1996, 2000). Realized heritability estimates over the successive 10-generation periods, 1–10, 11–20 and 21–30 were 0.31, 0.27 and 0.24, respectively (Nestor et al. 2000). There was no consistent difference between intended and realized selection differentials. There were, however, unfavorable changes in the selected line in egg production and hatchability. We have not seen information on σ_P and other properties of the distributions.

3. Japanese Quail. From a control population established in the 1960s from a wide genetic base, H. L. Marks and colleagues at Athens, Georgia have now continued selection for 100 generations. Selection for high and low 4-week body weight (BW4wk) was conducted on two nutritional environments: an adequate 28% CP diet (P line) and one with a low protein, 20% CP diet, supplemented by 0.2% thiouracil (T line),

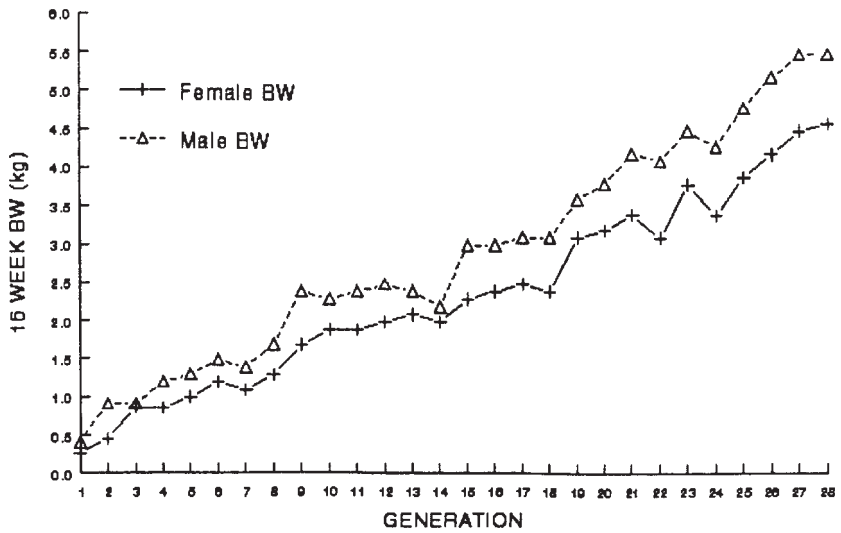

Fig. 6.6. Response to long-term selection for body weight in turkeys: mean body weight of turkeys at 16 weeks (BW16wk) of the line selected for BW16w expressed as a deviation from the unselected control. (Source: Nestor et al. 1996)

which depresses the function of the thyroid gland. The P line showed continued response, more rapidly over the first 40 or so generations than subsequently, and more rapidly from generation 81 than during 41–80 (Fig. 6.7). The T line responded at a similar rate to P initially, but then little from generations 21–60. At generation 90, the thiouracil in its diet was withdrawn, and there is some evidence of further response (Marks 1996). In the P line, the responses for generations 1–20, 21–40, 41–60, 61–80, 81–97 were 3.3g (ca. 4% of the initial BW4w), 1.9, 1.3, 1.3 and 1.8g per generation, respectively, and the corresponding realized heritabilities were 0.29, 0.17, 0.12, 0.07 and 0.11. In the T line the realized heritabilities were lower, indeed negative for generations 81–90. There have been unfavorable correlated changes in fitness traits consequent on the selection, particularly age at first egg, fertility, and hatchability.

Marks (1996) showed a distribution of body weights in the P line following 80 generations of selection. In the control line, the mean is 88g, the range was approximately 60–112g, and the distribution appears symmetric. In the high line, the mean was 251g, the range approximately 130–300g, and the distribution appears to be highly skewed downwards. This total response is of the order of 15–20 σ_P (assuming σ_P is 10g in the control), or 30–40 σ_G, ignoring any changes in scale.

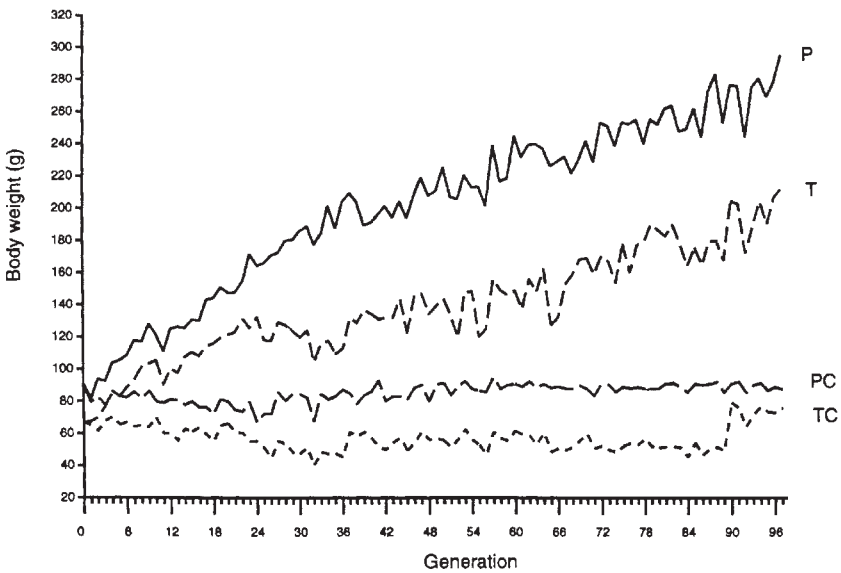

Fig. 6.7. Response to long-term selection for body weight in Japanese quail: mean body weight at 4 weeks (BW4wk) of two lines of quail selected for BW4w in different environments, and corresponding unselected controls. (Source: Marks 1996)

Marks and colleagues also conducted divergent selection on body weight at 4 weeks under complete and split diet environments, in which birds could self-select high or low energy feeds, but for "only" 30 or so generations. The high and low lines diverged approximately symmetrically and similarly on both diets through generation 30, by which time the high and low lines differed 4-fold in BW4wk. Response rates have slowed somewhat, and realized heritability declined during the course of the experiment (Anthony et al. 1996). In a 30-generation experiment, Nestor and colleagues obtained a very similar pattern of response (Anthony et al. 1996), such that Nestor's and Marks' lines appear almost as replicates of the same experiment. Egg production was reported for Nestor's experiment, and has declined relative to the control in both the high and low body weight selected lines.

B. Egg Production

1. Chickens. Several selection experiments for egg production traits in chickens have been conducted, starting in the last century, and the oldest have been reviewed by Lerner (1957). R. S. Gowe and colleagues at Ottawa, Canada conducted the most extensive long-term selection experiment for egg production and associated traits relevant to the efficiency of egg production. Results have been published for 30 generations of selection to 1980 (Gowe and Fairfull 1985; Fairfull and Gowe 1990). Two strains from different White Leghorn base populations were selected for part-year hen-housed production to 273 days of age (HHP273d). Some selection, however, was also practiced on full year record for the first few years, for egg weight, fertility, and hatchability almost from the beginning, and on egg quality and then low body size from mid-way through, increasingly mirroring commercial objectives. The selected lines were maintained with nearly 30 males and 300 females per generation on average. Over the 30-year period the response in HHP273d was substantial and similar in both lines, increasing from a mean of about 70 to 115 eggs, and there was no indication of a plateau having been reached (Fig. 6.8). Although this part record trait is close to neither commercial objectives nor overall fitness, total production over the laying year also rose, while mortality was reduced and egg weight increased. Estimates of heritability of HHP273d from the sire component of within generation analyses averaged 0.08 and 0.11 for the two lines in 1953–64 and 0.11 and 0.14 in 1971–80 (Gowe and Fairfull 1985). Although heritability appeared to change little in the selected lines, it was substantially lower than the average over 1953–80 of 0.28 in the control strain. (The values in the selected lines are, however, biased downwards by selection, i.e., the "Bulmer" effect).

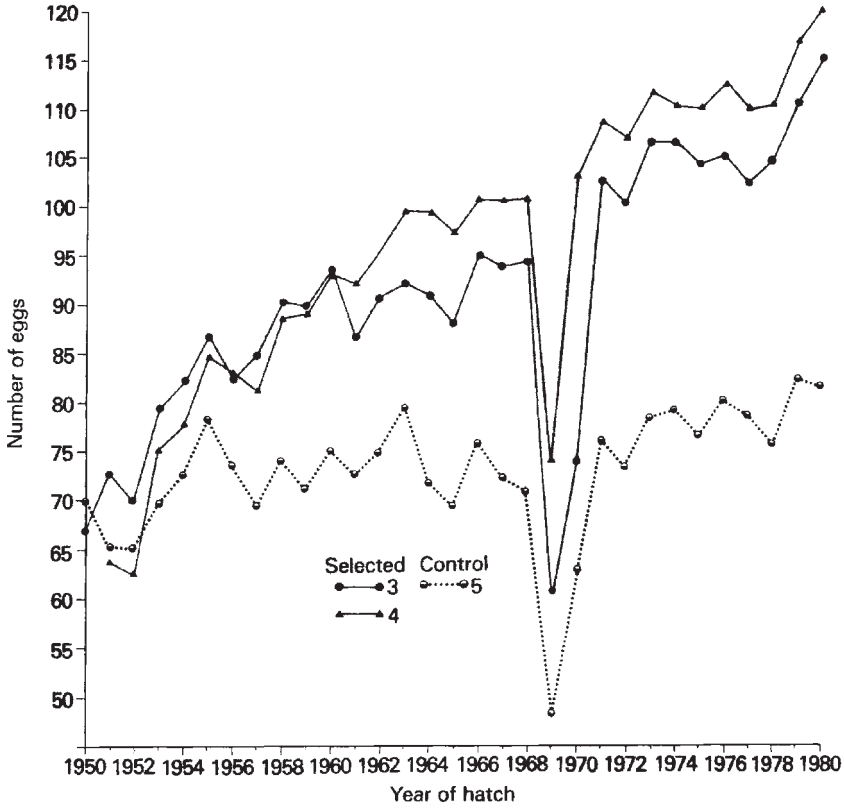

Fig. 6.8. Response to long-term selection for egg number in chickens: mean hen housed egg production to 273 days (HHP273d) of two lines of chicken selected mainly for HHP273d, together with a contemporary control (note: severe Marek's disease incidence ca. 1969). (Source: Gowe and Fairfull 1985)

2. Turkeys. Nestor and colleagues have also continued selection for egg production in turkeys for over 30 generations. Selection was started from the same base population as the growth lines referred to previously, but was based on number of eggs produced by individual dams in 84 days of lay (generations 1–3), 180 days (4–26), or 250 days (27–340) (Nestor et al. 1996). The selected line was maintained with 48 males and 96 females (generations 1–2), then 36 pairs (3–5), and 72 pairs subsequently. At generation 34, the inbreeding coefficient was 0.47 in the selection line and 0.15 in the unselected contemporary control population. Expressed as a deviation from the control, there was a fairly steady increase in 84-day production, the deviation being 30 eggs at generation

34. Production over longer periods also increased markedly, such that 250-day production was higher by some 130 eggs. There was no evidence of any plateau being reached, and the realized heritability for 250-day production was 0.26 in the last eight generations. There were substantial, but non-regular, reductions in body weight in the selection line.

C. Comment

The long-term selection experiments conducted in avian species all show very substantial and continued responses. Most have covered only 30 or so generations, but that of Marks with quail spanned 100 generations, and in no case was a plateau unequivocally achieved. Selection for juvenile body weight in three species of poultry give a fairly consistent pattern of responses: substantial change such that high and low lines diverge several fold in the selected trait, with the continued responses, despite much smaller population sizes, found in commercial birds. Heritabilities have generally fallen during the course of the experiment, to a level below that of the typical 0.25 for commercial broilers, but population sizes have been much smaller. Where data are given, in most cases phenotypic variances have been maintained, albeit with some evidence of non-normality of distributions. There have been some reported reductions in fitness, but not necessarily greatly impacting on response. Egg production has been shown to respond to selection, not withstanding the low heritability and diurnal dependency of the trait. The main conclusions we can draw are that many genes influence the trait and that associated fitness effects are not large; and that most genes have very small effect or that mutations are contributing substantially to the continuing response.

V. LONG-TERM SELECTION EXPERIMENTS ON GROWTH IN LABORATORY MICE

A. Responses and Limits

Roberts (1966a, 1966b, 1967) investigated limits to artificial selection for body weight in mice obtained in the very early selection experiments, using seven strains selected for growth (Table 6.2, rows 1–3). The clearest pattern of response was obtained in Falconer's N-lines (Falconer 1953), selected for increased body weight at 42 days (BW42d), with an initial mean of 21.5g and a continuous response in the high line to about

Table 6.2. Selection response or limits in some experiments on mice. (Source: Bünger et al., 2001b)

Base population	Trait selected	N_e	Trait	Gen.	Start (g)	End (g)	Resp. (g)	Resp. (%)	σ_P^z (g)	σ_A (g)	h^{2y}	HL[u] (gen)	References[x]
OB[w]	BW60+[v]	30	BW60 m	23	23.2	40.0	16.8	72	6.6	13	0.25		MacArthur, 1944,
(6 × IB[v])	BW60-/												1949; King, 1950;
	BW42-	20	BW42	38	19.0	10.0	8.0	53	4.2			4	Roberts, 1966a
OB	BW42+	15	BW42	52	21.6	28.0	6.4	30	3.4	7.1	0.35	8	Falconer, 1953,
(4 × IB)	BW42-	15	BW42	42	21.6	11.0	10.6	49	5.6	11.8	0.35	9	1955; Roberts, 1966a
OB	G 21-42+/												Falconer, 1953,
	BW42	17	BW42	53	24.5	35.0	10.5	43	4.6	8.1	0.33	7	1955, 1960;
	G 21-42-/												
	BW42	19	BW42	53	24.5	14.0	10.5	43	4.6	8.1	0.33	10	Roberts, 1966b
OB (ICR)	G21-42+	41	G21-42	24/27	13.6	24.7	11.1	82	4.7	8.2	0.35	12	Eisen, 1975
OB (4 × IB)	G21-42+	33	G21-42 m	34/43	13.8	26.6	12.8	93	7.4	16.5	0.20	15	Barria & Bradford, 1981

OB	BW60+	< 108	BW60 m	83	25	43.4	18.4	74	7	11	0.36	15	Goodale, 1938; Wilson et al., 1971
OB (Cpb:SE)	BW56+	43	BW56	43	25.6	51.6	26.0	102	10	14	0.53	16	Bakker, 1974
OB	P70/ BW70+	100/50	BW70 m	52	32.5	51	18.5	57	6	8	0.50	24	Sharp et al. 1984;
(2 IB × 1 OB)	P70/ BW70-	(approx.)	BW70 m	52	31.8	17	14.8	47	5	7	0.50		Bünger & Hill 1999
OB	BW42+	60	BW42 m	100	31.8	68.7	36.8	116	17	24	0.53	77	Bünger et al., 1983, 1998; Renne et al., 2002
(4 IB × 40B)													

[z]If σ_p was not given, CV%=10% was assumed and σ_p was calculated from the initial mean.

[y]Some h^2 values were published for within-family deviations, but assuming a full-sib intraclass correlation of 0.5, the within family and (overall) h^2 are the same.

[x]For details and full references (other than Renne et al. 2003) see Bünger et al., 2001b).

[w]OB: outbred, [v]IB: inbred; S: selection line.

[v]e.g. BW60+: individual increased body weight at 60 days; m: male; f: female; G21-42: gain between 21 and 42 days of age.

[u]HL: half-life of response.

generation 30, with no further response afterwards and an assumed limit at 28g. The low line also displayed a non-linear development with little response from generation 25 to 42, when it reached about 11g. The pattern of response to increased weight over 31 generations in another set (the C-lines, with an initial mean for BW42d of 24g) (Falconer 1960), was, apart from a drastic and unexplained decrease in one line, fairly constant. A second high line, CRL, showed an approximately asymptotic response pattern over the course of the experiment and apparently approached a limit at about 32g. The selection response in the low line (CFS) was almost linear up to generation 17, when the response seemed to cease at about 14g. Both lines probably experienced an improved environment after generation 22 as weights increased by 2–3g in both lines. Generalizing these findings, Roberts (1966a) concluded that: selection limits do not necessarily stay stable over prolonged time periods; high and low limits for body weight at 42 days were in the region of about 30 and 12g, respectively; the time to reach this limit varied from 10 to 30 generations; the response was $2 - 6\ \sigma_P$ or $3 - 12\ \sigma_G$; and up to 20 loci were estimated to have contributed to the selection response, each with an effect of about $0.5–1\ \sigma_P$.

The genetic nature of these limits was subsequently explored using continued, backward, and relaxed selection in lines CRL and CFS (Roberts, 1966b). The large line, after an apparent limit lasting 20 generations, showed a sharp increase in body weight, for which a rare recombinational event was suggested to be the most likely explanation. Whereas exhaustion of additive genetic variance seemed to be the reason for the limit in the high line, this variance appeared to remain in the low line and natural selection seemed to oppose the fixation of some alleles. In a further experiment, Roberts (1967) intercrossed four high lines and three low lines, and found that crossing produced new genetic variance in the former, such that body weight at 42 days increased to about 42g, but there was little selection response in the low line. Roberts (1966a) suggested that the limits found in these early experiments "set the standards for further experimental attacks on the limits."

In later experiments, these apparent limits were greatly exceeded, as shown in a recent in-depth review of a number of long-term selection experiments for body weight (Bünger et al. 2001b). This included a detailed analysis of four, those undertaken by Goodale and by Bakker, our experiment at Edinburgh, and that by our colleagues at Dummerstorf, Germany, the last of which has been continued for over 100 generations. Some information on experimental design and results are given in Table 6.2, with limits to response based partially on data fitting. The review focused mainly on the absolute range of body weights that can be

obtained by selection, on estimates of numbers of genes involved, and on changes in variance and CV. Here we mainly consider the shape of the response curve, changes in the variability, and distribution of the trait under selection.

The classic experiment for increased BW60d by Goodale (1938) commenced in 1930 and was continued for 84 generations (Wilson et al. 1971). There was rapid and fairly linear response for about 30 generations, but little response after 40 generations, the plateau being at a little under twice the initial weight. Bakker (1974) selected on high and low BW56d. The low line had become less than half the initial weight when this selection line had to be terminated at generation 27 due to low fertility, following a period of apparently no response over the last almost 10 generations. In the high line, where selection was continued for 44 generations, animals weighed almost double the initial value. Whilst rates of response in that line had also slowed down, there was no indication that a plateau had been reached.

Our lines at Edinburgh were continuously selected for high (EDH) and low (EDL) BW70d in males (initially on an index of lean mass, which is highly correlated with body weight) for 53 generations. There was a close to linear response, but some irregularities and indication of slowing down, especially in the high line (Fig. 6.9). Following 5 generations of relaxed selection (for management reasons), selection was continued for another 7 generations. There was some indication of further response in the high line but none in the low line, which seemed to show no response from generation 40 to 65 (Bünger and Hill, 1999a).

The longest continuous selection experiment for body weight in mice, that conducted at Dummerstorf, now exceeding 100 generations, has produced an over two-fold change in BW42d (Renne et al., 2002). The animals are heavier than those from any other selected line (Fig. 6.10). Rates of response gradually reduced during the course of the experiment, and at various times plateaus appeared to be approached, but response has continued subsequently and an over 10g response has been made between generations 70 and 100 (as much response as was made in Falconer's early experiments started from an unselected base, albeit confounded with scale change).

Considering all the experiments (Table 6.2), the selection responses reported by Bünger et al. (2001b) (plus the update on the Dummerstorf experiment) represent changes of 5–17 σ_P. This is equivalent to about 24 σ_G, based on the estimates of realized heritability for body weight at 42 to 70 days in the range 0.36 to 0.53 (σ_G estimated in the base populations or from realized heritabilities in the first period of the experiments). Upward selection was shown to increase the mean up to 2.4-fold and

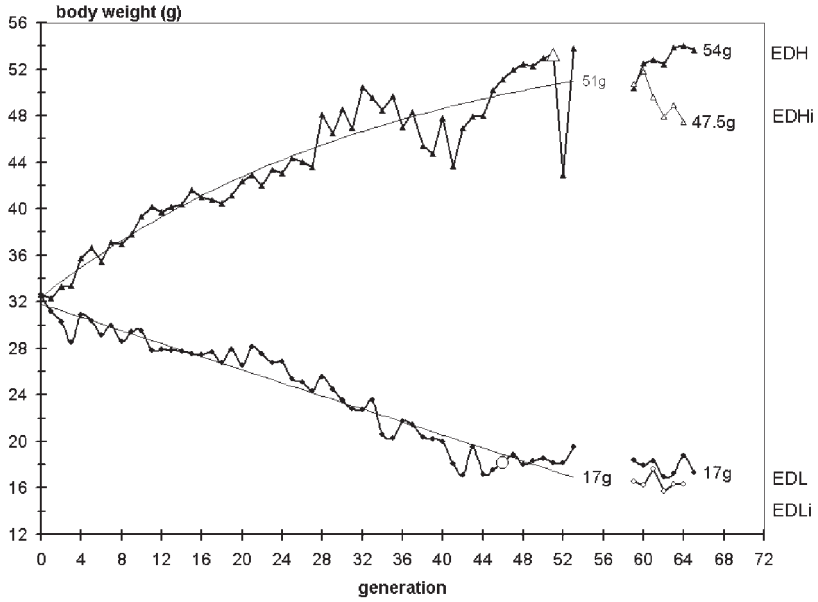

Fig. 6.9. Response to long-term divergent selection in Edinburgh for 70-day body weight in mice: mean 70 day body weight in males of the Edinburgh P mouse lines, EDH and EDL, and of inbred lines derived from them (open symbols).

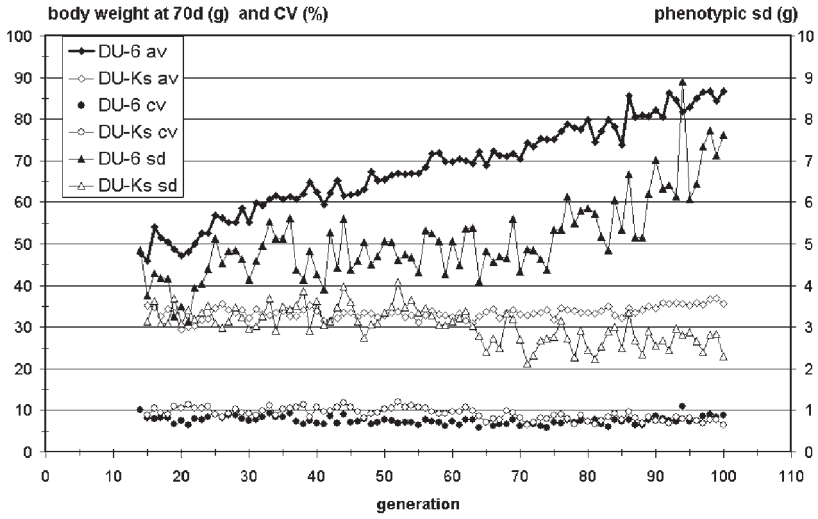

Fig. 6.10. Mean and variability of response to long-term selection in Dummerstorf for 42-day body weight in mice: means, phenotypic standard deviations and coefficients of variation for male 70-day body weight of the Dummerstorf high selected (DU-6) and unselected control line (DU-Ks). (Data for the latest generations were kindly provided by Dr. Ulla Renne, Research Institute for the Biology of Farm Animals, Dummerstorf, Germany.)

downward selection to reduce it to about 50%, such that the heaviest and smallest mouse lines developed by selection seem to have a body weight at 42 days of about 70g and 10g, respectively, approximately a 7-fold divergence. This divergence could be further enhanced by the introgression of the growth hormone deficient *lit* gene, which reduces the body weight independently of the genetic background by approximately 50% on average (Bünger and Hill, 1999b).

The higher long-term responses observed in the more recent experiments, and particularly that at Dummerstorf, are associated with larger population sizes (see Table 6.2). A direct experiment in which responses to selection for post-weaning gain were compared at different population sizes, albeit on rather shorter time scales than we have considered here, was conducted in mice by Kownacki and Zuk (1986), revealing a positive, but non-linear, association. Eisen (1980) reviewed earlier experiments and concluded that indeed larger population sizes led to greater long-term response and limits. This outcome is expected, both as a higher probability of fixation of increasing alleles in the base population (Robertson 1960) and from a higher rate of mutation (Hill 1982).

B. Variability and Distributions

Let us consider further the changes in variability as a consequence of selection, described in the review by Bünger et al. (2001b). What is striking about most of the experiments is that while (phenotypic) standard deviation (σ_P) has generally changed substantially, the (phenotypic) CV has not. In Goodale's experiment, the CV of BW60d increased only marginally, from a little under to a little over 10%, during which time σ_P approximately doubled. Similarly, in both the Dummerstorf (high) selected line (see spread of data in Figs. 6.10 and 6.11, Table 6.3, and Bünger et al. 2001b), and in the Edinburgh lines where selection was in both directions, the CV has remained almost constant. In contrast, in Bakker's experiment, the CV remained almost constant at approximately 10% in the high line, but increased to about 14% in the low line in which σ_P fell a little.

Previously in this review, we discussed winning times for dogs and horses and highlighted that these are based on top performers, that is, extremes rather than population means, but the question arises as to how representative are the maxima of the means. To examine this, maximum, minimum, and mean values for the selection trait of BW42d in males in the growth selection at Dummerstorf (Bünger et al. 1983, 1998) are shown in Fig. 6.11. Animals from the selection line were on average 2.2-fold heavier than their controls and the curve fitted to the maximum values in the selection line appears closer to the generation means than that

Table 6.3. Statistics of male body weights[z] (g) at 70d in the Dummerstorf control (DU-Ks) and selection line (DU-6) and the Edinburgh low (EDL) and high (EDH) growth lines, all maintained as outbreds.[y]

	DU-Ks	DU-6	EDL	EDH
N[x]	880	865	387	559
Mean (g)	35.7	84.3	17.7	52.6
SD (g)	2.66	7.03	1.62	4.90
Min (g)	26.3	57.8	13.5	37.2
Max (g)	44.0	102.1	22.4	65.7
Kurtosis	0.12	0.55	0.01	–0.07
Skewness	0.09	–0.40	0.11	0.00
CV%	7.5	8.3	9.2	9.3

[z]Body weights were corrected for generation effects.

[y]For line details see Bünger et al. (1998) and Bünger and Hill (1999a).

[x]Generations 90 to 100 were pooled for the Dummerstorf data and generations 59 to 65 for the Edinburgh data.

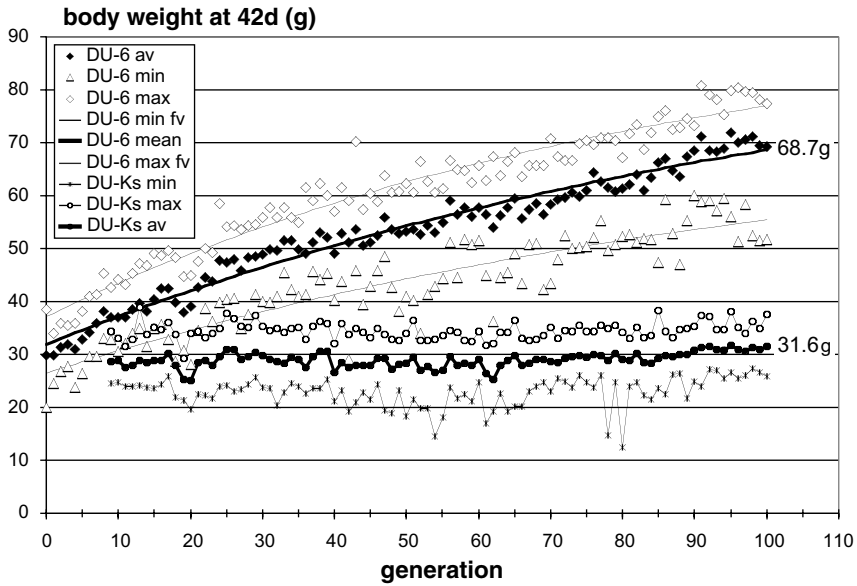

Fig. 6.11. Influence of long-term selection in Dummerstorf for 42-day body weight in mice on its distribution: means and weight at 42 days of the heaviest and lightest mouse each generation in males of the Dummerstorf high selected (DU-6) and unselected control line (DU-Ks) (Data for the latest generations were kindly provided by Dr. Ulla Renne.)

fitted to minimum values, indicating that the distribution has changed. Summary parameters for the last 10 generations of the Dummerstorf line (designated DU-6 in its home lab and DUH in the Edinburgh lab), are given in Table 6.3, showing that body weights in the control line are symmetrically distributed (and close to normal), whereas there is a downward skew (coefficient = –0.4) in the high weight selected line. In contrast, body weights for the last 10 generations of the Edinburgh selection experiment, when the EDH and EDL (former P-lines) were kept as outbreds, do not show such skew, and approximate a normal distribution in both high and low lines (Table 6.3).

To investigate the level and distributions of variability further, we consider other growth lines, selected for high or low growth in their home laboratory for at least 20 generations, and brought to Edinburgh a few years ago. (For more detail, see Bünger et al., 2001a.) From generation 8 onwards, we subsequently derived inbred lines from each by brother-sister mating. Statistics are given in Table 6.4 for three distinct periods: prior to inbreeding when some mass selection was being practiced (generations 4–7); during inbreeding, when homozygosity was becoming high, but sublines were still present in some cases (generations 11–14); and after at least 8 generations of inbreeding, by which time only one subline was being maintained (generations 15–20). Contemporary results for the non-inbred Edinburgh control and the outbred Dummerstorf selected lines (kept in the Edinburgh lab) are included, together with those on the Edinburgh inbreds (EDHi and EDLi) that had already undergone substantial full-sib inbreeding.

Although the differences among the lines in body weight are enormous, ranging from 16g (EDLi) to 90g (DUHo), the CV is never far from 10%. Disregarding the second period, when inter-subline variation may be present, the CV in the low lines varies from 6 to 11%, averaging about 9%, and is only a little greater in the high lines, from 6% to 15%, averaging about 10.5%. The high values in the BEH line could be due to the *compact* mutation (Varga et al., 1997), which was fixed in that line but could interact with segregating modifier genes to produce higher variability. Also, selection for high growth in some mouse lines is accompanied by a substantial increase in fatness, which itself increases the CV for body weight. Thus the CVs of BW98d of males from our fat (F, 21.7% body fat) and lean line (L, 4.2% fat) (Bünger & Hill, 1999a) in generations 45–52 were 14.5% and 10.2%, respectively.

Comparison of the first and the last periods shows that there has been no substantial trend for the CV in response to the inbreeding, but the values for the outbred Edinburgh lines (Table 6.4) seem a little higher than those of the inbred in all three periods, and lower than their outbred

Table 6.4. Statistics of male body weight at 70 days of growth lines before and after inbreeding[z] and of a control line (Edinburgh data).[y]

	EDCo	EDLi	EDHi	BEL(i)	BEH(i)	MUL(i)	MUH(i)	ROL(i)	ROH(i)	DAH(i)	RAH(i)	DUH(i)	DUHo
Generation	4–7												
N	167	108	232	168	145	143	111	160	257	113	175	246	246
Mean (g)	31.7	16.3	46.3	22.6	53.7	21.2	43.5	19.9	42.2	65.9	58.9	76.6	76.6
SD (g)	3.52	1.00	3.74	2.14	7.97	2.23	4.35	2.04	4.74	7.12	5.90	8.43	8.43
Kurtosis	0.83	–0.15	–0.30	1.88	–1.09	–0.05	–0.57	0.59	–0.43	–0.21	–0.09	–0.58	–0.58
Skew	0.56	0.03	0.19	1.01	0.09	–0.07	–0.05	–0.28	0.21	–0.15	0.15	0.17	0.17
CV%	11.1	6.1	8.1	9.5	14.8	10.5	10.0	10.2	11.2	10.8	10.0	11.0	11.0
Generation	11–14												
N	333	100	328	128	156	85	173	86	185	202	142	308	199
Mean (g)	33.4	16.0	43.2	19.8	53.3	18.7	44.0	19.0	42.8	59.7	62.8	70.9	89.9
SD (g)	3.81	1.20	4.23	1.93	8.16	1.78	3.50	2.08	6.45	8.08	5.83	12.28	8.60
Kurtosis	0.00	–0.37	–0.64	–0.21	–0.31	0.09	–0.04	3.36	0.03	–0.42	–0.47	–0.66	–0.13
Skew	–0.05	0.04	–0.15	0.16	–0.01	–0.36	–0.06	0.07	0.50	0.09	–0.16	0.04	–0.14
CV%	11.4	7.5	9.8	9.7	15.3	9.5	8.0	11.0	15.1	13.5	9.3	17.3	9.6
Generation	15–20												
N	427	139	199	180	67	150	148	158	287	154	260	189	340
Mean (g)	34.3	17.6	47.6	18.5	58.6	18.9	46.3	19.4	49.5	64.7	65.5	81.6	89.4
SD (g)	3.82	1.43	2.98	1.97	4.25	1.53	4.63	1.82	5.71	6.65	7.72	7.62	8.99
Kurtosis	1.21	–0.34	0.19	0.15	0.91	–0.12	1.84	0.02	0.22	–0.36	1.02	0.33	0.79
Skew	0.64	–0.36	–0.19	–0.11	–0.73	–0.37	–0.83	0.27	0.53	–0.07	–0.54	–0.10	–0.15
CV%	11.2	8.1	6.3	10.6	7.3	8.1	10.0	9.4	11.5	10.3	11.8	9.3	10.1

[z]o: outbred population throughout, i: inbred population throughout, (i): Initially outbred population, becoming increasingly inbred.

[y]The lines are described by Bünger et al. (2001a). **EDC**=Edinburgh control line (contemporaneous generations used, 62–65, 66–72, 73–78; **EDL**, **EDH**=Edinburgh low and high growth selection line (P-lines), contemporaneous generations of inbreeding used 15–18, 22–25 and 26–31 for **EDL**, 4 generations less in **EDH** (Bünger & Hill, 1999); **BEL**, **BEH**=Berlin low and high growth lines with a fixed myostatin mutation in the BEH; **MUL**, **MUH**=Munich low and high growth line; **ROL**, **ROH**=Roslin low and high growth line; **DAH**=Davis high growth line; **RAH**=Raleigh high growth line; **DUH**=Dummerstorf high growth line kept as an outbred and as an inbred line. Lines denoted (i) were immigrated by embryo transfer and then kept as outbred populations with moderate selection on BW70 during generations 4–7; in generation 8 inbreeding by full-sib matings was started in a few families in parallel (generations 11–14); and in the last period (generations 15–20 = inbred for 8 to 13 gen) only one inbred family was maintained.

control line from the same base population (Table 6.3). Similarly, the CV for DUHi is only slightly smaller than that for DUHo in period 3, although the mean and the variance are reduced (Tables 6.3 and 6.4). It is clear that the inbred lines generally show little, if any, reduction in variability below the random mating lines from which they were drawn, albeit these were partially inbred after many generations of restricted population size. Further, CVs in the selected lines do not differ greatly from their initial, founder, populations.

The fact that phenotypic variabilities, expressed free of scale as coefficients of variation, have remained rather constant during selection indicates that there must have been increases in environmental variance to compensate for the reductions in genetic variance due to the selection and finite population size. Indeed, reductions in heritability have been noted in several of the experiments described. Furthermore, note the very small changes in CV that accompanied inbreeding in the lines of mice held at Edinburgh (compare generations 4–7 and 15–20 for the lines denoted (i) in Table 6.4, which were made highly inbred during this period). This also strongly indicates some replacement of genetic by environmental variance, as the lines were not previously fully inbred, and those still under selection when inbreeding started were certainly not all at an asymptote (e.g., the Du-6 line, Fig. 6.10).

No general picture for the skew emerges. (The ROH line should probably be dismissed, as a strong upward trend for the BW is suggestive of either a mutation or some contamination, which is being investigated). The negative skew in the Dummerstorf line DU-6 (Table 6.4) is less pronounced in the DUHo (Table 6.3), which is essentially the same line but kept in the Edinburgh lab. The average skew (excluding ROH) in periods 1 and 3 is 0.08 and –0.37, respectively, for the high lines and is 0.17 and –0.14 for the low lines. When the pairs of lines selected divergently from the base population are considered, there is no support for the hypothesis that selection for high growth is accompanied by negative skew, or vice versa. Thus, overall, the evidence that long-term selection induces skew in body weight is weak.

The constancy of coefficient of variation with change of mean that is consistently observed is indicative of multiplicative effects of genes and of environmental deviations. A log normal form of distribution, in which there is positive skew on the untransformed data, but no skew after log transformation, would therefore be expected. Yet we do not generally find this, thereby presenting a puzzle: how do we rationalize constancy of CV over a wide range of performance with normality of the distribution?

To check whether log normality ever applied in such traits in the mouse, we analyzed data on an indicator of fatness, gonadal fat pad

weight/body weight in males (GFPW/BW), which was used as a selection criterion in another set of lines selected from the Edinburgh base. In an analysis of selection response (Martinez et al. 2000) it had been found that, after log transformation, there was an excellent fit to the infinitesimal model over the first 20 generations of selection. These data, as expected, show positive skew in the high, low, and unselected lines, with the skew removed by log transformation (V. Martinez, pers. commun.).

C. Selection from an Inbred Base

In the experiments referred to previously, selection has been initiated from a segregating population, for example an outbred or a cross of inbred lines. Response in early generations is therefore, presumably, utilizing the initial genetic variability; in later generations, de novo variation from mutation may be contributing, and it is not easy to disentangle their relative contributions. Although attempts (S. Mbaga, pers. commun.) have been made to do so using REML technology for some of our Edinburgh mouse selection lines, insufficiently useful discrimination between original and de novo contributions to variance has been obtained.

Experiments have been undertaken in which selection has been initiated from a highly inbred or otherwise isogenic population, such that only mutation can contribute to the response (reviewed by Keightley 2004). Although several of these have been carried out in Drosophila, only one, conducted in Edinburgh, is known in the mouse (Keightley 1998). Selection was practiced for 50 generations for high and low 6-week body weight from a highly inbred base, using 12 mating pairs per generation. Response was initially very slow (as expected, until mutations arose) and erratic, and the high and low lines reached about 18g and 24g, compared to a control mean of 20g. This divergence of 6g or 30% of the mean is equivalent to approximately 4 σ_P (i.e., 4 σ_E). This experiment is in a small population compared to many selection lines started from an outbred base, and mutational contributions are expected to be highly dependent on, perhaps proportional to, population size (Hill 1982). Mutations are therefore likely to have contributed to the response observed in the long-term selected lines started from a heterozygous base population, many of which have been conducted in much larger populations.

D. Comment

Some long-term selection experiments for body weight in the mouse have shown a clear and long-lasting plateau (e.g., Goodale's experiment); but in others there were phases of apparent plateau, with no

response over several (10 to 30) generations. In general, reaching a certain plateau takes obviously much longer than had first been expected (e.g., Roberts, 1966a), where values of 11 to 28 generations are given to reach the limit (Table 6.2). In more recent experiments, mostly in populations of larger effective size, longer half-lives and higher total selection responses (up to 17 times the original σ_P) have been obtained. Ignoring some mutations (such as *lit*), the low and the high "ends" for body weight at 42 days, for example, seem to be close to 10 and 70g, respectively. We know from changes over evolutionary time that these limits, probably due mainly to the higher population size used in the more recent experiments, are only temporary; and it requires just time and numbers for new mutations to occur and such limits to be exceeded. Given such a 7-fold divergence (comparing data from different selection experiments) or the 5.6-fold divergence found in the same environment (Table 6.4), it seems very impressive that the variance (as expressed by CV) changes very little.

VI. INFERENCES

There is a large difference between the results for rates of progress in commercial livestock for food production and those for animals in the track events. There seems to be little evidence of attenuation of response in the broiler chickens or indeed in egg layers, whereas Thoroughbred horses and perhaps also Greyhounds are showing little or no evidence of phenotypic, let alone genetic, improvement (at least as assessed by winning times). In contrast to laboratory selection experiments where the environment has been kept fairly constant during the many generations of selection, there have been changes in diet, housing, and other practices in the commercial species. Whilst such changes may have affected phenotypic change, in some cases (broiler chickens) unselected controls have been used and experiments conducted to distinguish genetic and environmental trends.

Furthermore, results of selection in the laboratory for traits of poultry, either growth or production, do not appear to show attenuation of response, albeit generally a slowing down as would be expected in populations of finite size. Selection lines for growth in mice, all carried out in populations with effective size less than 100, have shown plateaus in some but not all experiments; but total changes have been great, up to 17 σ_P.

Even so, the magnitude of changes observed in the selection lines or selection programs is small by evolutionary standards. Within species,

there is an almost 100-fold range of body size in dogs; and across species there is a range of body weights of over 7 orders of magnitude in extant mammals. Even the largest strains of mice are less than a quarter of the weight of rats, despite 100 generations of continuous selection in the most successful experiment from Dummerstorf (see Bünger et al. 2001b for further discussion and analysis). These large changes reflect, presumably, the efficacy of ultra-long-term selection over evolutionary time in large populations with utilization of very many mutations, of which some probably had outstanding effects.

A general feature of the selection experiments is that phenotypic variability has been maintained. Indeed, a characteristic of those for growth in the mouse is that the coefficient of variation has remained very close to initial values of approximately 10%, despite the large changes in phenotypic mean (6-fold between growth lines in the same lab). Inbred lines derived from the selected lines also show similar levels of variability, in terms of CV. In the commercial broiler lines, at least, there is no evidence of substantial change in heritability, either. The constancy of phenotypic variance or CV (allowing for scale) when some or much of the genetic variance has been removed suggests both that there has been an increase in environmental variability, perhaps due to increased environmental sensitivity (increased "plasticity"), and that there is some underlying but unknown mechanism regulating the amount of phenotypic variability.

There is some, but not consistent, evidence of change in the shape of the distribution, with evidence of negative skew in high selected lines. The constancy of CV in the body weight of selected mouse lines is, however, indicative of multiplicative effects of genes and environmental effects. Yet in the Edinburgh selected lines and in our other immigrated growth lines, there is no evidence of departure from symmetry, in contrast to the positive skew expected of the log normal distribution. The limited data we have access to indicate substantial negative skew for body weight in a line of high weight selected quail, but not in a high weight selected line of broilers. We do not have information on the Thoroughbred horses (and have not tried to seek it, since commonly analyzed traits such as winnings have highly non-normal distributions, and there is no a priori reason to expect otherwise). The data on the Standardbred trotters show skew down in the direction of selection (i.e., finishing times skewed upwards).

In view of the evidence of substantial mutation rates to quantitative traits (Keightley 2004), including that on the mouse, the problem is not to explain why response continues but why it stops. One explanation might be that increasing (in the direction of selection) mutations become

an increasingly small proportion of the total, such that selection is required just to eliminate unfavorable mutations rather than facilitate progress of the population—a version of Lewis Carroll's Red Queen. The fact that some populations regress on relaxation of selection does not imply that this hypothesis is correct, for there are many other explanations. Among these is, for example, the impact of natural selection, whether acting on the mean as such as in a stabilizing selection model, or on genes that increase the trait but have a deleterious effect on fitness.

An observation that is frequently remarked on in descriptions of selection experiments is that plateaus appear to be reached, and then response continues. There are several explanations, none conclusive. The first is chance: individual and common environmental deviations or genetic sampling, the last of which has autocorrelated effects over generations. The second is that the response is "waiting" for mutations, and when these occur response continues. Evidence for this is likely to be unequivocal, however, only if the mutation has a large effect, say over 1 σ_P, so it produces jumps in response, peaks in variance, or clear nonlinearity of response. An alternative hypothesis is that recombination is releasing unfavorable linkage disequilibrium (Mather 1941; Roberts 1966a). This requires a counterbalanced effect of the linked genes on the trait, and as it seems unlikely to find this by chance for genes of large effect, the idea has been dismissed (Hill 1982; Keightley 2004).

What information can we obtain about numbers of genes that influence the traits and, for example, on their degrees of dominance? Calculations have been done by a number of authors, and, for example, most recently by Bünger et al. (2001b). The general basis of these calculations is the relation between initial variability and limit given by Wright: that for n additive genes of equal effect and frequency, the range and σ_G are both proportional to n, so range/σ_G is proportional to $\sqrt{n}$. The predictions are unreliable for many reasons, including the following. There is the simplistic assumption that all genes have equal effect and equal frequency (acceptable from an F_1 cross base). Proper account is not taken of new variation arising from mutations, or the need to allow for finite population size and thus loss of some favorable genes, particularly but not exclusively, those of small effect. Hill and Rasbash (1986a) have attempted more general models, in which, for example, a gamma distribution of gene effects and various distributions of gene frequencies were assumed. Their analysis unhelpfully indicates, inter alia, that very wide differences in assumptions could lead to quite similar patterns and variation in selection response, for example in the magnitude of the total response relative to the genetic standard deviation in the base population. Inclusion of the effects of mutations into the calculations shows,

for example, that mutation is likely to contribute an increasingly high proportion of the current selection response as the length of the experiment increases, and does so more quickly if the distribution of mutation effects is highly leptokurtic (i.e., a few have very large effect) (Hill and Rasbash 1986b). The half-life of response due to genes of infinitesimal effect in the base population is $1.4N_e$ generations and is very much shorter otherwise (Robertson 1960). Residual response after this time and certainly any response that does not accord with an exponential decline in rate by this stage must surely be due mainly to mutations, indicated for example in the Dummerstorf high line (Fig. 6.10).

We have made attempts in our laboratory to analyze changes in variance in selected lines using modern statistical methods such as REML, in which changes due to selection itself, the "Bulmer effect" (Bulmer 1971), could be eliminated. Basically these become a test of the fit of the infinitesimal model. We have not obtained very consistent results, but typically there is a good fit to the model in early generations and departures subsequently (Meyer and Hill 1991; Beniwal et al. 1992). These do not enable any clear estimate of gene numbers, for example. As noted above, we have also attempted to utilize the different time pattern of their contributions to partition variance between that present initially and that arising by mutation, but there is insufficient power to do so.

Quite independent information on actual QTL and potentially gene effects comes from QTL mapping studies (Falconer and Mackay 1996; Lynch and Walsh 1998; Barton and Keightley 2002). These have almost invariably identified QTL of significant effects, each contributing an appreciable proportion of the variance, although ascertainment bias implies the estimates of effects are likely to be too high. Indeed, in the two long-term selected lines discussed here in most detail, from Edinburgh and Dummerstorf (Figs. 6.9 and 6.10), such QTL of large effect have been identified (Rance et al. 1997; Brockmann et al. 1998, respectively), one on the X-chromosome accounting for about 20% of the difference between the EDH and EDL lines. Such analyses do not, however, exclude the likelihood that many other loci are contributing very little of the variability in standing populations.

In fact, our information on how variation is maintained in populations is not clear-cut, despite long continued work on the subject. Modern analyses are based on models of genes of variable effect and recurrent mutation, with variation lost through stabilizing selection or pleiotropic effects of genes (Falconer and Mackay 1966; Bürger 2000; Barton and Keightley 2002; Zhang and Hill 2002). If the hypothesis that a large proportion of the variation in quantitative traits in natural populations is

derived from such a balance, then it is likely that genes with small, nearly neutral, effect contribute much variability. There is a need to consider the response in populations started using such models in which gene effects and frequencies have a joint distribution determined by the previous evolutionary process, and thereby to test the relevance of the models. In the species we have considered here, however, natural populations do not form the base, so the analyses need to be taken further to consider the consequences of selection following previous selection, inbreeding, and crossing, for example.

LITERATURE CITED

Anthony, N. B., K. E. Nestor, and H. L. Marks. 1996. Short-term selection for four-week body weight in Japanese quail. Poultry Sci. 75:1192–1197.

Arnason, T. 2001. Trends and asymptotic limits for racing speed in standardbred trotters. Livest. Prod. Sci. 72:135–145.

Bakker, H. 1974. Effect of selection for relative growth rate and body weight of mice on rate, composition and efficiency of growth. Mededelingen-Landbouwhogeschool-Wageningen 74:1–94.

Barton, N. H., and P. D. Keightley. 2002. Understanding quantitative genetic variation. Nature Rev. Genet. 3:11–21.

Beniwal, B. K., I. M. Hastings, R. Thompson, and W. G. Hill. 1992. Estimation of changes in genetic parameters in selected lines of mice. 2. Body weight, body composition and litter size. Heredity 69:361–371.

Brockmann, G. A., C. S. Haley, U. Renne, S. A. Knott, and M. Schwerin. 1998. Quantitative trait loci affecting body weight and fatness from a mouse line selected for extreme high growth. Genetics 150:369–381.

Bulmer, M. G. 1971. The effect of selection on genetic variability. Am. Nat. 105:201–211.

Bünger, L., and W. G. Hill. 1999a. Inbred lines derived from long-term divergent selection on fat content and body weight. Mamm. Genome 10:645–648.

Bünger, L., and W. G. Hill. 1999b. Role of growth hormone in the genetic change of mice divergently selected for body weight and fatness. Genet. Res. 74:351–360.

Bünger, L., A. H. Laidlaw, G. Bulfield, E. J. Eisen, J. F. Medrano, G. E. Bradford, F. Pirchner, U. Renne, W. Schlote, and W. G. Hill. 2001a. Inbred lines of mice derived from long-term on growth selected lines: unique resources for mapping growth genes. Mamm. Genome 12:678–686.

Bünger, L., U. Renne, and R. C. Buis. 2001b. Body weight limits in mice—Long-term selection and single genes. p. 337–360. In: E. C. R. Reeve (ed.), Encyclopedia of genetics. Fitzroy Dearborn Publ., London, Chicago.

Bünger, L., U. Renne, G. Dietl, and S. Kuhla. 1998. Long-term selection for protein amount over 70 generations in mice. Genet. Res. 72:93–109.

Bünger, L., L. Schüler, B. Kupatz, and U. Renne. 1983. Selection for growth in model animals (laboratory mice). 2. Direct selection response. Arch. Tierzucht 26:281–293.

Bürger, R. 2000. The mathematical theory of selection, recombination and mutation. Wiley, Chichester, UK.

Dickerson, G. E. 1955. Genetic slippage in response to selection for multiple objectives. Cold Spr. Harb. Symp. Quant. Biol. 20:213–234.
Dudley, J. W., and R. J. Lambert. 2004. 100 generations of selection for oil and protein in corn. Plant Breed. Rev. 24:
Dunnington, E. A., and P. B. Siegel. 1996. Long-term divergent selection for eight-week body weight in White Plymouth Rock chickens. Poult. Sci. 75:1168–1179.
Eisen, E. J. 1980. Conclusions from long-term selection experiments with mice. Z. Tierz. Züchtungsbiol. 97:305–323.
Fairfull, R. W., and R. S. Gowe. 1990. Genetics of egg production in chickens. p. 705–759. In: R. D. Crawford (ed.), Poultry breeding and genetics. Elsevier, Amsterdam.
Falconer, D. S. 1953. Selection for large and small size in mice. J. Genet. 51:470–501.
Falconer, D. S. 1960. Selection of mice for growth on high and low planes of nutrition. Genet. Res. 1:91–113.
Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman, Harlow, UK.
Gaffney, B., and E. P. Cunningham. 1988. Estimation of genetic trend in racing performance of thoroughbred horses. Nature 332:722–724.
Goodale, H. D. 1938. A study of the inheritance of body weight in the albino mouse by selection. J. Hered. 29:101–112.
Gowe, R. S., and R. W. Fairfull. 1985. The direct response to long-term selection for multiple traits in egg stocks and changes in genetic parameters with selection. p. 125–146. In: W. G. Hill, J. M. Manson, and D. Hewitt (eds.), Poultry genetics and breeding. Longman, Harlow, England.
Griffing, B. 1960. Theoretical consequences of truncation selection based on the individual phenotype. Austral. J. Biol. Sci. 13:307–343.
Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and B. T. Larson. 1994a. Growth, livability, and feed conversion of 1957 vs. 1991 broilers when fed 'typical' 1957 and 1991 broiler diets. Poult. Sci. 73:1785–1794.
Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and R. D. Rives. 1994b. Carcass composition and yield of 1957 vs. 1991 broilers when fed 'typical' 1957 and 1991 broiler diets. Poult. Sci. 73:1795–1804.
Hill, W. G. 1971. Design and efficiency of selection experiment for estimating genetic parameters. Biometrics 27:293–311.
Hill, W. G. 1982. Predictions of response to artificial selection from new mutations. Genet. Res. 40:255–278.
Hill, W. G. 1988. Why aren't horses faster? Nature 332:678.
Hill, W. G. 2000. Maintenance of quantitative genetic variation in animal breeding programmes. Livest. Prod. Sci. 63:99–109.
Hill, W. G. 2002. Direct effects of selection on phenotypic variability of quantitative traits. Proc. 7th World Congr. Genet. Appl. Livest. Prod. CD-ROM comm. no. 19-02.
Hill, W. G., and J. Rasbash. 1986a. Models of long term artificial selection in finite population. Genet. Res. 48:41–50.
Hill, W. G., and J. Rasbash. 1986b. Models of long term artificial selection in finite population with recurrent mutation. Genet. Res. 48:125–131.
Jones, D. R., K. E. Anderson, and G. S. Davis. 2001. The effects of genetic selection on production parameters of single comb white leghorn hens. Poult. Sci. 80:1139–1143.
Keightley, P. D. 1998. Genetic basis of response to 50 generations of selection on body weight in inbred mice. Genetics 148:1931–1939.
Keightley, P. D. 2004. Mutational variation and long-term selection response. Plant Breed. Rev. 24: Part 1, 227–248.

Koerhuis, A. N. M., and R. Thompson. 1997. Models to estimate maternal effects for juvenile body weight in broiler chickens. Genet. Sel. Evol. 29:225–249.
Kownacki, M., and B. Zuk. 1986. Populations size and response to selection for postweaning gain in the mouse. Genetica Polon. 27:99–111.
Lerner, I. M. 1957. The genetic basis of selection. Wiley, New York.
Liu, G., E. A. Dunnington, and P. B. Siegel. 1994. Responses to long-term divergent selection for eight-week body weight in chickens. Poult. Sci. 73:1642–1650.
Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.
McGuirk, B. J. 2000. Genetic changes in ruminants: Historic trends and future prospects. p. 29–38. In: W. G. Hill, S. C. Bishop, B. J. McGuirk, J. C. McKay, G. Simm, and A. J. Webb (eds.), The challenge of genetic change in animal production. Occ. Publ. Brit. Soc. Anim. Sci., no. 27.
McKay, J. C., N. F. Barton, A. N. M. Koerhuis, and J. McAdam. 2000. The challenge of genetic change in the broiler chicken. p. 1–7. In: W. G. Hill, S. C. Bishop, B. J. McGuirk, J. C. McKay, G. Simm, and A. J. Webb (eds.), The challenge of genetic change in animal production. Occ. Publ. Brit. Soc. Anim. Sci., no. 27.
McMillan, I., R. W. Fairfull, R. S. Gowe, and J. Gavora. 1990. Evidence for genetic improvement of layer stocks of chickens during 1950–80. World's Poult. Sci. J. 46:235–245.
Marks, H. L. 1996. Long-term selection for body weight in Japanese quail under different environments. Poult. Sci. 75:1198–1203.
Martinez, V., L. Bünger, and W. G. Hill. 2000. Analysis of response to 20 generations of selection for body composition in mice: fit to infinitesimal assumptions. Genet. Sel. Evol. 32:3–21.
Mather, K. 1941. Variation and selection of polygenic characters. J. Genet. 41:159–193.
Merks, J. W. M. 2000. One century of genetic changes in pigs and the future needs. p. 8–19. In: W. G. Hill, S. C. Bishop, B. J. McGuirk, J. C. McKay, G. Simm, and A. J. Webb (eds.), The challenge of genetic change in animal production. Occ. Publ. Brit. Soc. Anim. Sci., no. 27.
Meyer, K., and W. G. Hill. 1991. Mixed model analysis of a selection experiment for food intake in mice. Genet. Res. 57:71–81.
Nestor, K. E., J. W. Anderson, and R. A. Patterson. 2000. Genetics of growth and reproduction in the turkey. 14. Changes in genetic parameters over thirty generations of selection for increased body weight. Poult. Sci. 79:445–452.
Nestor, K. E., D. O. Noble, J. Zhu, and Y. Moritsu. 1996. Direct and correlated responses to long-term selection for increased body weight and egg production in turkeys. Poult. Sci. 75:1180–1191.
Preisinger, R., and D. K. Flock. 2000. Genetic changes in layer breeding: Historic trends and future prospects. p. 20–28. In: W. G. Hill, S. C. Bishop, B. J. McGuirk, J. C. McKay, G. Simm, and A. J. Webb (eds.), The challenge of genetic change in animal production. Occ. Publ. Brit. Soc. Anim. Sci., no. 27.
Rance, K. A., W. G. Hill, and P. D. Keightley. 1997. Mapping quantitative trait loci for body weight on the X chromosome in mice. I. Analysis of a reciprocal F2 population. Genet. Res. 70:117–124.
Rauw, W. M., E. Kanis, E. N. Noordhuizen-Stassen, and F. J. Grommers. 1998. Undesirable side effects of selection for high production efficiency in farm animals: a review. Livest. Prod. Sci. 56:15–33.
Renne, U., M. Langhammer, E. Wytrwat, G. Dietl, and L. Bünger. 2003. Analysis of growth in selected and unselected mouse lines. J. Expt. Anim. Sci. 42:218–232.
Roberts, R. C. 1966a. The limits to artificial selection for body weight in the mouse. I. The limits attained in earlier experiments. Genet. Res. 8:347–360.

Roberts, R. C. 1966b. The limits to artificial selection for body weight in the mouse. II. The genetic nature of limits. Genet. Res. 8:361–375.

Roberts, R. C. 1967. The limits to artificial selection for body weight in the mouse. III. Selection from crosses between previously selected lines. Genet. Res. 9:73–85.

Robertson, A. 1960. A theory of limits in artificial selection. Proc. Roy. Soc. Lond. B153:234–249.

Tharrington, J. B., P. A. Curtis, F. T. Jones, and K. E. Anderson. 1999. Comparison of physical quality and composition of eggs from historic strains of single comb white leghorn hens. Poult. Sci. 78:591–594.

Varga, L., G. Szabo, A. Darvasi, G. Müller, M. Sass, and M. Soller. 1997. Inheritance and mapping of compact (cmpt), a new mutation causing hypermuscularity in mice. Genetics 147:755–764.

Walsh, J. B. 2004. Selection theory and limits. Plant Breed. Rev. 24, Part I.

Wei, M., A. Caballero, and W. G. Hill. 1996. Selection response in finite populations. Genetics 144:1961–1974.

Wilson, S. P., H. D. Goodale, W. H. Kyle, and E. F. Godfrey. 1971. Long term selection for body weight in mice. J. Hered. 62:228–234.

Zhang, X.-S., and W. G. Hill. 2002. Joint effects of pleiotropic selection and stabilizing selection on the maintenance of quantitative genetic variation at mutation-selection balance. Genetics 162:459–471.

7

Long-term Selection for Pupal Weight in *Tribolium castaneum*

W. M. Muir, D. Miles, and A. E. Bell
Department of Animal Sciences
Purdue University
W. Lafayette, Indiana 47906

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-46892-4

I. INTRODUCTION

Concern for the limits to selection is central to all plant and animal breeding, that is, can selection continue indefinitely? Evolutionary biology suggests the process is endless, primarily as a result of new mutations. However, selection in nature is normally of low intensity, that is, only the very lower end of the distribution is culled, resulting in large effective population sizes, selection is directly on fitness, and time is counted in the millions, if not billions, of generations. These conditions minimize effects of random genetic drift for the trait of selection because genetic drift is inversely proportional to the effective population size, is opposed in one direction by selection (for increased fitness), and both population size is large enough and time sufficient for new mutations to be a significant factor in maintaining genetic variation. Also, natural selection is the result of endless natural development of isolated lines, allowing both within and between line selection. This process allows for utilization of epistatic variation through co-adapted gene complexes and exploration of fitness surfaces. With artificial selection, time is counted in many orders of magnitude fewer generations than with natural selection, selection is intense, the traits of selection are for performance or physical characteristics not fitness, and selection is usually within line, all of which results in small effective population sizes, little or no opposition to drift, minimal impact of new mutations, and utilization of only additive genetic variance. With these conditions, favorable alleles and new mutations may be lost by random genetic drift and fitness can deteriorate to the point where the lines are lost to reproductive failure.

The importance of population size and selection intensity in natural as opposed to artificial selection is most clearly seen with resistance factors, either herbicide, insecticide, anti-bacterial or anti-viral agents. Classic quantitative genetic theory states that the greatest progress for multiple traits will be made using selection index as opposed to tandem selection for independent culling (Turner and Young 1969). However, in the field, natural resistance to multiple insecticides develops more quickly using tandem release of insecticides than simultaneous (Roush 1998). Similar observations are also true for viral resistance to new drugs for AIDS patients (pers. commun. Dr. John Coffin, Tufts University School of Medicine). These results are contrary to classic theory where most resistance (progress) should occur with either index or independent culling selection. The reason for these contrary results is effective population size, existing genetic variation, and the mutation rate. With multiple resistance factors, the population sizes are reduced to a size,

often zero, such that selection response by small increments based on polygenic inheritance cannot occur. As such, for resistance to develop a new mutation must occur that offers multiple resistance in one step, an exceedingly rare event. For such a rare event to occur, the population size must be much greater than the mutation rate, that is, greater than a million. Because laboratory or even commercial populations rarely if ever approach these numbers, results observed in natural population are often different than those observed in artificial breeding programs.

To determine if limits to selection are indeed a concern in artificial selection programs, Dr. A. E. Bell initiated a long-term selection program in 1954, shortly after he came to Purdue University, using the model organism *Tribolium castaneum*, the red flour beetle. The utility of a model organism is to biologically examine these issues within a reasonable time frame (Brown and Bell 1961, 1980).

II. METHODS AND MATERIALS

A. Material

This study of long-term selection for pupal weight utilized four populations of *T. castaneum*. These were designated Purdue Large I, Large II, Large III, and Small. Large I and Large II originated from a study begun in 1954 with the formation of a heterogeneous randomly mated population (Purdue +) from eight diverse laboratory populations (Bell 1981, 1982) and originated as Replications I and II of Purebred Selection. Large III was initiated in 1961 from the same Purdue + Foundation and was the selected non-irradiated line in an X-radiation study (Bartlett et al. 1966). Two populations selected for small body weight were part of a study begun in 1963 (Bell and McNary 1963) to study the symmetry of response to high-low selection in different environments. Small 1 and 2 were combined after 8 generations of selection for low pupal weight. Selection was then resumed in the merged Small Line. Additional studies involving these four long-term selected populations were summarized by Bell (1981).

B. Selection Methods

Large I, II, III, and Small have been maintained as closed populations undergoing selection for either increased or decreased pupal weight. Selection was accomplished by weighing 200 random pupae of each sex to the nearest $\mu g \times 10$ on a Mettler M5 balance and keeping the largest or

smallest 50 males and 50 females. These were mass mated in standard medium (whole wheat flour with 5% dried brewer's yeast) in plastic boxes and held at 33°C and 70% relative humidity to yield the next generation.

Varying periods of relaxed selection interrupted the selection procedure. The periods of relaxed selection varied from one to 60 generations. Usually poor reproduction and fitness mandated relaxed selection, but sometimes other experiments were a higher priority.

C. Methods to Examine Limits

1. Selection Differentials. Selection differentials were recorded in the last 150 generations of selection. The selection differential for each line was computed as the difference between the mean of the entire parental population and the mean of the parents selected to yield the next generation.

2. Reverse Selection. After 340 generations, reverse selection was applied to each line in each of the next 20 generations, starting over each time from the selected population, thus 20 single generation selections were made in the reverse direction each round. Reverse selection was accomplished using the largest or smallest 50 males and 50 females in the opposite direction of selection for each line. Mass weight of all pupae from each of these single generation reverse selection experiments was recorded.

3. Correlation with Fitness. The correlation between progeny number of the parent, the primary fitness trait that natural selection operates on, and pupal weight of that parent before emergence was examined. At the termination of the experiment, after 360 generations, Large I and Small populations sizes were expanded by transferring the selected adults of the previous generation to new media every four to five days. When their adult progeny were mature (8–10 days after emergence) they were set on new standard media to obtain a 48 hour egg collection. Commencing with day 17 for the large populations and day 14 for the small population, the mass collections were screened daily for pupae that were then sexed, individually weighed, and held in separate creamers for eventual single pair matings. This procedure was replicated 4 times using different sets of parents. When mature, the adults were assortatively mated based on their pupae weight. For the matings, 200 (or all available) emerged males and females were arrayed by pupae weight and mated using positive phenotypic assortative mating. When the mated pairs

were 7–10 days of age, one 48 hour egg collection was taken. The parents were then removed and discarded. The rational for assortative mating was to increase the range of possible mating types and thus obtain a more precise estimate of the correlation coefficient.

Mass progeny weights were taken every other day, commencing on day 14 for the Small population and day 16 for the Large I population, the populations were screened for pupae which were then counted, and after the mass weighing, were discarded. Sire and dam weights and mass progeny weights and numbers were recorded.

D. Statistical Methods

1. Selection Response. Depending on the line, there were between 6 and 10 periods of continuous selection where there was no more than one generation of relaxed selection. These periods were called *Runs*. The last Run of all lines was a continuous period of over 120 generations. Data for the Runs were sequentially fit to a model with separate intercepts for each Run, a common slope for generations (G), and an interaction term (G × Run) to test for lack of fit. The Proc GLM procedure of SAS was used with Run fit as a classes variable and Generations as a continuous variable. When significant lack of fit was observed, a separate slope was fit for the last Run. This was accomplished by fitting a second dummy variable with a value of 0 prior to the generation of transition and a 1 thereafter (Draper and Smith 1966, p. 139). This result only occurred for the last Run of all lines.

2. Selection Differentials. Selection differentials were averaged across replicate lines within each direction of selection. Relative selection differentials were also computed as the percent response, that is, the selection differential was divided by the previous generation's mean and multiplied by 100. Selection differentials and relative selection differentials were regressed on generation number with both linear and quadratic effects. If quadratic was not significant, the term was removed and results fit to a simple linear model.

3. Single Generation Reverse Selection. In each generation for 20 generations, there was a single generation of reverse selection that was measured and discarded. There was also a generation in the desired direction of selection. The generation of selection in the desired direction was used as the basis of the next generation, for which these two directions of selection were again repeated. The difference between the mean of the parents and the next generation, regardless of direction of selection,

was the response. The response in the desired direction of selection was considered the normal direction of selection and the other the reverse direction. Although residuals are correlated from one generation to the next due to random genetic drift, this correlation is removed by taking sequential differences and can be treated as independent observations (Muir 1986, 1991). A two-tailed t-test was used to compare response in the normal vs. reverse direction.

4. Relationship of Size with Fitness. Fitness can approximately be measured by the average number of progeny produced by each mating pair. If that fitness is related to the weight of the parents, then an association can be detected using regression; the null hypothesis would be no association. Therefore, for lines within each direction of selection, offspring number was regressed on average parental pupal weight and the regression coefficient tested for significance.

III. RESULTS AND DISCUSSION

A. Selection Response

For Large I, the first 8 runs fit a common slope of 118.5 μg/generation (Fig. 7.1). The last run was fit to two lines with progressively lower slopes of 47.4 and 12.8 μg/generation. For Large II, the first 9 runs and part of the 10th run could be fit to a common slope of 92.6 μg/genera-

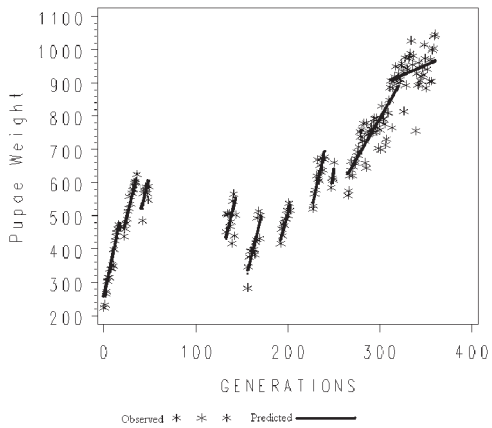

Fig. 7.1. Observed and predicted pupae weight (μg × 10) for the Large I line.

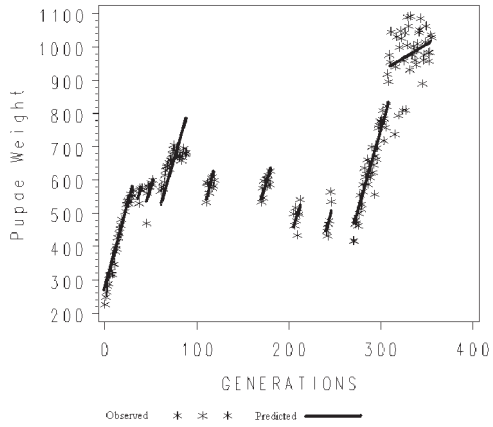

Fig. 7.2. Observed and predicted pupae weight (μg × 10) for the Large II line.

tion (Fig. 7.2). The last part of the 10th run fit a slope of 17.0 μg/generation. For Large III, the first 5 runs and part of the 6th run fit a common slope of 116 μg/generation (Fig. 7.3). The last part of the 6th run fit a slope of 16 μg/generation. For the Small line, the first 6 runs could be fit to a common slope of –54.7 μg/generation (Fig. 7.4). The last run was fit to two lines with progressively lower slopes of –.85 and +0.50 μg/generation.

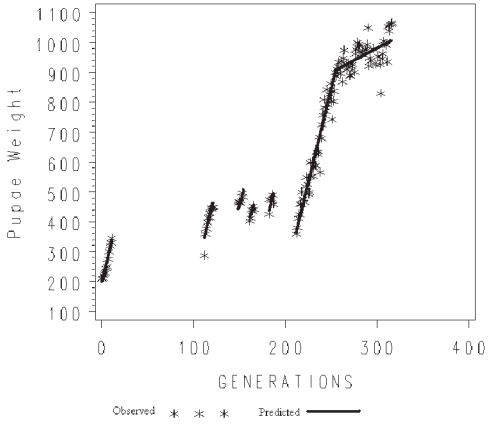

Fig. 7.3. Observed and predicted pupae weight (μg × 10) for the Large III line.

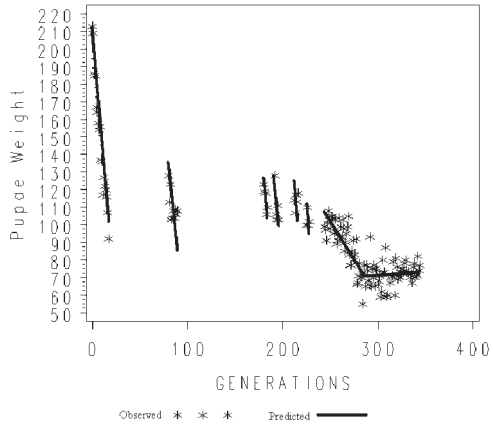

Fig. 7.4. Observed and predicted pupae weight (μg × 10) for the Small line.

Common to all selected lines, relaxed selection quickly reversed the progress made, indicating that natural selection and artificial selection were in opposite directions. Because both the Large and Small lines reversed their direction of selection when selection was relaxed, an intermediate pupal weight must confer optimum fitness. Only with sustained selection could real progress be made. However, the declining responses in advanced generations indicates that either genetic variability was being exhausted or a physiological limit in either direction was being approached, or both. The physical differences in the lines were dramatic, as shown in Fig. 7.5, with approximately a 1600% difference between the large and small lines, about the same percent difference as between the Chihuahua and Rottweiler.

B. Selection Limits

The declining responses to selection in advanced generations could be due to (1) loss of selection differential, (2) loss of genetic variability, (3) a strong negative correlation between fitness and pupal weight (loss of effective selection differential), or (4) an approaching physiological limit. These alternative explanations were examined during the final stages of selection.

Fig. 7.5. Photograph of representatives of adults from Large and Small lines.

1. Loss of Selection Differential. The per generation selection differentials, given in Fig. 7.6, show that the absolute value of the selection differentials actually increased in the large line with generations, but decreased in the small line. Thus, loss of phenotypic variation, at least in the large line, was not a factor. However, for traits such as weight, the mean and variance are often correlated. This effect can be adjusted for by either transforming by logs or by standardizing the selection differential by the mean of the previous generation. Fig. 7.7 shows that when the selection differential was standardized by dividing by the previous generation's mean, the proportionate selection differentials remained constant for the last 150 generations with the slope not significantly different from zero for either log transformed (not given) or as a percent of selection differential. Thus adequate selection differentials existed in both directions of selection and do not account for the slowing down of selection response.

2. Loss of Genetic Variability. Loss of genetic variability can easily be tested by reverse selection. If the population responds to reverse selection, genetic variability still exists, but cannot be utilized in the intended

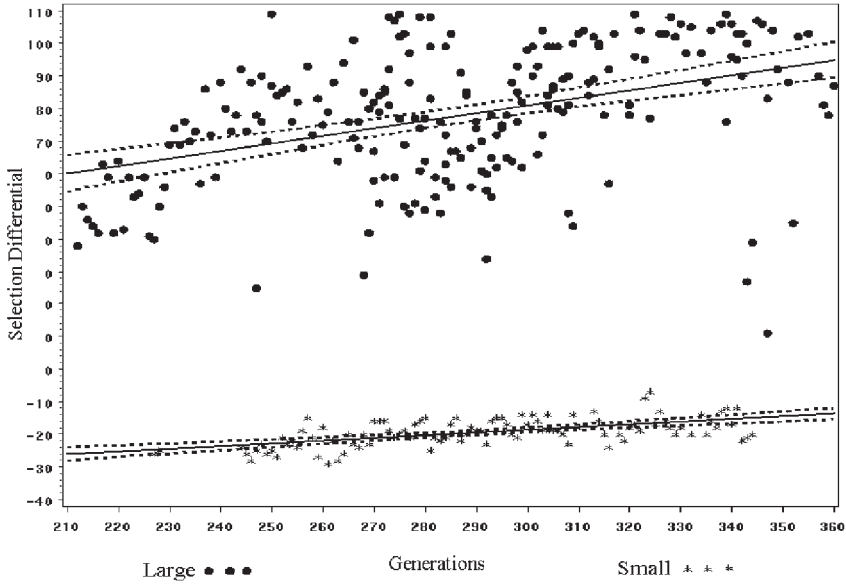

Fig. 7.6. Selection differentials (μg × 10) by generation for the Large and Small lines.

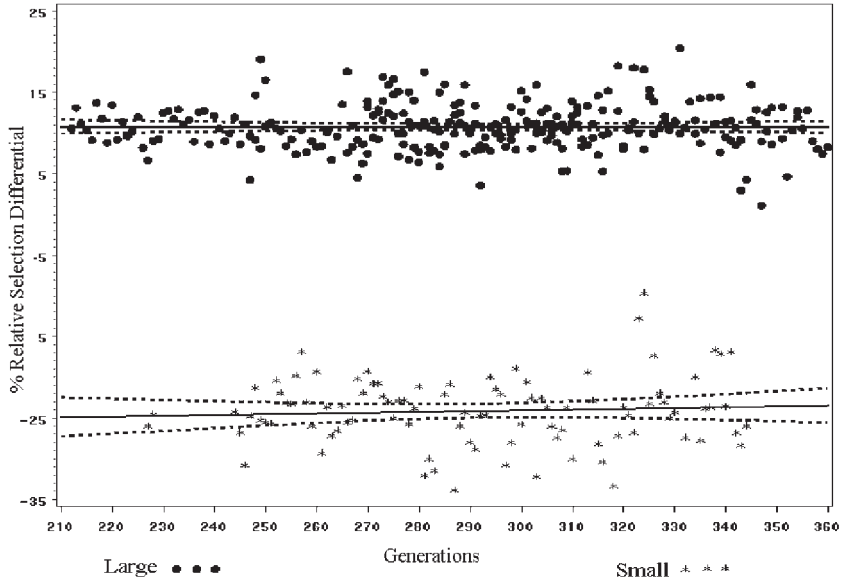

Fig. 7.7. Percent relative selection differentials by generation for the Large and Small lines.

direction of selection. In the large lines, selection in the up and down directions gave responses of .89 ± 140μg and –531 ± 166μg respectively, the latter being significantly different from zero. In the small line, selection in the up and down directions gave responses of 4.09 ± 15μg and –0.0 ± 13μg respectively, neither being significantly different from zero. Also, during the long-term selection, the final 50 generations of down selection in the small line actually resulted in a slight and non-significant positive response. Thus, significant genetic variability existed in the large line, as evident by response in at least the down direction, but does not appear to have been present in the small line due to lack of response in either direction.

3. Correlation with Fitness. The relationship between fitness and pupal weight for the Large line is given in Fig. 7.8. The significant regression shows a strong negative relationship between progeny number (N) and pupal weight (–0.0044 ± 0.0004 N/μg), indicating that natural selection opposes artificial selection in the direction of selection (up). The relationship between fitness and pupal weight for the Small line is given in

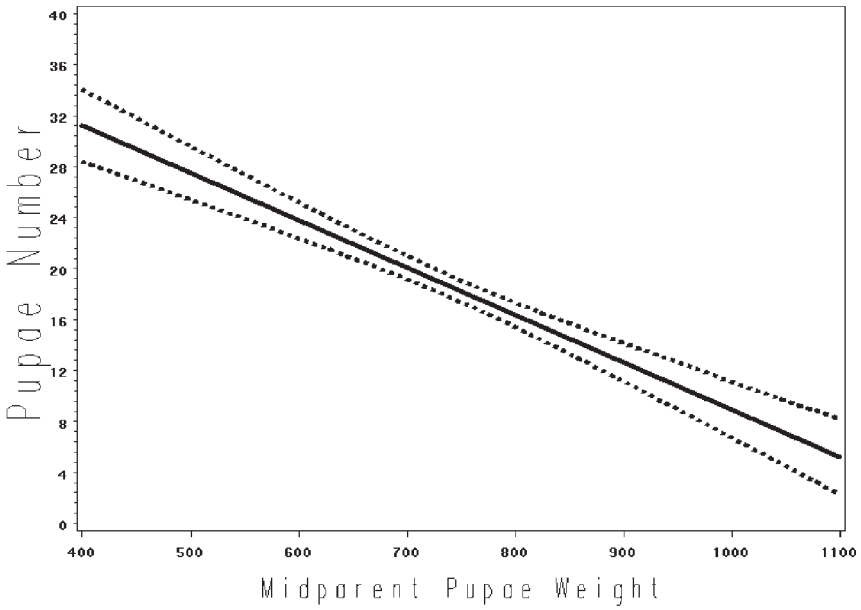

Fig. 7.8. Predicted progeny number regressed on average parental pupal weight (μg × 10) and 95% confidence interval for the Large line.

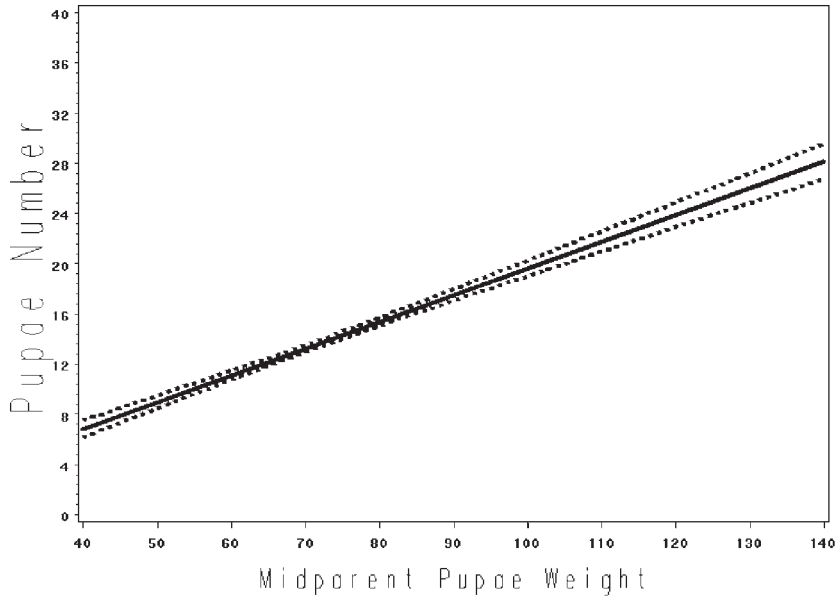

Fig. 7.9. Predicted progeny number regressed on average parental pupal weight (μg × 10) and 95% confidence interval for the Small line.

Fig. 7.9. The significant regression shows a strong positive relationship between progeny number (N) and pupal weight (0.0213 ± 0.001 N/μg) indicating that natural selection again opposes artificial selection in the direction of selection (down). The percent sterile matings (those producing eggs but with zero hatchability), given in Fig. 7.10, supports this conclusion, particularly in the Large line where sterility in the direction of selection was three times that of the opposite direction (35% vs. 12%). Thus, while the selection differentials remained constant, the effective selection differential was greatly diminished in the direction of selection for both lines.

4. Physical Limits. A physiological limit is a barrier imposed by the restrictions of physiology and/or physics, such as surface area to weight ratio needed for respiration, weight that can be carried by a structure, such as limbs or exo-skeleton, or maximum egg size possible for oviposition and growth. These limits can be tested using natural mutations

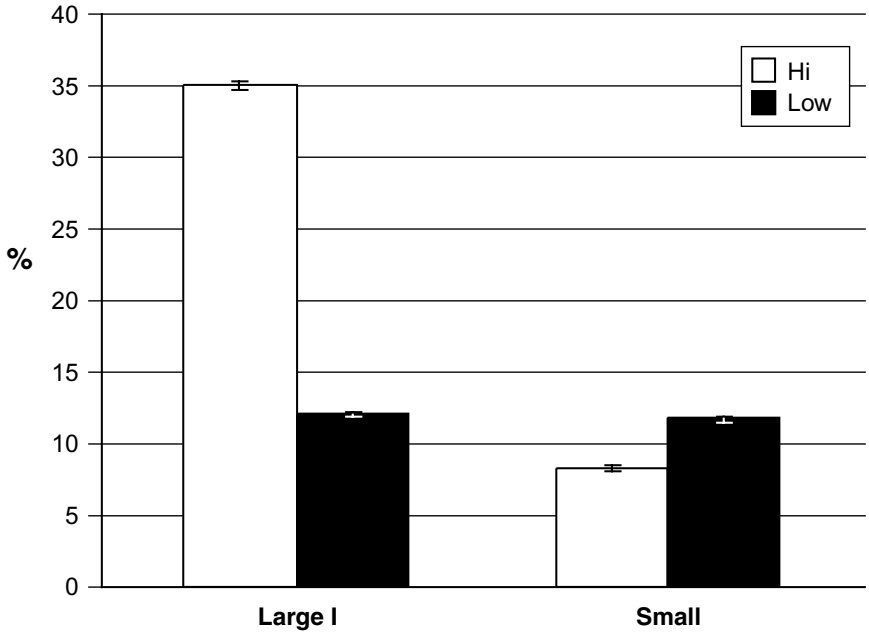

Fig. 7.10. Percent sterile matings in each direction of selection and line.

that exist for both gigantism and dwarfism, termed the Goliath and Pigmy mutants respectively. These mutants were not observed in our lab nor have they been reported to occur naturally. Out of curiosity, our lines were crossed to those mutants in another lab. When either of these mutants are crossed into the large or small lines, the effects on the progeny were cumulative, that is, they were either much larger or smaller respectively (Dr. J. Stuart, Department of Entomology, Purdue Univ., pers. commun.). Hence, we must conclude that the limits to selection were not physical.

IV. CONCLUSIONS

Long-term selection for pupal weight, over 360 generations, was applied in the red flour beetle, *Tribolium castaneum*, resulting in approximately a 1600% difference between the large and small lines, about the same

percent difference as between the Chihuahua and Rottweiler. Limits to selection were investigated and found to be due to loss of genetic variability in one direction (small) and negative correlations with fitness in both lines in the direction of selection. These results are unusual in that limits in one direction (down) were due to both loss of genetic variability and opposition from natural selection, while that in the high line was due only to opposition from natural selection. This result raises the question of why new mutations may contribute to maintenance of genetic variability in one direction (large) and not the other (small).

LITERATURE CITED

Bartlett, A. C., A. E. Bell, and V. L. Anderson. 1966. Changes in quantitative traits of *Tribolium* under irradiation and selection. Genetics 54:699–713.

Bell, A. E. 1981. Direct and correlated responses to long-term selection in *Tribolium*. Proc. 30th National Breeders Roundtable, p. 1–32.

Bell, A. E. 1982. The *Tribolium* Model in animal breeding research. 2nd World Congress on Genetics Applied to Livestock Production. Vol. 5:26–42.

Bell, A. E., and H. W. McNary. 1963. Genetic correlation and asymmetry of the correlated response from selection for increased body weight of *Tribolium* in two environments. XI Int. Genet. Congr., Proc. I:256.

Brown, W. P., and A. E. Bell. 1961. Genetic analysis of plateaued populations of drosophila melanogaster. Genetics 46:407–425.

Brown, W. P., and A. E. Bell. 1980. An experimental comparison of selection alternatives to plateaued response. Genetics 9:477–496.

Draper, N. R., and H. Smith. 1966. Applied regression analysis. Wiley, New York.

Muir, W. M. 1986. Optimal utilization of control populations for additional information and accuracy. Biometrics 42:381–392.

Muir, W. M. 1991. Estimation of response to selection in non-replicated populations. p. 95–121. In: Proc. 41st National Breeders Roundtable (May 7–8, St. Louis, MO).

Roush, R. T. 1998. Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? Philosophical Transactions Royal Society London Series B-Biological Sciences 353:1777–1786.

Turner, H. N., and S. S. Y. Young. 1969. *Quantitative Genetics in Sheep Breeding*. Ithaca, N.Y. Cornell University Press.

8

Phenotypic and Genomic Evolution during a 20,000-Generation Experiment with the Bacterium *Escherichia coli*

*Richard E. Lenski**
Center for Microbial Ecology
Michigan State University
East Lansing, Michigan 48824

*Acknowledgments: Many individuals have contributed to this long-term experiment. Rather than trying to list everyone here, I will let the literature cited record many of the contributions of the graduate students, postdoctoral associates, and colleagues who have worked on this project. However, I want to emphasize the contributions of a few individuals in particular. Michael Rose played an important role in getting this project started, as his own long-term study selecting for delayed senescence in fruitflies provided an elegant model to emulate. The *E. coli* experiment has depended on the dedication of three outstanding technicians over the years: Sue Simpson, Lynette Ekunwe, and Neerja Hajela. And Madeleine Lenski has tolerated my long-term "affair" with this experiment. This research has been supported by the National Science Foundation (currently grant DEB-9981397) and by Michigan State University. James Crow and Jules Janick provided many helpful comments on this paper.

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-46892-4

I. INTRODUCTION

This paper reviews another long-term selection experiment, one that is both shorter and longer than the 100 years of selection for oil and protein content in maize that is the main focus of this volume. In 1988, 12 populations of the bacterium *Escherichia coli* were founded from the same strain, and these have been propagated in a simple, defined laboratory environment ever since. Each day, the populations are diluted in fresh medium, undergo about 6.6 generations of binary fission before they exhaust the limiting resource, and then must wait until their "springtime" appears again the next day.

This review summarizes some interesting changes and dynamics, both phenotypic and genomic, that occurred in these populations through generation 20,000. For an annual plant 20,000 generations would of course require some 20,000 years; for humans 20,000 generations would span 400,000 years, assuming an average generation of 20 years. This ability to observe evolution in action over many generations is an obvious benefit of studying bacteria. In fact, the experiment recently passed 30,000 generations, but the bacteria evolve faster than we can study them, and generation 20,000 represents the last milestone at which we systematically studied the populations. Rather than taking the same measurements at intervals, we have repeatedly pushed our analyses in new directions. Thus, some changes were analyzed through 2,000 generations, others through 10,000 generations, and still other changes through 20,000 generations. Another wonderful feature of bacteria for evolutionary research is that we can store entire populations frozen, and resurrect them whenever we wish, such that we can gather more data about earlier generations if we later find other aspects of the evolving bacteria that we want to measure and study.

A. Motivating Questions

This long-term evolution experiment with *E. coli* has several goals. First, the experiment aims to measure the dynamics of evolutionary change. Is evolution strictly gradual, or are there episodes of more rapid change even in a constant environment? Does the initial rate of change continue indefinitely, or is some limit eventually reached? And how might the dynamic patterns depend on which particular phenotypic or genetic traits are measured?

Second, this experiment seeks to examine the repeatability of evolution by having 12 replicate populations, all of them founded from the same ancestor and maintained in the same environment. Which phenotypic and genomic aspects of evolutionary change are repeatable in this system, and which are haphazard? How can we understand the causes of parallelism and divergence of replicate lines? The issue of the predictability of evolutionary change—or lack thereof—has long been of interest. The question was well captured by the late paleontologist Steven Jay Gould (1989) in a thought experiment: "*I call this experiment 'replaying life's tape.' You press the rewind button and, making sure you thoroughly erase everything that actually happened, go back to any time and place in the past—say to the seas of the Burgess Shale. Then let the tape run again and see if the repetition looks at all like the original . . .*" Gould went on to say, however, that "*The bad news is that we can't possibly perform the experiment.*" Of course, we could never run an experiment on the vast temporal and spatial scales imagined by Gould. But on much smaller scales, our experiment with *E. coli* allows us to address the same question. We do so by allowing 12 scenarios to play out simultaneously, rather than sequentially as the notion of replay implies, but the issue of repeatability is fundamentally the same.

Third, the long-term evolution experiment offers the opportunity to integrate data on phenotypic and genetic changes. Are the dynamics of phenotypic and genomic change concordant? If there are discrepancies, why do they occur? If certain phenotypic traits evolve in parallel across the replicate populations, does this imply parallelism at the level of mutations, genes, or pathways? Have the bacteria become ecological specialists as they adapted to the monotonous selective regime? If so, what are the roles of pleiotropic tradeoffs versus accumulation of neutral mutations in causing specialization? How much phenotypic and genomic evolution can occur in 20,000 generations, and how well do the observed rates accord with rates inferred from comparative data? It may seem that linking genomic and phenotypic changes should be quite easy in a model system like *E. coli*, with its relatively small (and now

totally sequenced) genome, unicellular structure, and decades of service as a model system for molecular genetics. But linking phenotypic and genomic changes remains a real challenge for several reasons: (1) we are looking for a relatively few mutations in a genome that is several million base-pairs long; (2) most phenotypic effects of interest in this experiment are subtle quantitative changes, as opposed to the knock-outs causing losses of function usually studied by geneticists; (3) the absence of genetic markers and Mendelian recombination in the base population (which was clonal) force us to perform precise genetic manipulations to establish the effects of particular mutations on phenotypes of interest; and (4) the functions of many genes remain unknown and, even for genes that have been well studied, the extent of interactions with other genes is poorly known. Despite these difficulties, we have made substantial progress in linking genomic and phenotypic changes in the long-term *E. coli* lines. Some of this work has been published in the primary literature, but much more is still in progress. To avoid precluding later publication of this on-going work, the present review will present only those findings that have been published while describing on-going analyses in general terms.

An important aspect that impinges on all these questions is that our experimental system differs profoundly from the more familiar plant and animal models used in most other selection experiments. Bacteria are haploid, and they reproduce asexually. Many bacteria in nature have parasexual processes that allow varying degrees of recombination, but the *E. coli* strain used in this experiment lacks the potential to engage in these processes. Hence, these populations are strictly asexual. [In a separate experiment, we examined some of the consequences of allowing one of these parasexual processes to occur in association with introducing genes from another strain of *E. coli* (Souza et al. 1997).] Moreover, each replicate line in the long-term experiment was founded from a single clone (in fact, one cell) and hence there was no standing variation at the start of the experiment. New mutations thus provide the sole source of genetic variation available to selection. Therefore, any parallelism that we observe necessarily involves both the independent origin and fate of variants, as opposed to mere sorting of preexisting variants that are identical by descent. This dependence on new variation is one reason I prefer to call this an evolution experiment rather than a selection experiment. Also, selection in this long-term *E. coli* experiment is natural selection, as opposed to artificial selection as practiced by plant and animal breeders. By that I mean the environment selects; we only provide the environment (in this case, a simple and artificial one), but we do not choose particular individual cells to reproduce on the basis of their

phenotypes. Finally, I would mention that bacteria are of tremendous importance, not only as pathogens but as fundamental players in every natural and managed ecosystem. Yet, until recently bacteria have been largely ignored by evolutionary biologists, who perhaps regarded them as tools of molecular biology and lacking in the obviously colorful phenotypes of many plants and animals. The importance of bacteria in nature, along with their very different genetic systems, combine to make them an interesting area for evolutionary research.

B. Overview of the Experimental System

This section describes some important features of the experimental system. I have tried to limit the microbiological jargon and tedious details, which can be found in the various primary papers cited in this review. Instead, I want to give the reader a feel for how the experiment is performed and how the resulting evolutionary changes are discerned. Let me begin by summarizing several features that make bacteria in general, and *E. coli* in particular, powerful for studying evolution by an experimental approach. (I have chosen to pursue an experimental approach to evolution for two reasons: I enjoy experiments, and I feel that evolutionary biologists tend to underutilize the experimental approach. Of course, I realize that evolutionary biology is, first and foremost, an historical science and must rest primarily on comparative and paleontological data.)

1. Experimental Advantages of *E. coli*. First, *E. coli* are very easy to grow and count. Second, they grow in simple environments that are easy to control and manipulate, for example by varying culture temperature or resource supply. Third, *E. coli* have rapid generations, allowing experiments to last hundreds or thousands of generations; and they can have large populations, providing a substantial input of mutational variation. The resulting supply of mutations is quantified in Section I.B.4 below.

Fourth, one can preserve cells in an ultra-low freezer, and later resurrect them as needed. Thus, one can compare ancestral and derived forms directly, at any time, without relying on the possibility that protocols or conditions have subtly changed over the years. Not only can one store the original ancestor, but one can store samples from intermediate times in the experiment. These samples contain not mere clones but, in effect, the entire population (less the fraction used to propagate the population). Therefore, if one discovers that something interesting has changed in some later generation, then one can go into the "frozen

fossil record" to discover when the difference emerged, what allele frequencies were at various times, and so on. And when an accident disrupts the experiment (say, a malfunctioning incubator or contamination), one can simply restart the populations from the most recent samples. (When I switched from studying insects to bacteria, I was obviously attracted to the rapidity of bacterial generations. But I realize now that the ability to store and revive organisms and populations is just as important for this type of research.)

Fifth, the *E. coli* strain used in this experiment is strictly clonal. As mentioned earlier, many bacteria undergo parasexual processes, but the strain used in this study lacks the requisite mechanisms to do so. As a consequence of this clonality, one can place a genetic marker in a particular background and have it remain there. By placing different states of an immobile marker in the replicate populations, we protect against the possibility that inadvertent cross-contamination could go undetected and thus not be corrected by re-starting lines. Also, the immobile marker allows us to mix two populations, which brings us to the next advantage. Sixth, one can directly measure the mean fitness of derived bacteria relative to their ancestor. Fitness assays are head-to-head competition experiments, typically between a derived population and its ancestor, except the ancestor carries a neutral genetic marker that allows it to be readily distinguished on an appropriate medium. Section I.B.3 provides more detailed information on how we define and measure relative fitness.

Seventh, *E. coli* has long served as a model organism in genetics, genomics, molecular biology, biochemistry, and cell physiology. Hence, there is a wealth of information about this organism that can potentially help us interpret our own findings (Neidhardt et al. 1996; Blattner et al. 1997).

2. Experimental Design and Conditions. The experiment includes 12 replicate populations, all founded from the same ancestor, except for a single genetic marker described below. To start the experiment, the bacteria were plated as single colonies, each derived from a single cell, and then a separate colony was used to inoculate each population. Thus, each population was founded from a single cell, and hence each population has depended entirely on new mutations for its subsequent evolution (Lenski et al. 1991).

Six of the populations are unable to catabolize the sugar arabinose. The other six have a point mutation in the *ara* operon that allows them to grow on arabinose. The Ara marker is selectively neutral under the conditions of our experiment (as shown by many control fitness assays),

but it serves two purposes. First, as described in Section I.B.3, it allows us to distinguish two genotypes or populations when they are deliberately mixed to measure their relative fitness. Second, in the course of propagating our lines, we always alternate between Ara^- and Ara^+ lines. Therefore, any inadvertent cross-contamination event would introduce the wrong marker state, which can then be detected and corrected by restarting the affected populations from frozen samples. This protection has allowed us to be confident that even parallel changes are in fact evolutionarily independent (although it has become less critical as our genetic analyses have now identified other molecular markers that evolved during the course of the experiment and uniquely identify each population).

The bacteria live in a liquid, buffered, minimal-salts medium supplemented with glucose as the sole source of carbon and energy (Lenski et al. 1991). Glucose is limiting to bacterial density, and it is supplied at a concentration of 25 μg/ml which, although much lower than that used in most microbiological experiments, allows the bacteria to reach a density of about 5×10^7 cells per ml when the glucose is depleted. Each culture is 10 ml, so that the final population size is about 5×10^8 cells. The cultures are held in small Erlenmeyer flasks and incubated at 37°C.

Every day, each population is serially transferred by diluting 0.1 ml into 9.9 ml of fresh medium. This basic rhythm has continued, day in and day out, for over a decade now (with a few interruptions as described below). The 100-fold dilution and re-growth allow $\log_2 100 = 6.64$ generations of binary fission per day. In fact, the bacteria grow and deplete the available glucose in the first eight hours or so, and then spend the remainder of the day in stationary phase. (In principle, we could transfer the bacteria more often, or dilute them more than 100-fold each day, and thereby increase the number of generations. However, daily transfers are logistically simpler, and imposing a larger dilution would reduce the effective population size.) To the extent that there is cell death after the glucose is depleted, the number of generations might be even more than 6.64 per day; in fact, however, the founding strain experiences no appreciable mortality over the course of a day of starvation (Vasi et al. 1994). Fifteen days, therefore, allow about 100 generations, and some 2,400 generations occur in a year.

Samples of all the evolving populations have been stored at intervals, initially every 100 generations and later at 500-generation intervals. Samples are stored at –80°C, with glycerol added as a cryoprotectant. We have always been readily able to revivify bacteria, even after more than a decade in storage. There is no evidence from either performance measures or genomic analyses that the bacteria have mutated during their

storage. An important feature of our storage regimen is that we freeze entire populations, and not just individual clones (although we sometimes store clones as well). That is, after a population has been serially transferred, most of the population is left behind; we then add glycerol to that almost complete population (less the 1% that was transferred) and store it away. Thus, we can recover the entire population, not just individual clones, for later analysis or to restart the population if needed.

Over the course of many years, accidents and disruptions of various sorts can and do happen. For example, the experiment was halted for some months when I moved to Michigan State University from the University of California, Irvine, where it began. The experiment has also been restarted from the frozen samples on several occasions when contamination occurred (including cross-contamination of one line by another). Despite disruptions, the bacteria have undergone more than 30,000 generations since the experiment began on 15 February 1988. We are limited more by the time and effort required to analyze the bacteria in meaningful and new ways than by the number of bacterial generations elapsed.

Prior to making any measurements, all clones or populations that will be studied are stored in the freezer, removed simultaneously, and then acclimated to the culture conditions under which the assays will be performed. In effect, all measurements are therefore performed in a "common garden" following several generations of acclimation that eliminate any non-heritable effects of prior growth conditions. In this way, we can be confident that significant differences we observe between clones or populations, whether in fitness or some other property, reflect underlying genetic differences (even before we have identified the responsible mutations).

I am often asked about the ancestral strain and what environment it had evolved in prior to the start of the long-term experiment. The so-called B strain used in the long-term evolution experiment had already been used in laboratories for several decades, during which time it was grown in many different media and stored for periods, either under starvation conditions at room temperature (the old-fashioned method) or in a freezer where cells are metabolically inactive (the modern approach). Some adaptive evolution undoubtedly occurred in the laboratory prior to the start of the long-term experiment, although the opportunity for adaptation to these conditions was certainly small compared with the millions of years that its *E. coli* ancestors spent in, and moving between, the digestive tracts of their vertebrate hosts. More importantly for our purpose here, the environment in the long-term experiment is

clearly novel from the perspective of the bacteria, not in the sense that any one aspect (e.g., presence of glucose) has been encountered for the first time, but rather in the combination of all the factors, including their uniformity from day to day.

3. Defining and Measuring Fitness. We measure relative fitness by competing two clones or populations against one another. These competitions are performed as separate experimental assays, and do not impinge on the continuing propagation of the long-term lines themselves. By counting the number of the two competing types at the beginning and the end of a competition experiment, we can calculate the net growth rate that the bacteria of each type achieved while they competed with the other type for the common pool of resources (Lenski et al. 1991). In our standard serial transfer regime, differences in net growth rate could reflect, in principle, not only differences in exponential growth but also differences in the duration of the lag prior to the commencement of growth, the effect of diminishing resources on growth, and survival after the resources have been depleted. Relative fitness is then simply defined as the ratio of the two competitors' net growth rates.

Using hypothetical numbers for simplicity, consider the following example. An evolved population and genetically marked ancestor (see below) are mixed at a 1:1 ratio, with the initial density of each 2×10^5 cells per ml. Over the course of a day, they collectively grow 100-fold because the assay is performed using the same glucose-limited medium and dilution factor as in the long-term evolution experiment. However, the ratio of the two competitors changes during the competition, such that at the end of 24 h the ratio is 3:1, with final densities of the evolved and ancestral types being 3×10^7 and 1×10^7 cells per ml, respectively. The realized net growth rate of the evolved competitor is calculated as $\log_e [(3 \times 10^7)/(2 \times 10^5)] \cong 5.01$ per day, while the corresponding rate for the ancestor is $\log_e [(1 \times 10^7)/(2 \times 10^5)] \cong 3.91$ per day. Thus, the fitness of the evolved population relative to the ancestor is $5.01/3.91 \cong 1.28$, which is a dimensionless quantity because the units cancel. Notice that this quantity is smaller than the 3-fold change in the relative abundance of the two competitors from start to finish, because the competition assay compounds the difference in growth rates over several generations.

In this review, I will often discuss the mean fitness of an evolved population relative to the ancestor, usually as measured under the same environmental conditions as used in the long-term evolution experiment (unless otherwise stated). However, one can also compete an evolved population against the ancestor under some other conditions than those

used in the evolution experiment, and thereby examine how adaptation to one environment produced correlated changes in fitness measured in a different environment.

A key operational issue is how we distinguish our two competitors. For example, when we mix and compete an evolved population against the ancestor, how can we tell them apart in order to calculate their net growth rates and hence fitness? The Ara genetic marker that was mentioned earlier provides the key. Ara^- and Ara^+ cells produce red and white colonies, respectively, on an appropriate agar medium. Thus, we can tell any two competitors apart provided that one is Ara^- and the other Ara^+. For example, when measuring the fitness of an evolved population relative to the ancestor, we always compete the evolved population against the ancestral clone that carries the opposite marker. The six Ara^- populations compete against the Ara^+ ancestor, and the six Ara^+ populations compete against the Ara^- ancestor. The Ara marker has been shown repeatedly to be selectively neutral in our standard glucose-limited environment, and whenever we run experiments in other environments suitable controls are included. Finally, although we distinguish the two competitors on an agar-based medium, that medium is used only to distinguish and count the bacteria; the actual competition occurs in the liquid environment. (In principle, two competitors might differ in their plating efficiency, that is the fraction of cells in liquid that produce colonies on the agar medium. In fact, however, any such difference has no effect on calculated growth rates, and hence relative fitness, provided only that each competitor's plating efficiency is the same in the initial and final samples. To ensure that this condition is fulfilled, we estimate both initial and final densities at the same fixed point in the population cycle, when all cells are in stationary phase.)

4. Number of Mutations per Population. Just how many mutations have occurred in any one of the evolving populations over the course of 20,000 generations? To answer this question, it is critical to distinguish between the number of mutational events, the vast majority of which are lost from the population, and the number of mutations substituted in the population by the combined effects of natural selection and random drift.

Taking each definition in turn, the number of mutational events across the entire genome is equal to the product of the population size, number of generations, base-pair mutation rate, and genome size. The size of each population fluctuates daily between about 5×10^6 and 5×10^8 owing to the dilution and subsequent re-growth. The effective popula-

tion size, expressed in terms of the process of origin and substitution of beneficial mutations, is approximately equal to the minimum (bottleneck) population size times the number of generations between minimum and maximum sizes: $5 \times 10^6 \times \log_2 100 \approx 3 \times 10^7$ (Lenski et al. 1991). The mutation rate for *E. coli*, assuming normal DNA repair and editing (which are functional in the ancestral strain), has been estimated to be about 5×10^{-10} per base-pair (Drake et al. 1998), although evidence from our own experiment suggests a somewhat lower ancestral rate of about 1×10^{-10} per base-pair (Lenski et al. 2003). The genome length for *E. coli* is about 5×10^6 base-pairs (Blattner et al. 1997). Thus, the total number of mutations per population during 20,000 generations is expected to be approximately 1.5×10^9 using the higher mutation rate, and about 3×10^8 using the lower rate. That is, several hundreds of millions of point mutations have appeared in each population, even after adjusting for the transfer bottleneck. In addition to these point mutations, other types of spontaneous mutation can also occur, including insertions, deletions, and inversions; many of these larger events involve insertion sequences, or IS elements, that are present in most bacteria.

That is certainly a lot of mutations. In fact, with a genome length of 5×10^6 base-pairs and three alternative base-pairs per position, only 1.5×10^7 point mutations are even possible. Thus, each population has had most point mutations represented many times over. Of course, this calculation does not imply that every possible sequence has ever existed—far, far from it—because each mutation occurs against a relatively few genetic backgrounds. The backgrounds change as the population evolves, but as we shall see below, that vast majority of the genome does not change in the majority of cells.

At first glance, this mutational redundancy might suggest that each population should have "tried" and substituted any mutation that is beneficial generally across all backgrounds. But while the populations should repeatedly try all beneficial mutations, that does not ensure their substitution. As Haldane (1927) showed, most beneficial mutations are lost by random drift before they become common enough for selection to drive them to fixation; the probability of substitution of a beneficial mutation is only about $2s$, where s is its selective advantage. Thus, a mutation that confers a 10% advantage will be lost by drift about 80% of the time, whereas one with only a 0.5% advantage will disappear 99% of the time. Twenty-fold mutational redundancy should overcome drift loss in the former case, but not in the latter. The situation is made even more complicated, and the fixation probabilities further reduced, by the fact that the populations studied here are asexual, which leads to a

phenomenon called clonal interference (Muller 1932; Gerrish and Lenski 1998). In essence, clonal interference occurs because beneficial mutations may arise in two or more different clones but, in the absence of recombination, only one of them can ultimately go to fixation. In general, mutations with only weak beneficial effects, even those lucky enough to escape drift loss, require a very long time to become fixed or even numerically dominant, and thus a clone bearing a weakly beneficial mutation will usually be out-competed by some more beneficial mutation that appears on another background. Thus, the 2*s* probability of fixation calculated by Haldane is, in fact, an upper limit.

Given that a billion or so mutations appear in each population during 20,000 generations, how many are likely to have been substituted? It is difficult to give a precise answer, but we can consider two special cases in order to give us a rough sense of the number of fixations that could reasonably occur by selection and random drift in this amount of time. Let us consider selection first. We will assume that the maximum selective benefit of any mutation is 10%, which accords well with experimental findings that will be given later. Given that a mutation first appears in a single individual, and considering the case where it is lucky enough to survive drift loss or clonal interference, it will take about 250 generations for the mutant genotype to achieve a frequency of 50% in the population, at which point it is more likely to get the next beneficial mutation that comes along instead of competing with it (Lenski et al. 1991). Assuming that beneficial mutations are neither so rare as to require a very long waiting period given the population size, nor so common that beneficial mutations often occur in minority subpopulations, then we can imagine "stringing together" 20,000/250 = 80 beneficial substitutions, each of 10% effect, in a 20,000-generation period. In fact, however, the real number of beneficial substitutions is almost certainly much less than this number, as evidenced by waiting periods between substitutions and a pronounced deceleration in the rate of adaptation over the course of the experiment. I would hazard the estimate that between 10 and 20 beneficial mutations have been substituted in each population.

Turning to drift, the steady-state rate of substitution of neutral mutations is equal simply to their genome-wide rate of occurrence (Kimura 1983). Using the genome size and even the higher base-pair mutation rate given earlier, and assuming that most mutations are neutral, one expects a neutral substitution rate of only about 0.0025 per generation. Over 20,000 generations, that rate would allow about 50 neutral substitutions per evolving population. The number would be somewhat lower if a substantial fraction of mutations are deleterious, but that does not appear

to be the case (Kibota and Lynch 1996). The situation is complicated (but less so than it first seems) by the fact that a drift substitution requires on the order of N generations, where N is population size. Given that the bacterial population size is in the millions, and the experiment has run for only thousands of generations, there has not been enough time for a drift substitution starting from a homogenous initial state. But on-going selection of beneficial mutations actually simplifies things, because each selective substitution traces back to an individual cell in an asexual population, and hence the drift-effective size of these populations is much smaller than their nominal size. (This drift-effective population size is a different quantity from the effective population size that matters for the origin and fate of beneficial mutations themselves.) These selective substitutions participate in driving out neutral mutations that occur in other clones, but the occasional neutral mutation will hitchhike to fixation with the beneficial mutation that appears in the same genome. By lowering the drift-effective population size, this selective process dramatically shortens the time required for a particular neutral mutation to be substituted. However, this same selection does not affect the overall rate of neutral substitutions, which is independent of population size (Kimura 1983).

Combining beneficial and neutral substitutions, it seems probable that fewer than 100 mutations could have been substituted in each population, out of the billion or so that occurred. Thus, the bacterial genomes will have changed very little in the broad scheme of things, which is reassuring given that a decade is a mere "drop in the bucket" in terms of molecular evolution. It is also why the hunt for mutations is difficult in the context of evolving populations that started with no standing variation. Finally, let me mention in advance that several, but not all, of the populations evolved genetic defects in their DNA repair pathways that caused a large increase in their mutation rate. Therefore, the number of mutations, especially neutral ones, substituted in those populations should be greater than suggested by the calculations above. We will return to this interesting complication in Section II.C.

II. PHENOTYPIC AND GENOMIC EVOLUTION

In principle, it might be nice to separate sections on phenotypic and genomic evolution, but such separation is not feasible or appropriate for two reasons. First, the distinction is imprecise because certain mutations are discovered by virtue of having observed particular phenotypic changes. Second, one of the main goals of the genomic analyses is to

examine the coupling between phenotypic and genomic changes, in terms of both overall dynamics and the effects of particular mutations on phenotypic changes. Nonetheless, the overall trend of this main section will be from the phenotypic to genomic levels.

A. Relative Fitness

In a number of experiments over the years, we have measured the temporal trajectory for fitness of the evolving populations relative to their common ancestor. Several important features of the data are as follows. First, as shown in Fig. 8.1, the average fitness gain for the 12 populations after 20,000 generations is about 70% (Cooper and Lenski 2000). That is, during competition between an evolved population and its ancestor, the evolved bacteria undergo approximately 1.7 cell doublings for every cell doubling by the ancestor.

Second, the rate of improvement in fitness has tended to decelerate over time. Lenski et al. (1991) found that the rate of improvement was lower between generations 1,000 and 2,000 than it was between 0 and 1,000 generations in all 12 populations. The average gain in the earlier period was about twice that in the later period. Lenski and Travisano

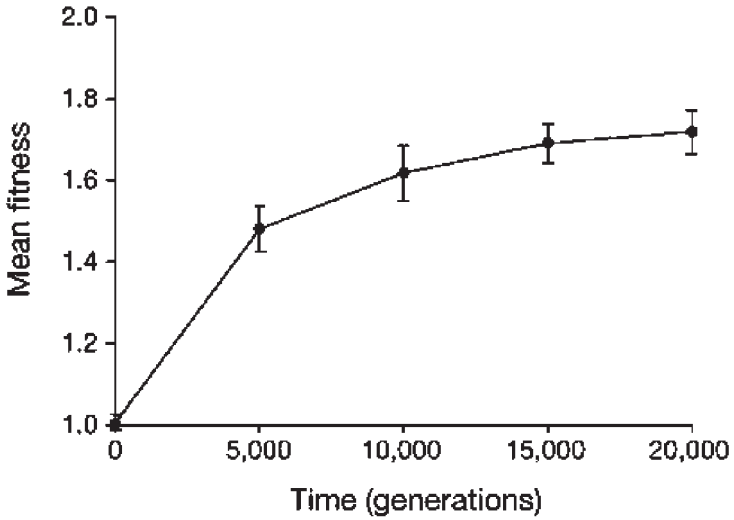

Fig. 8.1. Trajectory of mean fitness, averaged over 12 evolving populations of *E. coli*, through 20,000 generations. Fitness is expressed relative to the ancestor, and it measures the ratio of growth rates realized during direct competition in the selective environment. Error bars are 95% confidence intervals. Source: Cooper and Lenski 2000. Reprinted with permission of *Nature.*

(1994) extended this finding of decelerating adaptation to 10,000 generations. Most recently, Cooper and Lenski (2000) reported fitness trajectories through 20,000 generations, and calculated that the average rate of improvement relative to the ancestor between 15,000 and 20,000 generations was only about one-tenth the average rate during the first 5,000 generations. Nonetheless, there was significant improvement even during this last interval, indicating that adaptation had slowed down but not stopped.

Third, the fitness trajectory for individual populations is not a smooth curve, but instead follows a step-function (Lenski et al. 1991; Lenski and Travisano 1994), as illustrated in Fig. 8.2. The step-like increases are produced when beneficial mutations sweep through an evolving population, and the observed dynamics accord well with mathematical models of the process of genetic adaptation in asexual populations (Lenski et al. 1991; Gerrish and Lenski 1998; see also Orr 1998). [The step-like trajectory also shows that punctuated evolution can occur very simply, although it has been debated whether these data are relevant to the punctuated-equilibrium model based on the fossil record (Gould and

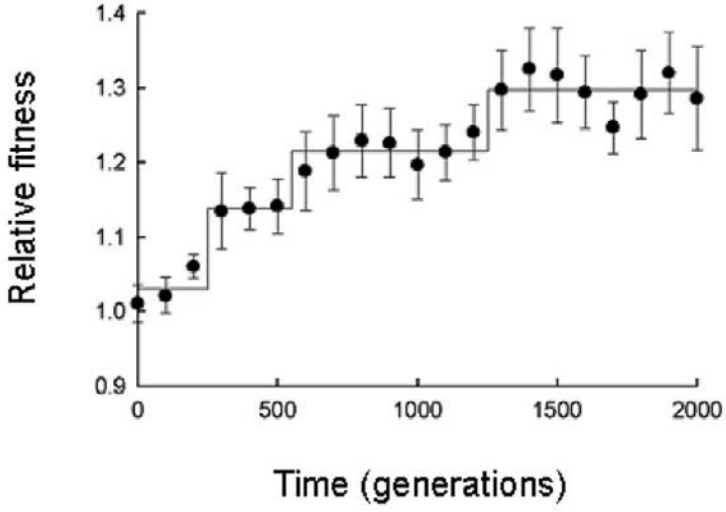

Fig. 8.2. Trajectory of mean fitness in one evolving population through 2,000 generations. The step-like function provides a better fit than simpler models. Each step presumably represents the spread of a beneficial mutation of large effect. The overall trajectory appears step-like owing to the underlying dynamics of mutation and selection in asexual populations, and given the 100-generation sampling interval. Error bars are 95% confidence intervals. Source: Lenski and Travisano 1994. Copyright 1994 National Academy of Sciences, U.S.A.

Eldredge 1977; Elena et al. 1996; Coyne and Charlesworth 1996; Gould 2002).] The steps are observed only in the trajectories for individual populations, because beneficial mutations occur stochastically and replicate populations are not synchronized. Also, resolving individual steps requires intensive replication of the fitness measurements at a relatively fine temporal scale, and the larger steps that occur early in the evolution experiment are therefore easiest to discern. The early steps produce fitness gains of roughly 10% relative to the ancestor. Given the population size in the experiment, a mutation that appears in an early generation and confers a 10% advantage (and escapes loss by genetic drift) will still be a small minority after 200 generations, but by 300 generations it will comprise the vast majority. Thus, if fitness assays are made every 100 generations, the trajectory will appear to jump between 200 and 300 generations. Longer periods for ascendancy are required if there is a substantial waiting time for a beneficial mutation to appear, and for mutations that confer smaller benefits. Also, owing to the asexuality of the evolving populations, each beneficial mutation that is substituted effectively purges the standing genetic variation in the population, as noted before in the context of neutral mutations. This purging includes other beneficial mutations that appeared in other backgrounds, but which have smaller beneficial effects than the one that ultimately prevails, a phenomenon called clonal interference (Muller 1932; Gerrish and Lenski 1998).

Fourth, the fitness trajectories for the 12 replicate populations are very similar in their overall form and the extent of improvement, but they are not identical. Lenski et al. (1991) showed that, during the first 2,000 generations, the among-population variance component for fitness was significant and corresponded to a standard deviation of about 3%, or approximately one-tenth the change in mean fitness over that period. Lenski and Travisano (1994) also found fitness variation of several percent among the replicate populations through 10,000 generations. Although Cooper and Lenski (2000) did not specifically address this issue, an analysis of their data indicates that significant among-population variation in mean fitness persisted throughout 20,000 generations. Thus, there exists subtle variation in performance among the 12 replicate populations, despite their similar trajectories.

Fifth, in addition to the variation in mean fitness among the replicate populations, there also exists significant variation among clones sampled from the same population. Lenski et al. (1991) quantified the within-population variance for fitness in the first 2,000 generations. They found that the observed variance was not significantly different from the variance that must be produced by the on-going substitution of

beneficial mutations, a quantity that they calculated using Fisher's fundamental theorem. Elena and Lenski (1997) performed a similar analysis at 10,000 generations. They found that the within-population variation in fitness had not changed much since 2,000 generations. However, because the rate of adaptation had decelerated so much over that time, the on-going substitution of beneficial mutations no longer provided a sufficient explanation for the observed level of variation. They considered two alternative explanations, according to which the within-population variation in fitness was maintained by the balance between deleterious mutations and selection, or by frequency-dependent selection. The former also was insufficient to account for the observed variation. However, they observed a tendency for clones to have advantages when rare relative to other clones in their population, which could account for this variation. In five of the six populations examined, the average advantage-when-rare tended to be very small, about 1–2%. However, in one population the average clonal advantage-when-rare was near 7%. Rozen and Lenski (2000) studied this population in detail and found that it contained two distinct ecotypes that stably coexisted. One of the two ecotypes was superior at exploiting the glucose that was provided in the medium, but it also secreted a metabolite that the other ecotype could better use. Thus, frequency-dependent selection enters into the dynamics even in this very simple system. However, it should also be emphasized that the magnitude of these frequency-dependent effects are usually quite small compared with the large improvements relative to the ancestor.

Sixth, and finally, one can ask whether the pronounced deceleration in the rate of fitness improvement is an artifact of performing competitions between the evolved lines and their ancestor. For example, in a study of evolving yeast populations, Paquin and Adams (1982) uncovered non-transitive competitive interactions. In their study, the fitness of evolved yeast clones measured relative to the original ancestor sometimes declined, whereas a clone's fitness measured relative to its own immediate predecessor always increased. Various indirect lines of evidence argue against the importance of this phenomenon in the long-term experiment with *E. coli*, including the relatively weak and negative frequency-dependent effects described above, as well as the observation that absolute fitness components, including exponential growth rate and the duration of the lag prior to growth, consistently improved during the evolution experiment (Vasi et al. 1994). We also performed some 300 competitions between samples from various generations of the same population, to test the possible contribution of non-transitive effects to the fitness trajectory (De Visser and Lenski 2002). In short, these data

accord very well with transitive interactions. Therefore, the decelerating trajectory of mean fitness measured relative to the common ancestor indicates a real and pronounced decline over time in the rate of adaptation, as opposed to an inability to detect further adaptation based on competitions with an increasingly distant ancestor.

B. Cell Size and Yield

One of those most conspicuous changes in the evolving *E. coli* populations is the average size of individual cells. Without knowing the direction of change in advance, one would probably guess that individual cells have become smaller. The evolved cells are growing and dividing much faster; and smaller cells have a higher surface-to-volume ratio, all else equal, which would be favorable for nutrient transport into the cells. In fact, however, the average cell size substantially increased over time in all 12 populations (Lenski and Travisano 1994; Vasi et al. 1994; Elena et al. 1996; Lenski and Mongold 2000). After 10,000 generations the grand mean cell volume was about twice that of the ancestor, as shown in Fig. 8.3.

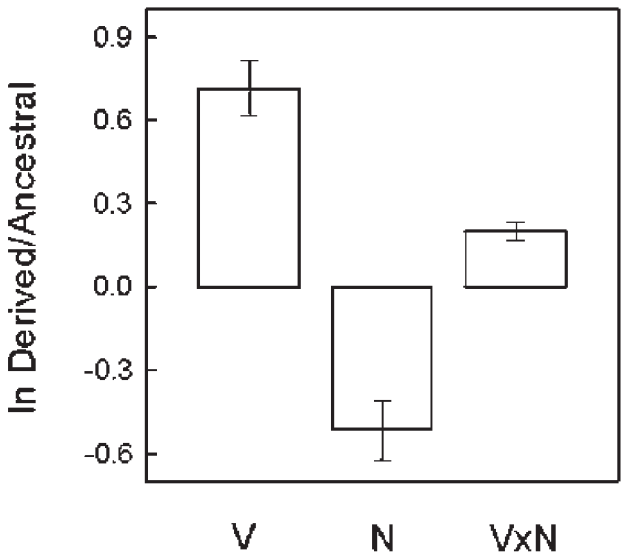

Fig. 8.3. Evolved changes after 10,000 generations in average cell volume (V), cell number at stationary phase (N), and biovolume yield (V×N). Each value is the mean over 12 populations of the ln-transformed ratio of evolved to ancestral values. Error bars are 95% confidence intervals. Source: Lenski and Mongold 2000. Used by permission of, and copyright 2000 by, Oxford University Press.

The trajectories for cell size and relative fitness are quite similar in their general form, in both cases changing much faster early in the experiment than late. Moreover, the step-like gains in fitness are temporally associated with corresponding changes in average cell size, implying that the mutations responsible for adaptation often caused a correlated increase in cell size (Elena et al. 1996). However, the evolved populations are more variable in their average cell volume than in their fitness (Lenski and Travisano 1994). Also, a few populations produce cells that are more spherical than the ancestor, although in most populations the overall rod-shape is similar to the ancestor (Lenski and Mongold 2000).

What can account for these unexpected changes in cell size and shape? We do not fully understand the relationship between the changes in cell morphology and fitness, although we can exclude several plausible explanations and thereby gain some understanding that may guide future work. Our initial cell-volume measurements were made on stationary-phase populations, when the bacteria have exhausted the glucose and ceased growth. Therefore, one plausible hypothesis for the larger cell size in the evolved populations is that the cell cycle has been altered such that cells stop completing their divisions as glucose becomes limiting and thereby produce more "doublets" at this stage. This hypothesis is rejected by the observation that the evolved cells are also larger in exponential-phase growth (Vasi et al. 1994) and, moreover, the evolved populations do not contain any more doublets at stationary phase (Lenski and Mongold 2000). A second plausible hypothesis is that the cells, while larger in volume, may not actually contain more biomass but instead might be larger sacks of water. Such an effect could result from some change in osmoregulation, for example. However, this hypothesis was rejected by measurements of the three main macromolecular constituents—protein, RNA, and DNA, which comprise >75% of the cell dry weight—that showed their concentrations are similar in the ancestral and evolved cells (Lenski et al. 1998).

It has been known for many years that any given strain of *E. coli* (ignoring evolution for the moment) produces larger cells when it is growing fast than when it is growing slow. This effect may seem counterintuitive but nonetheless it occurs, apparently because faster-growing cells are stuffed with more ribosomes and more copies of their replicating genome. (*E. coli* is genetically haploid, with a single circular chromosome, but an individual cell has several copies of its chromosome when it is growing under favorable conditions.) Hence, a third plausible hypothesis for the larger cell size of the evolved populations is that this change is simply an extension of the phenotypic correlation between growth rate and cell size into a genetic context. In other words, the long-term experiment

selected faster-growing cells, faster-growing cells tend to be larger, and thus their larger size is another manifestation of this phenotypic correlation. At first, it was not obvious to us how we could test this hypothesis, which requires disentangling the phenotypic and genetic correlations. However, it was possible to test it using a chemostat, which is a continuous-culture device that allows one to vary growth rate by changing flow rate through an open system. In particular, we could force evolved and ancestral clones to grow at the same rate in separate chemostats, and ask whether they achieved the same cell size, as this hypothesis would predict. We performed this experiment across a range of different growth rates and saw the expected positive phenotypic correlation between growth rate and cell size in both ancestral and evolved clones (Mongold and Lenski 1996). Nonetheless, the evolved cells were larger even when they grew at the same rate as the ancestor. This phenotypic correlation is thus consistent with, and contributes to, the increased size of the evolved cells. However, it is not sufficient to account for even half of the observed evolutionary change in cell size, based on the allometric relationship between growth rate and cell size measured for the ancestral genotype.

A fourth hypothesis is that we somehow selected inadvertently for larger cells. However, there is nothing in our procedures that would directly select cells on the basis of their size; for example, there is no filtration that could differentially retain larger cells, nor did we ever inspect cells visually as a basis for propagation.

A fifth possibility is that larger cells can acquire more resources. Although a larger cell has greater exposed surface than a small one of the same shape, the larger cell also has a reduced ratio of surface area to volume, which is allometrically unfavorable for resource acquisition in a constant environment. That is, a smaller cell that produces two daughter cells of the same small size should be able to acquire the necessary resources faster than a larger cell that produces two daughter cells of the same large size, all else being equal. However, the culture conditions in the long-term evolution experiment included a daily cycle of feast and famine as populations were diluted into fresh medium and then exhausted the glucose. It is possible that, in such fluctuating conditions, larger cells may have an advantage by virtue of their greater total surface area, which could allow them to acquire and sequester more glucose than they can immediately use, and then convert that surplus into progeny as the resources became depleted. Or perhaps larger cells, by virtue of having greater metabolic reserves, can respond more quickly to the sudden availability of resources and thereby commence growth more quickly (Lenski and Mongold 2000).

A sixth hypothesis is that the optimal cell size of *E. coli* in its natural environment is smaller than the optimum under the experimental regime, and hence the populations evolved larger cells. Unfortunately, the forces that shape cell size in either case are unknown, although a speculative scenario may serve to illustrate this hypothesis. In nature, *E. coli* is subject to attack by lytic viruses and other consumers, and one could imagine that size-selective predation favors cells that are smaller than would otherwise be optimal. But in our experimental regime, these consumers are absent, which would release the bacteria from size-selective predation and thereby favor larger cells. Let me reiterate that this scenario is only speculative, and it has not yet been tested in any way.

The three previous hypotheses imply selection on cell size per se. A seventh hypothesis is that the cell volume changes are merely a correlated response to some other more fundamental physiological change, despite the consistent pattern of increases in all 12 populations. (The third hypothesis, based on the physiological relationship between growth rate and cell size, is perhaps a specific example of this more general hypothesis.) For example, as will be described in Section II.D, the evolving populations have tended to become ecological specialists by down-regulating various catabolic pathways that are unnecessary in the experimental environment. Such changes are advantageous because they channel cellular metabolism to more productive pathways. Graña and Acerenza (2001) have recently produced a model of the bacterial cell cycle that suggests this reduction in wasteful expression can explain the observed evolution of larger cells. Their model fits nicely with several features of this system, although some independent test of its predictions would be desirable (for example, showing an evolutionary reduction in cell volume in a complex environment that favored expanded physiological capabilities). In any case, the model of Graña and Acerenza provides a plausible example of how changes in cell size may arise from selection on some general aspect of cell physiology and performance.

Whatever the reason (or combination of reasons) for the evolution of larger cells, this outcome appears to be closely connected with another change that also seems counterintuitive. The evolved populations yield fewer cells than the ancestor when they are grown separately under the standard conditions of the evolution experiment (Fig. 8.3). One might think that the evolved populations should produce more cells than the ancestor; after all, they have become more fit. Evolved cells do generally out-number the ancestors at the end of a competition assay, but not when they are grown separately. This difference has nothing to do with frequency-dependent selection or other complex interactions. It arises

because relative fitness does not measure the difference in numbers of organisms per se, but rather it measures the difference in the net rates of change in those numbers. All 12 evolved populations produce fewer, but larger, cells. The evolved cells each acquire and consume more of the limiting glucose, at a rate that is evidently disproportionate even to their larger volume. As a consequence, the larger evolved cells can exclude their smaller ancestors in the scramble competition for this limiting resource.

These two rather unexpected changes, larger cells that are fewer in number, combine to produce another change that is more readily understood. The product of average cell volume and numerical yield—that is, total biovolume produced—increased significantly in all 12 populations (Fig. 8.3). Coupled with the fact that concentrations of the main cell constituents remained nearly constant during the evolution experiment, this increased biovolume implies improved efficiency in the physiological conversion of the limiting glucose to biomass. Also, although the evolved populations are quite variable in both their average cell volume and numerical yield, these two traits vary inversely, such that total biovolume is almost constant (Lenski and Mongold 2000). This constancy suggests that the evolved populations have reached some limiting efficiency, but have done so by different routes.

C. Mutation Rate

Mutation is sometimes discussed as though it were an unavoidable consequence of copying mistakes during DNA replication. In fact, however, most organisms have exquisite molecular machinery that can identify and correct incipient errors, before they become mutations, using enzymes that proofread and repair DNA (Friedberg et al. 1995). When an organism's repair pathway malfunctions, such as by a mutation in one of the underlying genes, then its genomic mutation rate increases, often dramatically. [As an aside, it is interesting that many genes involved in DNA repair in bacteria have homologous genes in humans, and mutational defects in these homologous genes have been associated with certain cancers (Friedberg et al. 1995).]

One of the most interesting changes that has occurred in the long-term *E. coli* experiment is that several populations have evolved defects in their DNA repair (Sniegowski et al. 1997). These defects were demonstrated as follows. First, Luria-Delbrück fluctuation tests were run to measure the rate of spontaneous mutation at two or three genetic loci in which mutations produce a phenotypic change that is easily scored.

After 10,000 generations, three of the 12 populations exhibited greatly elevated mutation rates at each locus tested, whereas the other nine retained mutation rates similar to the ancestor, as shown in Fig. 8.4 for one locus. Across the loci tested, the increase in mutation rate in the "mutator" lines was about 100-fold. Second, such elevated mutation rates generally result from a loss of function, and thus genetic complementation tests were performed. Clones that possessed the mutator phenotype were made mero-diploid by introducing, one at a time, plasmids that encode functional copies of one of the genes involved in DNA repair. These tests revealed that the three hypermutable lines had become defective in the methyl-directed mismatch repair pathway, although in two different loci (Sniegowski et al. 1997). These genes were later sequenced to identify the precise mutations in the mutator lines (Shaver et al. 2002). Finally, clones isolated from earlier time points were tested to determine when hypermutability had evolved. In two populations, the mutator type became numerically dominant fairly early (around generations 2,500 and 3,000), and in the other population somewhat later (around 8,500 generations). All three lines that became mutators retained this phenotype through generation 20,000. A fourth population evolved a mutator phenotype after 15,000 generations (Cooper and Lenski 2000). The affected locus and other properties of this

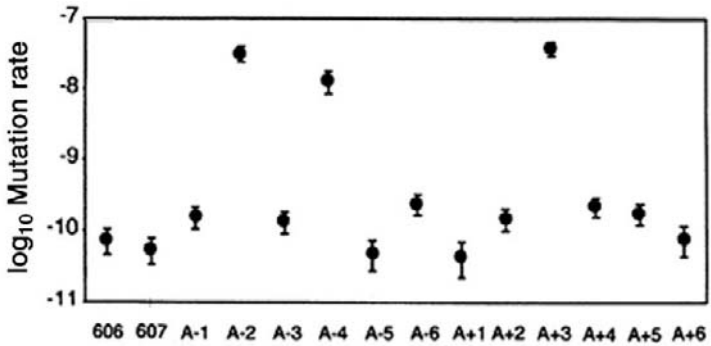

Fig. 8.4. Three of the 12 long-term *E. coli* populations evolved hypermutable phenotypes by 10,000 generations. These data show mutation rates for resistance to an antibiotic, nalidixic acid, measured in the ancestor (606 and 607) and evolved populations (A-1 through A+6). Error bars are approximate 95% confidence intervals. Notice that three evolved populations have rates that are about 100-fold higher than the ancestor or other evolved lines. Genetic analyses showed that these mutators had become defective in the methyl-directed mismatch repair pathway. Source: Sniegowski et al. 1997. Reprinted with permission of *Nature*.

late mutator have not yet been as thoroughly studied as the three that became mutators earlier.

The discovery that several populations evolved hypermutability raises some intriguing questions about both the causes and consequences of this phenomenon. How did the mutations that eliminated DNA repair become common? Their spread is puzzling because most mutations are deleterious; any mutation that increases the rate of other mutations should tend to increase genetic load and would thus be detrimental. Given that mutators did spread in some populations, but not in others, how does this difference affect subsequent phenotypic and genomic evolution? Do the mutator populations, by virtue of increased genetic variation, reach higher fitness than those populations that retained the ancestral mutation rate? And how does the increased mutation rate affect the rate of molecular evolution?

Let us begin by considering the consequences of elevated mutation rates, which will also give us some insight into understanding how the mutations that produced the mutator phenotype spread in their populations. We can assume, to a first approximation, that the rates of neutral, beneficial, and deleterious mutations are equally affected by the increase in mutation rate caused by defective DNA repair. Because the rate of substitution of *neutral* mutations depends only on the corresponding mutation rate (and not on population size or background selection), then we should clearly expect the lines that became mutators to accumulate many more neutral mutations than the other lines.

With respect to *beneficial* mutations, and the resulting rate of adaptation, the expectation is more complex. On the one hand, the waiting time for beneficial mutations to appear will be shorter if the mutation rate is higher. On the other hand, large populations may not spend much time waiting for a beneficial mutation, and the time required for a beneficial mutation to spread to fixation thus becomes important. As noted earlier, the most highly beneficial mutations in this experiment confer about a 10% selective advantage; such a mutation would (assuming it is lucky enough to avoid extinction by drift when it is still rare) require some 250 generations to increase from a single cell to the majority type (Lenski et al. 1991). Beneficial mutations that conferred a lesser advantage would take even longer to become the majority type. That long period spent as a minority, coupled with the clonal nature of the populations, means there is ample opportunity for a *more* beneficial mutation to occur on another background and ultimately out-compete the one that occurred first. As a consequence of this clonal interference, the rate of adaptation in a large asexual population shows diminishing returns

with an increasing supply rate of beneficial mutations. This effect has been confirmed both theoretically (Gerrish and Lenski 1998) and in a separate experiment with bacteria in which mutation rate and population size were manipulated (De Visser et al. 1999). These studies indicate that, for the parameters most relevant to the long-term experiment, the increase in the rate of genetic adaptation caused by a 100-fold increase in mutation rate should be rather small. Consistent with these expectations, the populations that became mutators evolved only slightly higher fitness than the other populations, and even this difference is not statistically significant. The important point here is that any acceleration in the rate of adaptation is far less than proportionate to the increase in mutation rate. Hence, the increase in mutation rate has very different consequences for the substitution rates of neutral and beneficial mutations.

Finally, one would not expect *deleterious* mutations to be substituted by pure drift in such large populations as studied here, unless a mutation has a truly negligible effect. However, a slightly deleterious mutation could be substituted by hitchhiking with a beneficial mutation that occurred in the same background. But, as explained in the previous paragraph, the mutator lines do not undergo many more beneficial substitutions than the other lines. Thus, there are not many more chances for deleterious mutations to hitchhike to fixation in the mutator populations. More important than the substitution of deleterious mutations, however, is the effect of hypermutability on the genetic load resulting from deleterious mutations that have not been fixed. This load will reduce the mean fitness of the mutator lines (a consequence) as well as impede the substitution of mutations that cause the mutator phenotype (a cause that will be examined below). In a haploid population, the theoretical genetic load caused by deleterious mutations is approximately equal to the genomic rate of deleterious mutation, and thus should increase proportionately with the mutation rate (Sniegowski et al. 2000). But because the total genome-wide rate of deleterious mutations was quite low in the ancestor, the load associated with a 100-fold increase in mutation rate is still not very large. The genome-wide mutation rate in repair-proficient *E. coli* is about 0.0025 mutations per genome per generation (Drake et al. 1998), perhaps even lower (Lenski et al. 2003). This includes neutral mutations, and the rate of deleterious mutation estimated from mutation-accumulation experiments is still lower, only about 0.0002 per genome per generation (Kibota and Lynch 1996). Even a 100-fold increase in this rate would produce a genetic load of only 2%. Hence, the mutators do not suffer a huge reduction in mean fitness

associated with an increased genetic load, nor will selection against deleterious mutations be sufficiently strong to impede the spread of mutator alleles that are linked to beneficial mutations of large effect.

Having examined the likely consequences of the hypermutability that evolved in some of the long-term *E. coli* populations, let us return to the question of how a mutation that produced this effect could be substituted given its increased load of deleterious mutations. First, there is no evidence that loss of DNA repair confers any direct fitness advantage (Chao and Cox 1983; Shaver et al. 2002). This result is based on direct competitions between strains that differ only by a mutation in a DNA repair gene, and the small fitness effect that is measured is consistent with the slight increase in genetic load calculated in the preceding paragraph. Second, the idea that hypermutability might be favored because it increases evolvability fails in several respects. Evolution is not a goal-directed process. Group selection may sometimes give rise to features that appear goal-directed, but the large population size and lack of spatial structure in the long-term experiment (populations were constantly dispersed by shaking the liquid medium) provide no meaningful opportunity for group selection favoring greater evolvability to overcome even a 2% higher load of deleterious mutations. What can explain the substitution of mutations causing defects in DNA repair?

The answer appears to rely on hitchhiking, but with an important twist. The mutator allele is deleterious, but it can hitchhike with a beneficial mutation that more than offsets its disadvantage. That statement is true for any deleterious mutation, especially one that causes little harm. The twist is that the mutator allele is much more likely to generate a beneficial mutation in its own background than is the wild-type allele that encodes functional DNA repair. In essence, the mutator promotes its own hitchhiking (Taddei et al. 1997).

Consider the following "back-of-the-envelope" calculations. There are six or so genes in *E. coli* that encode DNA repair functions which, if eliminated individually, would cause an increase in the mutation rate of similar magnitude to what evolved in several of the long-term lines. Together these genes comprise about 12,000 base-pairs that are at-risk for mutation and perhaps one-third of them would, if mutated, disrupt DNA repair. If the base-pair mutation rate in repair-proficient *E. coli* is about 5×10^{-10} per generation, then the overall rate of mutations to produce a mutator is perhaps 2×10^{-6} per generation. In fact, one of the genes involved in DNA repair contains a repeated motif that appears prone to mutations (Shaver et al. 2002), and this rate may therefore be an underestimate. Given this rate of production and a 2% fitness cost of

being a mutator, then the equilibrium frequency of mutators in a haploid population, under mutation-selection balance, should be roughly 1×10^{-4}. This mutator sub-population has a 100-fold higher rate, per capita, of generating the next beneficial mutation that eventually goes to fixation, which would bring the mutator allele along as a hitchhiker. Therefore, the mutator allele should have a probability of about 1% of being substituted along with the next beneficial mutation. That may not seem like much, but remember that each population substituted perhaps 10 or 20 beneficial mutations. Any one of these, at least among those providing a benefit much greater than 2%, would have allowed the mutator sub-population a chance to generate the next winner and then hitchhike along with it. The fact that 4 of the 12 populations became mutators is reasonably consistent with this scenario, especially given the uncertainties associated with exact values.

The main conclusions are paradoxical. Mutators are associated with rapid adaptation, but not because they accelerate genetic adaptation to any great extent in a large population. Instead, rapid adaptation, such as occurs in a new environment, provides more opportunities for a mutator to hitchhike with a beneficial mutation, which the mutator subpopulation may produce just a bit sooner than would otherwise occur. Over a long time, one could imagine this process reversing itself. That is, the populations may eventually be so well adapted to the experimental regime that the cost of deleterious mutations exceeds the benefit of any remaining advantageous mutation. At that point, a mutation that restored DNA repair function would provide a selective advantage, and there would be no beneficial mutations in other clones to interfere with its spread. However, the advantage of this anti-mutator allele would be proportional to the reduction in genetic load, about 2%, and hence its spread would be fairly slow. Most other accessible beneficial mutations conferring a greater advantage would have to be substituted in the population before this process of reversal could effectively commence. Hence, the mutators that have become substituted will probably be difficult to displace.

D. Ecological Specialization

It is clear from the fitness trajectories that the evolved *E. coli* lines have substantially improved their performance in the selective environment. But what happened to their performance in other environments as they adapted to this particular set of conditions? Have they become highly specialized, or do they retain their ancestral performance levels in

other environments? If the bacteria did become more specialized, was this a consequence of pleiotropic tradeoffs associated with the mutations that adapted them to the experimental environment? Or was specialization a consequence of decay by random drift of mutations in genes encoding physiological functions that were unimportant in the selective environment?

The first set of experiments that we performed to address these questions used bacterial clones sampled at generation 2,000 (before any of the populations had become hypermutable). We tested the fitness of these clones relative to the ancestor in a series of environments that were identical in every respect to the selective environment, except that the sole carbon and energy source, glucose, was replaced by one of 11 other substrates (Travisano et al. 1995; Travisano and Lenski 1996). The evolved lines exhibited significant correlated declines in performance on two substrates, and significant correlated gains on five substrates. Interestingly, four of the substrates that showed correlated gains were among the five substrates that share with glucose their route of transport into the cell through both inner and outer membranes. In addition to these differences between substrates in average correlated response, the among-line genetic variance component also differed between substrates. The among-line variance was lower in glucose than in any of the other 11 substrates, indicating that the direct response to selection was more parallel than the correlated responses. There was also a tendency for the correlated responses to be more uniform for the substrates that used the same basic mode of transport as glucose, with greater divergence among populations in their responses to substrates that entered the cell by other pathways.

The second set of experiments to address this general set of issues differed in approach in several ways (Cooper and Lenski 2000). First, the evolutionary time frame extended to 20,000 generations. Second, several time points were examined to allow the trajectory of correlated responses to be examined. Third, many more growth substrates were tested using 96-well plates. Finally, to accommodate the plate format as well as the larger number of samples and substrates, measurements were made of growth kinetics as opposed to competitive fitness per se. These data, summarized in Fig. 8.5, showed a significant trend toward ecological specialization, with "catabolic breadth" (a measure of overall performance on 64 informative substrates other than glucose) declining by 42%, on average, after 20,000 generations. Almost all of the performance losses were quantitative, not qualitative (absolute), with one conspicuous exception: all 12 lines completely lost the ability to grow on D-ribose, as will be discussed in the next section.

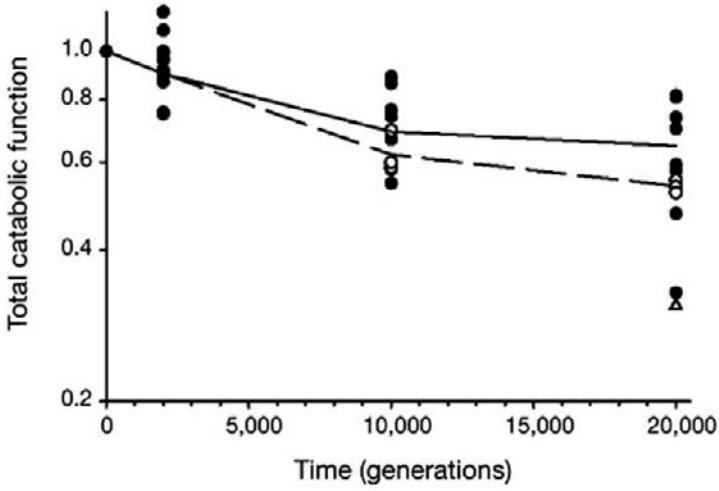

Fig. 8.5. Ecological specialization of the evolving populations indicated by declining catabolic breadth. The populations evolved in a minimal medium that contained glucose as the sole source of carbon and energy. Total catabolic function is a weighted average performance on 64 other substrates, standardized to an ancestral value of 1 and shown on a ln-transformed scale. Each point indicates one of the 12 populations. Closed circles represent populations that retained the ancestral mutation rate; open circles became mutators between 2,000 and 10,000 generations; and the open triangle became a mutator between 10,000 and 20,000 generations. The solid line indicates the mean of the nonmutator populations; the dashed line is the mean of the mutator populations excluding the one that evolved latest. Notice that the rate of decline decelerates in parallel to the rate of fitness gain (Fig. 8.1). Note also that the decline in total catabolic function is slightly faster in the populations that became mutators, but not nearly to the 100-fold extent that their mutation rates increased (Fig. 8.4). Source: Cooper and Lenski 2000. Reprinted with permission of *Nature*.

In principle, two distinct population-genetic processes could account for the trend toward specialization: antagonistic pleiotropy (AP) and mutation accumulation (MA). According to AP, the losses of performance on other resources result from tradeoffs, in which the same mutations that are beneficial in the glucose environment have detrimental effects in other environments. According to MA, the correlated losses of adaptation to the other environments were caused by mutations that drifted (or hitchhiked) to fixation but did not themselves contribute to adaptation to glucose.

Several lines of evidence indicate that AP was more important than MA for specialization that evolved in the long-term experiment (Cooper and Lenski 2000). First, the decay in catabolic breadth was faster early

in the experiment than late. This trajectory mirrors genetic adaptation, which was also more pronounced early than late, and hence supports AP. Second, the decay of catabolic breadth was not significantly greater in those lines that became hypermutable than in those that did not. The 100-fold higher mutation rate in the mutator lines should have led to a corresponding increase in MA and associated specialization by drift decay. The fact that specialization was not dramatically more pronounced in these mutator lines implies that the contribution of MA is secondary to that of AP. Third, much of the observed decay in catabolic breadth was concentrated in a subset of functions, a pattern more consistent with AP than with MA. Fourth, a separate study was performed by another group of losses of catabolic function in *E. coli* populations that were all mutators and, moreover, were subjected to severe bottlenecks (Funchain et al. 2000). Such bottlenecks promote MA by magnifying drift, which prevents the elimination of deleterious mutations and precludes the genetic adaptation that underlies AP. This other study observed no deceleration in the decay rate of catabolic breadth, nor was there much concentration of losses in a subset of catabolic functions. These differences in the trajectory and the pattern of specialization support the importance of AP in our long-term selection experiment. Fifth, as described in the next section, at least some of the mutations that contributed to glucose adaptation contributed to the decay of catabolic breadth, providing a direct demonstration of AP.

In summary, the extensive and largely parallel fitness gains in the glucose environment were accompanied by correlated changes in performance in other resource environments. These correlated responses were more divergent across the replicate lines than was the directly selected performance on glucose, and overall catabolic breadth tended to decline. The decay of catabolic breadth, and the resulting ecological specialization, are more consistent with the effects of antagonistic pleiotropy than with drift decay by mutation accumulation.

E. Ribose Catabolic Function

While performing the analyses of catabolic breadth, we discovered that all 12 populations had lost the ability to grow on D-ribose as a sole carbon source (Cooper and Lenski 2000). When we examined frozen samples to document the time course of these losses, we saw that the lines had invariably lost their ability to use ribose very quickly: seven in the first 500 generations, and all 12 of them by generation 2,000 (Cooper et al. 2001). Finding these rapid and parallel changes immediately suggested that the loss of the ribose catabolic function was highly advanta-

geous in the glucose environment. We therefore set out to discover the genetic bases of these mutations, and to measure their fitness effects independent of other genetic changes that occurred during the evolution experiment.

Early in this work, we made another discovery that surprised us: mutations that cause the loss of the ribose utilization function occur at a surprisingly high rate, about 5×10^{-5} per cell generation based on Luria-Delbrück fluctuation tests, even in a strain with functional DNA repair (Cooper et al. 2001). This rate is more than an order of magnitude higher than what one would expect for point mutations for the entire *rbs* operon. Molecular genetic analyses revealed that the losses of the ribose catabolic function in the evolved lines involved deletions of part or all of the *rbs* operon, as illustrated in Fig. 8.6. One endpoint of the deletion differed in every case, but the other deletion endpoint was precisely the same in all cases, and coincided exactly with the edge of a mobile element, called IS*150*, that happened to be immediately adjacent to the *rbs* operon in our ancestral *E. coli* strain. Evidently, this element was responsible for the genetic instability of the ribose catabolic function.

This localized hypermutability led us to question our initial assumption that such striking parallelism was caused by strong selection. Perhaps instead the parallel losses merely reflected the genetic instability of the *rbs* operon. To examine this issue further, we isolated several *rbs*-deletion mutations on the ancestral background, and we also engineered a deletion genotype not involving the nearby IS*150* element. When each of these Rbs^- genotypes was put in competition with the otherwise identical Rbs^+ ancestor, in the same glucose medium as used in the long-term experiment, the Rbs^- competitor had a fitness advantage of about 1–2% (Cooper et al. 2001). Thus, positive selection for loss of the ribose catabolic function and its genetic instability both contributed to the rapid and parallel losses. A mathematical analysis of the temporal spread of the Rbs^- genotypes, using the measured mutation rate and selection coefficient, showed that neither the hypermutability of that locus nor a 2% selective advantage alone was sufficient to account for the substitution of the Rbs^- mutants in only 2,000 generations. However, the two processes combined could explain the rapid evolutionary loss of the ribose-catabolic function.

F. More and More Genetics

The preceding section gave a concrete example of the type of research that we are most actively pursuing with the long-term *E. coli* populations. We seek not only to find mutations that were substituted in these

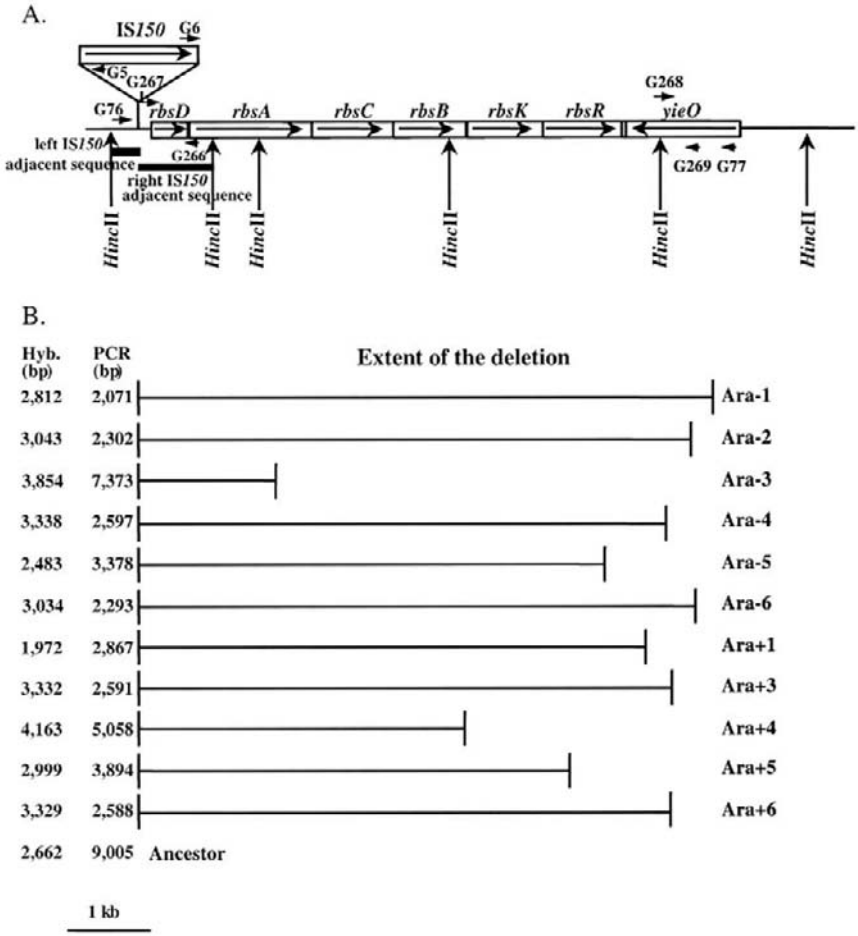

Fig. 8.6. Deletions in the evolving populations of the *rbs* operon led to parallel losses of their ability to grow on D-ribose. A. Map of the *rbs* operon shows an IS*150* element located upstream of the first gene in the ancestral strain. The boxes denote genes, and arrows within them show the direction of transcription. B. Physical extent of deletion mutations in 11 of the populations. All of the deletions have the IS*150* element as their left endpoint, but they have different right endpoints. Source: Cooper et al. 2001. Reprinted with permission of American Society for Microbiology.

populations, but we also want to understand how particular mutations spread and what other traits they influence. To do so, we must reconstruct genotypes that differ only by the mutation at hand, in order to isolate its effects from other mutations that were also substituted in the same evolving line. Are most of the mutations that were substituted neu-

tral or beneficial? Are beneficial mutations found in the same genes and pathways in many or all of the lines, or did each population discover a unique way of adapting to the selective environment? Can we integrate existing knowledge of the genetics and physiology of *E. coli* with the various findings of our long-term evolution experiment? Our work on these fronts is well underway, and we have many interesting results, but some are not yet published. Hence, in this section, I will sometimes speak in general terms about the approaches that we are using to discover mutations. I hope that readers who are interested enough to have read thus far into my review will also be interested in following our genetic discoveries and analyses of mutational effects as they are published over the coming years.

1. IS-Mediated Mutations. Insertion sequences, or IS elements, are mobile elements present in most bacterial genomes. IS elements encode functions that promote their own transposition, and hence they are mutagenic. Through recombination between homologous elements, IS elements also promote rearrangements, including deletions and inversions. The mutations caused by IS elements can be discovered much more readily than point mutations, because IS mutations cause discernible changes in the size of restriction fragments and because knowledge of their sequence allows one to make probes and primers for finding their genomic locations.

Two of the lines were chosen as focal material for investigating IS-mediated mutations. Many clones from several time-points through generation 10,000 were used as study material, and numerous IS-mediated mutations were indeed discovered (Papadopoulos et al. 1999), as shown in Fig. 8.7. The resulting genetic diversity was impressive: in one population all 11 clones tested at generation 10,000 had distinct genotypes based on IS elements, while in the other line testing of 13 clones revealed 10 different genotypes. Given all this variation, we chose to focus on those IS-mediated mutations that were substituted in one of these two populations in this period, reasoning that such mutations were more likely to be beneficial and hence of greater interest. In addition to the IS-mediated *rbs* deletions discussed in Section II.E, nine other IS substitutions were genetically characterized in the two focal populations (Schneider et al. 2000). These included six new insertions, two large inversions, and a deletion. The new insertions are of particular interest because they involve interesting genes, and because such mutations are easier to work with than inversions or deletions. We are presently performing studies in which we construct genotypes that are identical except for a mutation in the affected gene, in order to measure the mutation's effects on fitness

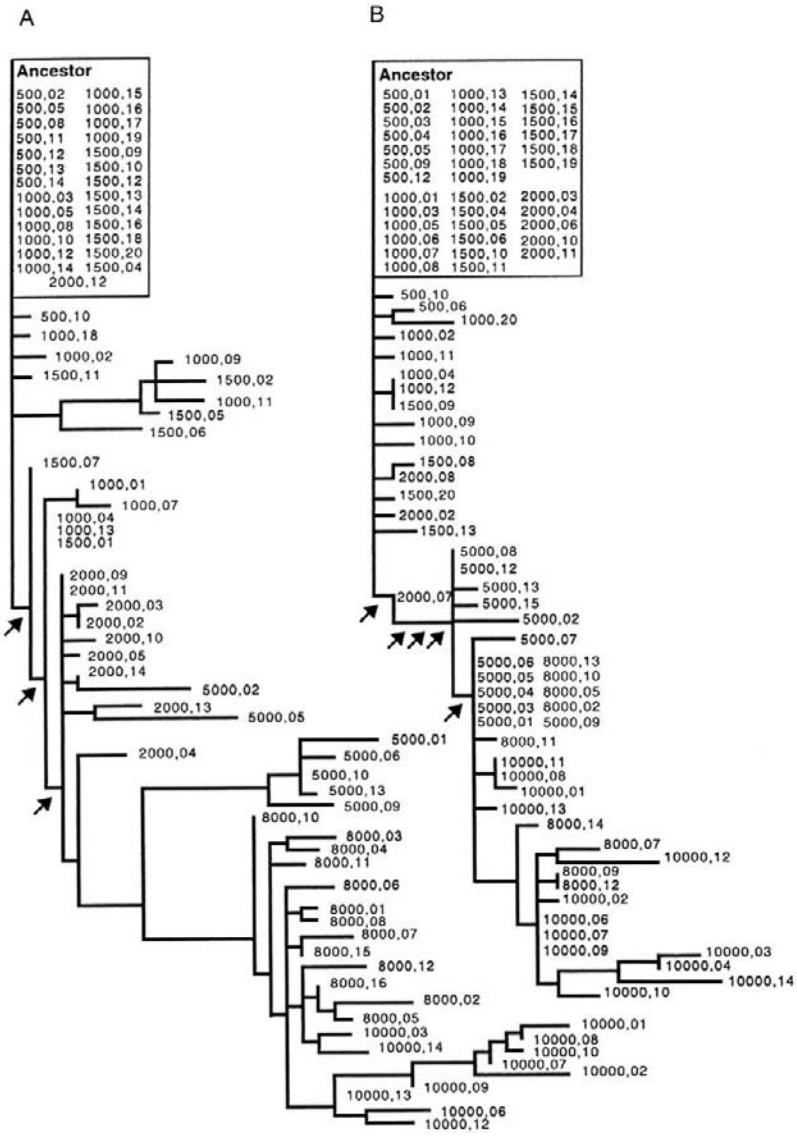

Fig. 8.7. Evolving DNA "fingerprints" in two of the populations, obtained using IS elements as probes. A and B show phylogenies constructed for two populations through 10,000 generations. The boxes represent the ancestral clone and clones sampled in later generations that were indistinguishable from the ancestor by this approach. Each number indicates a clone, with the first part showing generation number and the second an arbitrary designation for clones sampled in the same generation. Arrows indicate some of the IS-mediated mutations that were substituted in the population. Source: Papadopoulos et al. 1999. Cooyright 1999 National Academy of Sciences, U.S.A.

and other traits. If a particular IS-mediated mutation proves to be beneficial, then the affected gene becomes an interesting candidate for study in the other 11 lines to determine, for example, whether they also substituted similar mutations.

2. Transposon Tagging. Another approach to finding beneficial mutations analyzes the linkage between fitness-enhancing mutations and genetic markers. These markers must be deliberately introduced into the bacterial chromosome for this purpose, owing to the initial homogeneity of the material. This approach is elegant, but also laborious. Mark Stanek and I (ms. in prep.) used this approach to find a beneficial mutation as follows. Starting with a clone from one population at generation 10,000, we produced a library of more than 1,000 marked clones. Each clone carried a transposon that randomly inserted at a different genomic location, and that encodes a marker that can be readily selected. A viral transduction system was then used to move bits of DNA from the pooled library into the ancestral genotype, and selection was applied to the marker (to eliminate non-recombinants and other uninformative genotypes). We then had a pool of recombinant genotypes that carried bits of the evolved clone's genome, including the marker, in the ancestral background.

The challenge was to find an interesting "needle in a haystack," which we did as follows. First, we propagated the recombinant pool for about 40 generations, too little to allow much evolution de novo, but enough to enrich slightly those recombinants that acquired a beneficial mutation from the evolved clone. Second, we used the fact that cell size and fitness were strongly correlated during evolution, and the much greater ease of screening cell size than fitness, to isolate promising recombinant clones for further study. Third, after finding a recombinant clone that produced much larger cells than the ancestor, the two were placed in competition to confirm that the recombinant was indeed more fit. Fourth, having in hand a recombinant clone containing a beneficial mutation and a nearby marker, we more precisely quantified the linkage between the two by co-transduction. An analysis of these data indicated that the beneficial mutation of interest was approximately 3,000 base-pairs from the transposon that carried the marker. Fifth, we sequenced about 5,000 base-pairs in each direction from the transposon, and we found one and only one difference between the ancestral and recombinant clones. This mutation is therefore responsible for the differences in both fitness and cell size between these clones. Finally, we went back into the stored samples to determine the trajectory of that allele in the population in which it arose, and whether the same or similar mutations were substituted in other lines.

3. Expression Arrays. Section II.D described how we used 96-well plates to screen changes in many catabolic functions, which led us to identify deletion mutations in the *rbs* operon described in Section II.E. Another data-intensive approach we have recently pursued uses DNA arrays, which allow one to measure simultaneously the changes in expression for almost all the genes in the *E. coli* genome (Cooper et al. 2003). In brief, one harvests the mRNA from a cell, makes the corresponding cDNA that is radio-labeled, and hybridizes that cDNA to a membrane-bound array of all 4,290 of the organism's open-reading frames. We then compared the patterns of gene expression of the ancestor and evolved lines when they had been separately grown under identical conditions. To minimize the vexing statistical problem of avoiding false positives in such large data sets (with >4,000 genes and a 0.05 significance level, one could easily get >200 false positives), we have so far focused our attention on changes in gene expression that occurred in two independently evolved lines, which again serve as focal material, over 20,000 generations.

The overall gene-expression profiles of both the evolved lines were significantly more divergent from the ancestor than were controls, in which the genetically marked variants of the ancestor were compared to one another. The two independently evolved lines were also more divergent from one another than were the ancestral controls. If the two evolved lines had changed their patterns of gene expression in completely different ways, we would have expected them to be about twice as divergent from one another as they were on average from the ancestor. In fact, however, they were less divergent from one another than either was from the ancestor. This finding indicates strong (though not complete) parallel evolution of gene expression profiles.

We then identified 59 genes whose individual expression levels had changed significantly in both independently evolved lines. Remarkably, all 59 changed in the same direction in both lines (both lines with increased expression, or both with decreased expression), even though this concordance was not part of the statistical test used to find these 59 genes. At this point, it is very important to emphasize that these expression data are phenotypic data; they are not genetic data, despite the nucleic-acid based methods employed. For example, a single mutation in some regulatory gene could cause tens or more of coordinated changes in expression of other genes. By using the abundant knowledge of metabolic and regulatory pathways in *E. coli*, we inspected the parallel patterns of changes in gene expression in order to identify some candidate genes for sequencing and, if mutations were found, manipulation.

To make a long and complex story short and simple, we identified a mutation in one of the two focal lines in a gene called *spoT* (Cooper et

al. 2003). The encoded SpoT protein is a bi-functional protein that can add and remove a phosphate moiety from a molecule called ppGpp, an important effector whose intracellular concentration has cascading effects on gene expression. We then moved the *spoT* mutation we had discovered into the ancestral genetic background and showed that it conferred a competitive fitness advantage of almost 10%. Moreover, moving the evolved *spoT* allele into the ancestor produced many of the same changes in gene expression that we had used to discover that mutation. Interestingly, there was no *spoT* mutation in the other focal line, and some other (presently unknown) mutation evidently has similar effects on gene expression. Moreover, this unknown mutation evidently has similar effects on fitness because moving the evolved *spoT* mutation from one line to the other conferred no fitness advantage. However, sequencing revealed non-synonymous substitutions in *spoT* in seven of the other ten independently evolved populations. As we will see in the next section, finding mutations in the same gene in 8 of 12 lines is quite unlike what is seen in most of the genome, providing further evidence for the adaptive significance of these substitutions.

4. Random Sequencing. IS-mediated mutations can be found by screening the entire genomes of ancestral and evolved clones, because these mutations produce large changes in the size of restriction fragments that can be readily detected. By contrast, it is much more difficult to detect point mutations or other small mutations. Of course, it is possible now to sequence an entire bacterial genome, but at present the costs are too great to do this for all the populations, clones within populations, and sample time points that would interest us. Therefore, as a first step in this direction, we sequenced 36 randomly chosen gene regions in 50 clones, including the two variants of the ancestor and two clones from two time points for all 12 populations (Lenski et al. 2003). We found a total of only 10 mutations in these randomly chosen regions; all were found in populations that became mutators and, moreover, all had sequence signatures that were typical of mutator genotypes. Based on the subset of these mutations that were synonymous (hence presumably neutral) and had been substituted in their populations, and using independent data on the relative mutation rates of the mutator and repair-proficient states, we estimated the ancestral mutation rate as about 1.4×10^{-10} per base-pair per generation. This value is somewhat lower than a widely cited estimate based on genetic experiments (Drake et al. 1998), but it is higher than another estimate calculated from inter-specific sequence comparisons (Ochman et al. 1999).

These data from randomly chosen genes also provide a useful control for understanding how much background variation to expect in candidate genes where we have some phenotypic basis for sequencing them, such as the *spoT* candidate deduced from studying expression arrays. In none of the 36 randomly chosen genes did we find mutations in even two of the 12 evolved lines (Lenski et al. 2003), whereas eight of these lines had non-synonymous substitutions in *spoT* (Cooper et al. 2003).

In addition to sequencing random genes, Christine Borland and I are currently exploring methods that might allow more rapid discovery of single-nucleotide polymorphisms, or SNPs. Such methods are based on hybridizing fragments of genomic DNA derived from two different clones, followed by molecular methods to detect mismatches between the fragments. Perhaps in a few years we will also pursue complete genome sequences for our ancestral strain and several evolved clones.

III. CONCLUSIONS

For over a decade, my group has maintained 12 populations of the bacterium *Escherichia coli*, founded from the same ancestor, in a simple defined laboratory environment in which glucose provides the sole source of carbon and energy. The evolving populations have undergone more than 20,000 generations of binary fission. Each population had a billion or so mutations appear, although only a tiny fraction were actually substituted in the population.

Extensive phenotypic evolution has occurred, including substantial gains in competitive fitness. After 20,000 generations, the evolved bacteria on average grow about 70% faster than the ancestor when they compete in the same environment. Average cell size also dramatically increased in the evolving populations. The evolved bacteria have tended to become glucose specialists, with mostly subtle reductions in many other catabolic functions. This specialization reflects pleiotropic tradeoffs of mutations that are beneficial in glucose medium, much more than it does drift accumulation of neutral mutations in unused genes. Several populations evolved defects in DNA repair and became hypermutable as a consequence, but the mutator lines do not exhibit proportionately greater adaptation or specialization.

The rate of phenotypic evolution has decelerated over time. Deceleration in the rate of adaptation reflects each population's approach to an adaptive peak or plateau, not an inability to measure further adaptation owing to non-transitive competitive interactions. Evolution was often parallel across the replicate populations, not only for fitness in the selec-

tive environment but also for cell morphology and underlying physiology as reflected by performance on other substrates and patterns of gene expression. On the one hand, such parallelism is surprising given that the populations relied entirely on new mutations for genetic adaptation. On the other hand, the large population size and large number of generations ensured that almost any one-step mutation was accessible in every population.

We are now in the midst of finding mutations that have been substituted in these evolving populations. Several different approaches are being used, including sequencing both candidate and random genes. Once a mutation of interest is found, the gene must be manipulated, such as by moving the mutation onto an otherwise isogenic background, to test its effects on fitness and other traits of interest. For example, in the course of screening catabolic breadth, we found that all 12 populations had lost their ability to grow on ribose. Molecular genetic analysis found similar, although not identical, deletions of the *rbs* operon associated with an adjacent mobile genetic element. Both spontaneous and engineered mutations that eliminated this operon were produced in the ancestral background, and these mutations were shown to confer a selective advantage in the environment used in the long-term experiment. Similarly, evolved changes in gene-expression profiles led to the discovery of point mutations in the *spoT* gene in 8 of the 12 populations. By precisely moving an evolved *spoT* allele into the ancestral genome, we showed that this mutation provided a significant competitive advantage in the environment of the long-term evolution experiment.

LITERATURE CITED

Blattner, F. R., G. Plunkett, C. A. Bloch, N. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao. 1997. The complete genome sequence of *Escherichia coli* K-12. Science 277:1453–1462.

Chao, L., and E. C. Cox. 1983. Competition between high and low mutating strains of *Escherichia coli*. Evolution 37:125–134.

Cooper, T. F., D. E. Rozen, and R. E. Lenski. 2003. Parallel changes in gene expression after 20,000 generations of evolution in *E. coli*. Proc. Natl. Acad. Sci. (USA) 100:1072–1077.

Cooper, V. S., and R. E. Lenski. 2000. The population genetics of ecological specialization in evolving *E. coli* populations. Nature 407:736–739.

Cooper, V. S., D. Schneider, M. Blot, and R. E. Lenski. 2001. Mechanisms causing rapid and parallel losses of ribose catabolism in evolving populations of *E. coli* B. J. Bacteriol. 183:2834–2841.

Coyne, J. A., and B. Charlesworth. 1996. Mechanisms of punctuated evolution. Science 274:1748–1749.

De Visser, J. A. G. M., and R. E. Lenski. 2002. Long-term experimental evolution in *Escherichia coli*. XI. Rejection of non-transitive interactions as cause of declining rate of adaptation. BMC Evol. Biol. 2:19.

De Visser, J. A. G. M., C. W. Zeyl, P. J. Gerrish, J. L. Blanchard, and R. E. Lenski. 1999. Diminishing returns from mutation supply rate in asexual populations. Science 283:404–406.

Drake, J. W., B. Charlesworth, D. Charlesworth, and J. F. Crow. 1998. Rates of spontaneous mutation. Genetics 148:1667–1686.

Elena, S. F., and R. E. Lenski. 1997. Long-term experimental evolution in *Escherichia coli*. VII. Mechanisms maintaining genetic variability within populations. Evolution 51:1058–1067.

Elena, S. F., V. S. Cooper, and R. E. Lenski. 1996. Punctuated evolution caused by selection of rare beneficial mutations. Science 272:1802–1804.

Friedberg, E. C., G. C. Walker, and W. Siede. 1995. DNA repair and mutagenesis. ASM Press, Washington.

Funchain, P., A. Yeung, J. L. Stewart, R. Lin, M. M. Slupska, and J. H. Miller. 2000. The consequences of growth of a mutator strain of *Escherichia coli* as measured by loss of function among multiple gene targets and loss of fitness. Genetics 154:959–970.

Gerrish, P. J., and R. E. Lenski. 1998. The fate of competing beneficial mutations in an asexual population. Genetica 102/103:127–144.

Gould, S. J. 1989. Wonderful life: the Burgess shale and the nature of history. Norton, New York.

Gould, S. J. 2002. The structure of evolutionary theory. Harvard Univ. Press, Cambridge.

Gould, S. J., and N. Eldredge. 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology 3:115–151.

Graña, M., and L. Acerenza. 2001. A model combining cell physiology and population genetics to explain *Escherichia coli* experimental evolution. BMC Evol. Biol. 1:12.

Haldane, J. B. S. 1927. A mathematical theory of natural and artificial selection. V. Selection and mutation. Proc. Camb. Phil. Soc. 23:838–844.

Kibota, T. T., and M. Lynch. 1996. Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*. Nature 381:694–696.

Kimura, M. 1983. The neutral theory of molecular evolution. Cambridge Univ. Press, Cambridge.

Lenski, R. E., and J. A. Mongold. 2000. Cell size, shape, and fitness in evolving populations of bacteria. p. 221–235. In: J. H. Brown and G. B. West (eds.), Scaling in biology. Oxford Univ. Press, Oxford.

Lenski, R. E., and M. Travisano. 1994. Dynamics of adaptation and diversification: 10,000-generation experiment with bacterial populations. Proc. Natl. Acad. Sci. (USA) 91:6808–6814.

Lenski, R. E., M. R. Rose, S. C. Simpson, and S. C. Tadler. 1991. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. Am. Nat. 138:1315–1341.

Lenski, R. E., J. A. Mongold, P. D. Sniegowski, M. Travisano, F. Vasi, P. J. Gerrish, and T. Schmidt. 1998. Evolution of competitive fitness in experimental populations of *E. coli*: what makes one genotype a better competitor than another? Antonie van Leeuwenhoek 73:35–47.

Lenski, R. E., C. L. Winkworth, and M. A. Riley. 2003. Rates of DNA sequence evolution in experimental populations of *Escherichia coli* during 20,000 generations. J. Mol. Evol. 56:498–508.

Mongold, J. A., and R. E. Lenski. 1996. Experimental rejection of a nonadaptive explanation for increased cell size in *Escherichia coli*. J. Bacteriol. 178:5333–5334.

Muller, H. J. 1932. Some genetic aspects of sex. Am. Nat. 66:118–138.

Neidhardt, F. C., R. Curtiss, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (eds.). 1996. *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington.

Ochman, H., S. Elwyn, and N. A. Moran. 1999. Calibrating bacterial evolution. Proc. Natl. Acad. Sci. (USA) 96:12638–12643.

Orr, H. A. 1998. The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. Evolution 52:935–949.

Papadopoulos, D., D. Schneider, J. Meier-Eiss, W. Arber, R. E. Lenski, and M. Blot. 1999. Genomic evolution during a 10,000-generation experiment with bacteria. Proc. Natl. Acad. Sci. (USA) 96:3807–3812.

Paquin, C., and J. Adams. 1982. Relative fitness can decrease in evolving populations of *S. cerevisiae*. Nature 306:368–371.

Rozen, D. E., and R. E. Lenski. 2000. Long-term experimental evolution in *Escherichia coli*. VIII. Dynamics of a balanced polymorphism. Am. Nat. 155:24–35.

Schneider, D., E. Duperchy, E. Coursange, R. E. Lenski, and M. Blot. 2000. Long-term experimental evolution in *Escherichia coli*. IX. Characterization of insertion sequence-mediated mutations and rearrangements. Genetics 156:477–488.

Shaver, A. C., P. G. Dombrowski, J. Y. Sweeney, T. Treis, R. M. Zappala, and P. D. Sniegowski. 2002. Fitness evolution and the rise of mutator alleles in experimental *Escherichia coli* populations. Genetics 162:557–566.

Sniegowski, P. D., P. J. Gerrish, and R. E. Lenski. 1997. Evolution of high mutation rates in experimental populations of *Escherichia coli*. Nature 387:703–705.

Sniegowski, P. D., P. J. Gerrish, T. Johnson, and A. Shaver. 2000. Evolution of mutation rates: separating causes from consequences. BioEssays 22:1057–1066.

Souza, V., P. E. Turner, and R. E. Lenski. 1997. Long-term experimental evolution in *Escherichia coli*. V. Effects of recombination with immigrant genotypes on the rate of bacterial evolution. J. Evol. Biol. 10:743–769.

Taddei, F., M. Radman, J. Maynard Smith, B. Toupance, P.-H. Gouyon, and B. Godelle. 1997. Role of mutator alleles in adaptive evolution. Nature 387:700–703.

Travisano, M., and R. E. Lenski. 1996. Long-term experimental evolution in *Escherichia coli*. IV. Targets of selection and the specificity of adaptation. Genetics 143:15–26.

Travisano, M., F. Vasi, and R. E. Lenski. 1995. Long-term experimental evolution in *Escherichia coli*. III. Variation among replicate populations in correlated responses to novel environments. Evolution 49:189–200.

Vasi, F., M. Travisano, and R. E. Lenski. 1994. Long-term experimental evolution in *Escherichia coli*. II. Changes in life-history traits during adaptation to a seasonal environment. Am. Nat. 144:432–456.

Subject Index
Volume 24 (Part 2)

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-35316-7

Cumulative Subject Index (Volumes 1–24)

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-35316-7

G

H

Q

T

Cumulative Contributor Index (Volumes 1–24)

Plant Breeding Reviews, Volume 24, Part 2, Edited by Jules Janick
ISBN 0-471-35316-7