PART III

LEVELS OF OBSERVATION IN EXPERIMENTAL EVOLUTION
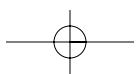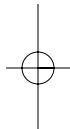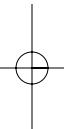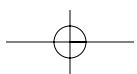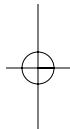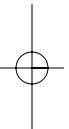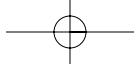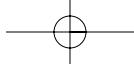
9

FITNESS, DEMOGRAPHY, AND POPULATION DYNAMICS IN LABORATORY EXPERIMENTS

Laurence D. Mueller

EARLY FOUNDATIONS OF POPULATION BIOLOGY

An important theme in this review will be the importance of a close balance between theory and experiments. Theory has as a goal the creation of simple and general principles. Understandably, therefore, theoreticians are often loath to spend too much time and energy worrying about the details of any specific organism's biology. However, experimental population biologists must determine if the assumptions of the specific theories that they wish to test are biologically unrealistic for the particular experimental organism that they wish to study. It is perilous for the experimental biologist to rely on the theoretician to determine if a particular organism or experimental design is appropriate for a test of the theory that they are both interested in.

Another important aspect of the work reviewed here is the interweaving of problems in ecology and evolution. Early in the history of these fields, topics like population dynamics were relegated to the realm of ecology, while the fields of evolution and population genetics considered problems like fitness components and methods for their estimation, ignoring ecological context. But over the course of the twentieth century, it became clear that population dynamics are affected by the organisms involved within what had been considered an "ecological" time frame. This idea was first treated in detail in the theoretical work of MacArthur (1962), but later the evolution of population growth rates became the focus of experimental studies (Mueller and Ayala 1981c). In a similar vein, the impact of life cycles and population regulation on fitness measurements and evolution was first clearly demonstrated by the work of Prout (1965, 1971a, 1971b, 1980). While this commingling of ecological and evolutionary research is still developing, there has already been significant progress over the last thirty years (see also Irschick and Reznick this volume).

Some of the earliest work in experimental population biology was carried out early in the twentieth century by Raymond Pearl using laboratory populations of *Drosophila*. His choice of fruit flies as a model system was in part an accident. A fire destroyed Pearl's laboratory and his mouse colony, forcing him to reconsider his plan to pursue questions in population biology using mice. On the advice of T. H. Morgan, he decided to study fruit flies. By 1919, Pearl had his experimental fly populations established, and experimental ecology was born.

The most enduring legacy of Pearl's work is not his experimental findings; in fact, his experimental methods are by today's standards unacceptably imprecise. Pearl's legacy was actually his focus on the interaction between experiment and theory. Pearl had interests in human population growth and so always viewed his fly experiments as models for other organisms. Pearl was also interested in using simple models to describe his experimental results. Because these theoretical models, like logistic population growth, were offered as general principles of population dynamics by Pearl, they caught the attention and interest of many people. Ultimately, they led others to consider more seriously the techniques used for determining population growth rates experimentally.
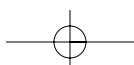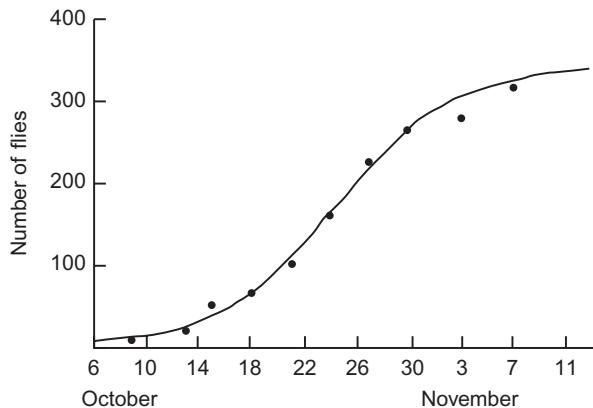
FIGURE 9.1
Population growth of a laboratory population of *D. melanogaster*. The circles are observed numbers and the line is the fitted logistic curve (taken from Pearl 1927).

Pearl was interested in human population growth, but he thought that there must be a universal law of population growth that would apply to all organisms, including humans and fruit flies. He began his experimental research with *D. melanogaster*. His experimental results apparently closely followed the simple logistic equation (figure 9.1). The techniques used to maintain Pearl's flies were somewhat haphazard, however. He would supply food to the flies at irregular intervals in varying amounts whenever it seemed as if food was needed. Pearl described his procedure this way: "The second type of experiment is one in which an attempt is made to add food as the supply is used up. The technical difficulties of doing this satisfactorily with a *Drosophila* population are considerable but by sufficient care they can be overcome in large degree" (Pearl 1927).

This procedure evidently lacked a proper protocol for the systematic renewal of resources, the replacement of bottle environments, or census taking. In fact, the experimental procedures are so vague that one can imagine that judgments about food addition could be unduly influenced by the numbers of flies produced in the most recent census. Clearly, the techniques cannot be replicated by another scientist. Many of these problems in experimental technique are discussed by Sang (1949). It would be another thirty-five years before *Drosophila* was again the subject of serious population dynamic experiments.

At the same time as Pearl was conducting his experimental research, many of the theoretical results for population genetics with and without overlapping generations were established. The connection between Mendelian genes and quantitative characters was developed by Fisher (1918). Selection in populations with discrete nonoverlapping generations was explored by Haldane (1927a). Two important papers (Norton 1928; Haldane 1927b) developed measures of fitness in age-structured populations. These papers demonstrated the importance of age-specific mortality and fecundity in determining fitness (see Rauser et al. this volume).

Pearl was not apparently directly motivated by the papers of Haldane and Norton, but he nevertheless started collecting experimental data on age-specific survival and
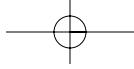
fecundity (Pearl et al. 1927). Pearl's work established that age-specific survival depends not only on the current total population density but also on the past history of population densities that an individual has experienced. A similar relationship between the current dynamics of a population and its past environments has also been recently described by Benton et al. (2006). Almost all theoretical work has ignored this biological finding, most likely because it greatly complicates the mathematical analysis. Fortunately, Pearl et al. (1927) did show that the current population density has a much greater impact on present survival then does past density. However, Pearl's work had shown that even an empirical problem as basic as the estimation of fitness in populations without age structure presented subtle complications that took some time for most population biologists to appreciate.

EMPIRICAL MEASURES OF FITNESS

Simple theory regarding the action of natural selection was well developed before experimental tests of this theory were even attempted. In keeping with the separation of ecological and evolutionary thought before 1960, little consideration was given then to the specifics of the life cycle of an organism when interpreting experimental data in evolutionary biology. Prout (1965) was the first to illustrate these complications and used a typical set of experimental data collected by Polivanov (1964). Polivanov used a simple model to interpret his experimental results. The model (figure 9.2A) assumes a single locus with two alleles and therefore three genotypes, A_1A_1, A_1A_2, and A_2A_2. Assuming random mating, zygote genotype frequencies will be in Hardy-Weinberg proportions, but after selection operates these frequencies are perturbed to X_{11}, X_{12}, and X_{22} for the genotypes A_1A_1, A_1A_2, and A_2A_2, respectively. If the fitness of the three genotypes are
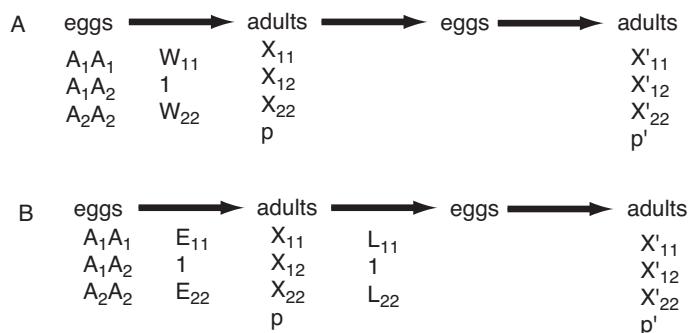

FIGURE 9.2

Two generations of a simple life cycle. A, Here the three genotypes at a single diallelic locus experience viability selection sometime between the egg and the adult census stage. B, In this example, selection at a single locus is affected by viability selection prior to the adult census stage and by fertility selection immediately after the adult census stage.
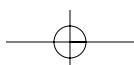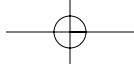
W_{11}, 1, and W_{22}, respectively, then it is relatively simple to show that the homozygote fitness (W_{11}), under this model, should be

$$2\frac{X'_{11}(1 - p)}{X'_{12}p},$$

where p is the frequency of the A_1 allele among the zygotes.

Prout (1965) pointed out that even simple laboratory populations of *Drosophila* can violate the implicit assumptions of these simple population genetic models, in turn leading to serious problems with the interpretation of fitness estimates. The major problem is that even under carefully controlled lab conditions, the life cycle of the fruit fly is still more complicated than these simple models assume (Prout 1965). Polivanov used two common third chromosome mutants stocks of *D. melanogaster* for his experimental estimates of fitness. *Stubble* is a dominant phenotypic mutant that causes the thorax bristles to be half their normal size. In addition, it acts as a recessive lethal. The recessive mutant *ebony* causes a dark coloration of the body. When dealing with these real genetic variants, there is no guarantee that the fitness effects of these deleterious alleles will be limited to egg-to-adult viability, which the simple population genetic model employed assumes. For instance, one possibility is that these mutants affect both egg-to-adult survival and male and female fertility (figure 9.2B). When this is true, the estimated fitness will typically be incorrect by a large amount, and there can be a spurious inference of frequency dependence (figure 9.3).

These observations led Prout (1969, 1971a, 1971b) to outline a detailed methodology for estimating fitness and its components. This methodology required that important
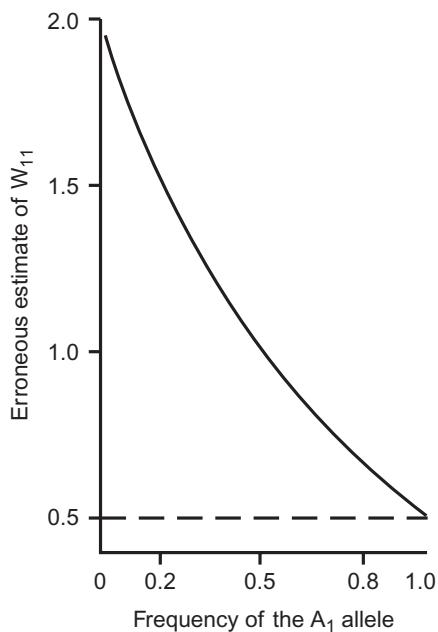

FIGURE 9.3
The erroneous value of fitness given by the equation, $2\frac{X_{11}(1 - p)}{X_{12}p}$, when there are actually neglected early and late components of fitness as shown in figure 9.2B. The correct value of fitness is 0.5. Adapted from Prout (1965).
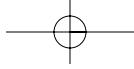
biological details of the life cycle of study organisms be taken into account. These techniques were further refined by Christiansen (Christiansen and Frydenberg 1973; Christiansen 1980). These methods have been applied to polymorphisms on the fourth chromosome of *Drosophila melanogaster* (Bungaard and Christiansen 1972) and the esterase polymorphism in the blenny *Zoarces* (Christiansen et al. 1973, 1978).

Before the advent of molecular techniques like protein gel electrophoresis, naturally occurring genetic variation was studied in experimental lines derived from specific crosses with specially constructed mutants. One method devised to study variation for fitness-related traits was to examine a collection of individual fruit flies that were all homozygous for the same second or third chromosome sampled from nature (Sved 1971; Sved and Ayala 1970; Tracey and Ayala 1974). While this technique resulted in large fitness differences that could be easily measured, these homozygous genotypes were hardly likely to be found in nature or for that matter in outbred lab populations. In addition to producing homozygosity for 20 to 30 percent of all genes, the chromosome extraction process usually resulted in genetic variation from marker stocks being introduced into the study lines.

Not surprisingly, these studies showed that making whole chromosomes in *Drosophila* homozygous resulted in large declines in fecundity (Marinkovic 1967), viability (Dobzhansky et al. 1963), and male virility (Britnacher 1981) relative to heterozygous genotypes. Sved and Ayala (1970) devised experimental techniques for allowing populations to complete their entire life span in a population cage, so that after many generations, net fitness estimates could be made from the equilibrium frequency of marked chromosomes. These studies revealed that viability, although often equated to fitness, only accounted for a small part of the reduced fitness of these homozygous genotypes. Adult fitness components, especially virility, contributed substantially to net fitness (Britnacher 1981).

These laboratory genotypes were also used to study the interactions between separate chromosomes on net fitness. For instance, Seager et al. (1982) estimated the fitness of effects of homozygosity on the second chromosome and the third chromosome of *D. melanogaster*. They found that the fitness of genotypes homozygous for both the second and third chromosome was generally higher than models of independent gene action predicted.

Recently, significant improvements in the techniques of chromosome extraction and fitness estimation have been made (Fowler et al. 1997; Barton and Partridge 2000; Gardner et al. 2001, 2005). These improvements include (1) studying the fitness of chromosomal heterozygotes, (2) backcrossing the extracted chromosome lines to an outbred population that has already been adapted to the lab environment, and (3) making replicate estimates of fitness for each chromosome. It is still hard to determine if the fitness variation detected by the techniques used by Partridge and her collaborators is representative of natural fitness variation. This is because these new techniques still suffer from several shortcomings, which include (1) the fitness effects of a multiply inverted marker
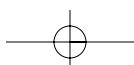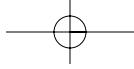
chromosome; (2) nonrandom sampling of chromosomes (most or all chromosomes had recessive lethal effects); and (3) populations maintained with overlapping generations, despite the use of a discrete-time model to estimate fitness, although Barton and Partridge outline conditions under which they argue this approximation might work.

Clark et al. (1981) studied fitness among a variety of two-locus mutant genotypes. Their analysis also attempted to estimate preadult and adult components of fitness, as shown in figure 9.2. Clark et al. noted that with two loci, ignoring the early or late components of fitness could lead to spurious estimates of epistasis, in addition to the potential for artifactual estimation of frequency dependence noted by Prout (1965). They found that estimates of epistasis for these laboratory mutant systems were often significantly different from zero, although the sign of these epistatic effects was not consistently positive or negative.

The primary goal of the chromosome extraction methods just described was to infer genetic variation for fitness or fitness components in natural populations. Population geneticists have also been interested in getting estimates of fitness for whole populations of wild-type individuals. These populations might be genetically variable but be adapted to different environments or have different geographic origins. Several surrogates of fitness, like biomass or productivity, have been proposed for whole population estimates (Carson 1961a, 1961b). *Productivity* usually refers to the total number of individuals produced by a genotype or population under controlled conditions, whereas *biomass* is simply the wet or dry weight of all the individuals. Productivity is still occasionally used as a measure of fitness (e.g., Houle et al. 1994). Productivity can provide a convenient index of fitness for a wide variety of genotypes or genetically differentiated populations. Haymer and Hartl (1983) tested the utility of such productivity measures of fitness by comparing them to more traditionally based fitness estimates. They measured fitness by measuring biomass, productivity, and the direct competition of genotypes for an array of extracted second chromosomes. The results show a very weak correlation between the more direct competitive estimates of fitness and biomass or productivity. In the next section, on population dynamics, I will discuss one possible explanation for this effect.

Perhaps the most sophisticated method for making fitness estimates of wild-type genomes is the cytogenetic cloning, or "hemiclone," method (Chippindale et al. 2001). This method requires the sophisticated array of attached-X and compound autosome stocks available in *D. melanogaster*. However, with these tools nearly the entire genome of a single fly (comprising X, second, and third chromosomes) can be placed into the genetic background of an appropriate stock population. This then permits fitness estimates of a single naturally occurring haploid genome against a wide array of natural genetic backgrounds.

Using the hemiclone technique for fitness estimation has permitted the estimate of fitness effects of genomes in males and female contexts. These studies have revealed disparate and often antagonistic fitness effects of genes in males and females, effects that may help explain the evolution of extreme secondary sexual characteristics in males (Chippindale et al. 2001; Pischedda and Chippindale 2006).
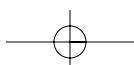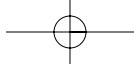
ECOLOGICAL AND EVOLUTIONARY STUDIES
OF POPULATION DYNAMICS

EXPERIMENTAL SYSTEMS

The commentary by Sang on Pearl's work emphasized the idea that any study of population dynamics in the laboratory will need a well-defined system for resource replacement or movement of individuals to new environments. Implicit in these experimental design issues are also decisions about whether the experimental population will have overlapping or discrete generations. One early experimental system for studying the population dynamics of *Drosophila* was the serial transfer system (Ayala 1965). This system consisted of a breeding adult population with overlapping generations, although it was first analyzed as if it were a discrete generation system. New recruits were collected at weekly intervals from cultures that had eggs laid in them one, two, three, and four weeks ago. Early attempts were made to use discrete-time models to study the adult population size (Hasting et al. 1981). However, the *Drosophila* serial transfer system cannot be modeled by first-order difference equations due to the complicated sampling structure used for collecting new recruits (Mueller and Ayala 1981a; Mueller and Joshi 2000, chapter 3). Nevertheless, it is not difficult to create discrete-generation populations of *Drosophila* (Mueller et al. 2000).

In contrast to the discrete-generation life cycles of *Drosophila*, most unicellular organisms used for ecological and evolutionary research are maintained on a continuous reproductive schedule. Bacteria (Lenski and Levin 1985; Bohannan and Lenski 2000) and communities consisting of unicellular algae and rotifers (Fussmann et al. 2000, 2003, 2005) are maintained in systems with a continuous flow of nutrients called *chemostats*. These systems permit continuous reproduction and thus can be modeled with continuous-time equations.

Tribolium is another well-studied experimental system that allows the maintenance of all life stages—eggs, larvae, pupae, and adults—in the same flour medium. At regular time intervals, often once a month, all life stages can be censused and the flour changed. Without additional experimental intervention, the adult population will have overlapping generations. The interesting aspect of ecological studies with *Tribolium* is the ease of obtaining simultaneous census counts for all life stages by using different size sieves to filter the flour. The duration and number of larval instars are affected by a number of genetic and environmental factors, especially temperature, humidity, and food (King and Dawson 1972).

One important model of *Tribolium* population dynamics is the larva-pupa-adult (LPA) model (Dennis et al. 1995). This model combines the egg and larval stages together and ignores the relatively weak density dependence of both larval and adult mortality as well as fecundity. Nevertheless, as discussed in the next section, this model has proven to be especially useful.

Nicholson (1954a, 1954b, 1957) pioneered the use of blowflies *(Lucilia cuprina)* as a model organism. Nicholson kept blowflies in large cages capable of supporting populations
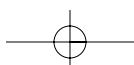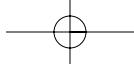
of ten thousand or more adults. The larval and adult food sources were separately controlled. Adults received both a sugar and protein food source and were allowed to live indefinitely. Thus, these blowfly populations consisted of overlapping generations that were counted at regular time intervals. Blowflies, *Tribolium*, and *Drosophila* suffer from the common liability that there is no simple way to assess the age of the adults. The next section considers models of population dynamics for these specific experimental systems.

ECOLOGICAL MODELS

Experimental studies of *Drosophila* population dynamics inevitably involve both adult and larval life stages. The effects of crowding on both life stages will ultimately be important for population dynamics. Larval crowding affects both survival to the adult stage and ultimate adult size (Bakker 1961). Since female fecundity is highly correlated with adult size, the effects of larval crowding will carry over into the next generation. In addition, there are substantial effects of adult crowding on female fecundity.

Bakker's work motivated the development of several theoretical models of competition and population dynamics (de Jong 1976; Nunney 1983; Mueller 1988a). Mueller's population dynamic model can be used to determine the conditions for stability of the population at its carrying capacity. In general terms, this model revealed that *Drosophila* populations may become unstable and enter fixed point cycles, or even chaos, if adults were provided with abundant resources and larvae were provided with low levels of food (Mueller 1988a; Mueller and Huynh 1994). These predictions about the dependence of stability on food provisioning were experimentally verified (Mueller and Huynh 1994), and later the model was used to study the evolution of population stability (Mueller and Joshi 2000).

Laboratory populations of blowflies often display strong and regular cyclic fluctuations with respect to population density (Nicholson 1954b, 1957). Blowflies show strong density-dependent fecundity and larval mortality. However, there is about a twenty-day time delay between the current adult density and its effect on future adult density. Blowfly population size cycles are strongly affected by the relative levels of larval and adult food levels, so they are in some aspects similar to *Drosophila* laboratory populations (Mueller and Joshi 2000, chapter 4). However, the conditions that are stabilizing for blowflies in the laboratory are low levels of food for both adults and larvae. High levels of larval food and low levels of adult food do not stabilize blowfly population dynamics as they do for *Drosophila*.

The growth of *Tribolium* populations is regulated by strong density-dependent cannibalism of eggs and pupae. Experimental manipulation of these rates of cannibalism leads to fine control of population stability and the strength of the underlying LPA model of *Tribolium* population dynamics (Costantino et al. 1995, 1997), a very different population-dynamic system from that of blowflies or *Drosophila*. While the level of ecological modeling for *Drosophila*, *Tribolium*, and blowflies is equally high, most evolutionary studies, described in the next few sections, have employed *Drosophila*.
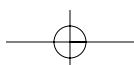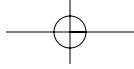
EARLY THEORIES OF DENSITY DEPENDENCE
IN EVOLUTIONARY ECOLOGY

The modern union of ecological theories of density-dependent population dynamics and natural selection was started by MacArthur (1962) and then refined by Anderson (1971), Charlesworth (1971), Clarke (1972), and Roughgarden (1971). Under this theory, genotypic fitness is taken to be density-dependent. For a single locus with multiple alleles, the fitness of genotype A_iA_j is given by

$$W_{ij} = 1 + r_{ij} - r_{ij}NK_{ij}^{-1},$$

where r_{ij} is the genotypic intrinsic rate of increase, K_{ij} is the genotypic specific value of the carrying capacity of the logistic equation, and N is the total population size. The outcome of natural selection inherently depends on the environment with this specification of fitness. In uncrowded environments, genotypes with the highest values of r are favored, while in crowded environments the genotypes with the highest values of K are favored. This theory can be used to explain variation in life-history traits if one assumes that there are trade-offs such that phenotypes with high values of r have low values of K and vice versa.

MEASUREMENTS OF THE DENSITY DEPENDENCE OF FITNESS

The relationship between density-dependent rates of population growth and fitness was studied with extraction lines like the ones reviewed in the previous section (Mueller and Ayala 1981b). Mueller and Ayala (1981b) measured density-dependent rates of population growth in twenty-five lines, each homozygous for a different second chromosome that had been sampled from nature. These growth rates were compared to the growth of an outbred population to determine relative fitness (Mueller and Ayala 1981b). At low density, the fitness-reducing effects of inbreeding are observed (figure 9.4). However, at high density, relative fitness of the average inbred line is no different from that of the outbred line using population growth rates as a measure of fitness. This is surprising, since the population cage studies that demonstrated the severe reduction in fitness caused by inbreeding were carried out at high population density (Seager et al. 1982).

These observations can be understood by looking at models that incorporate density-independent natural selection with density-dependent population growth (Prout 1980). This type of model doesn't require that there be no difference between isogenic lines in their density-dependent survival and fecundity, only that it be small relative to the density-independent fitness differences. Let's assume population dynamics are described by the discrete-time hyperbolic model,

$$N_{t+1} = \left[\frac{\widetilde{S}}{1 + sFN_t} \right] FN_t,$$
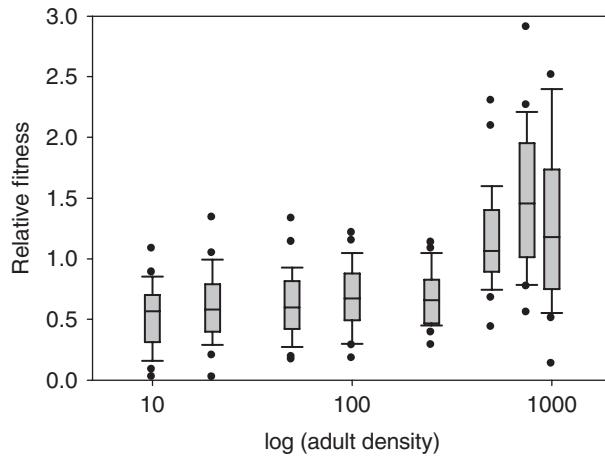
FIGURE 9.4

Box plots of the relative fitnesses of twenty-five inbred lines of *Drosophila melanogaster* based on density-dependent rates of population growth. From Mueller and Ayala 1981b. The growth rate of each line is divided by the growth rate of the genetically variable outbred line to get the relative fitness value. The box boundaries denote the 25th and 75th percentiles, the bars indicate the 10th and 90th percentiles, and the points show outliers that exceed the 10th and 90th percentiles. The median is indicated by a line through the box.

where N_t is the adult population size at time *t*, *F* is the per capita fecundity, and $\widetilde{S}$ and *s* measure density-dependent survival from egg to adult. Thus, after random mating, there are a total of FN_t eggs produced. A fraction

$$\frac{\widetilde{S}}{1 + sFN_t}$$

of these eggs survive to become adults in the next generation. Under this model, the equilibrium population size, or carrying capacity, is

$$\frac{\widetilde{S}F - 1}{sF}.$$

When fecundity is high, as it is for young *Drosophila* females, then the carrying capacity simplifies to approximately $\frac{\widetilde{S}}{s}$. In other words, the carrying capacity and, by extension, population growth rates near the carrying capacity are insensitive to changes in fecundity. Therefore, if differences in female fecundity are a large part of the fitness reduction of inbred *Drosophila* lines, it is not surprising that their growth rates at high density do not differ. However, large differences in fecundity will affect population growth rates at low densities, since nearly all eggs will survive to become adults. In *D. pseudoobscura* homozygous for whole second chromosomes, decreased fecundity accounts for about a 20 percent fitness reduction (Marinkovic 1967). This type of effect may also explain why productivity does not reliably reflect fitness differences among chromosomal homozygotes in *Drosophila*.
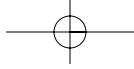
The major lesson from this work is that the fitness consequences of genetic variation may not be apparent in all population growth measurements. In the case of genetic variation affecting fecundity, it appears that this will at least affect population growth rates at low density. However, there can be other large differences in fitness components among genotypes that have no effect on population growth rates. In *D. melanogaster*, for instance, it is known that lines homozygous for second chromosomes suffer a substantial reduction in male virility relative to heterozygous males. Male virility is a frequency-dependent fitness component, and therefore in a population of equivalent males, as long as all females are fertilized—which is what we normally observe—we would not expect low male virility to have any effect on population growth rates at any density. In conclusion, we see that as a general surrogate for fitness, population growth rates have many limitations and will often incorrectly estimate the true fitness of a genotype.

The *Drosophila* population-dynamic model discussed here predicted that density-dependent natural selection would favor increases in competitive ability (Mueller 1988a). Crowding is also expected to increase the equilibrium adult numbers and under some circumstances could result in the evolution of smaller body size, contrary to verbal theories of *r*- and *K*-selection (Mueller 1988a). A simple analysis would suggest that larger body size should be favored under crowded conditions since this would buffer the organism against variations in food availability. However, a more detailed analysis of the relationship between size, fitness, and density in *Drosophila* reveals that in food-limited environments, the ability to pupate at a smaller size may be advantageous.

The relationship between fitness and density may be complicated and depend in nontrivial ways on the details of the organism's life history. These observations suggest that experimental work must be founded on theories that have taken the experimental organisms peculiar life-history traits into account. The use of general theories lacking biological specificity, like *r*- and *K*-selection, to predict the outcomes of evolution for specific experimental systems is unlikely to be successful.

EVOLUTION OF GROWTH RATES

Even if growth rates are not good measures of fitness, this in itself does not show that growth rates will not evolve as suggested by the theory of Roughgarden (1971) under the appropriate conditions. This idea has been tested experimentally with *Drosophila* (Mueller and Ayala 1981c; Mueller et al. 1991). These tests involved experimentally manipulating adult and larval densities and creating one set of replicate populations, called *r*'s, that were kept at low adult and larval density and another set, called *K*'s, that were kept at high adult and larval density. Each of these environmental treatments was replicated threefold, and natural selection was allowed to change the genetic composition of these populations.

After just eight generations, these populations showed trade-offs in their density-dependent growth rates at high and low densities (Mueller and Ayala 1981c), (figure 9.5). This experiment was repeated after 198 generations of *r*- and *K*-selection by rederiving two new sets
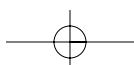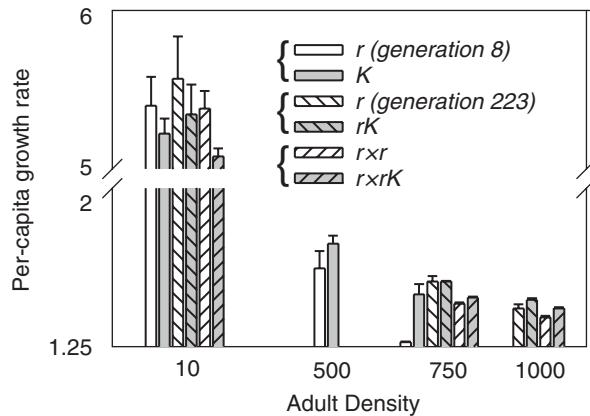
FIGURE 9.5

The per capita growth rates at four adult densities for populations cultured at low density (*r*, *r* × *r*) and populations cultured at high densities (*K*, *rK*, and *r* × *rK*; from Mueller and Ayala 1981c and Mueller et al. 1991). The bars are standard errors. The derivation of the various lines is described in the text. The measurements for the *r*- and *K*-populations shown as solid histograms were made after eight generations of selection. The measurements for the other populations were made after 223 generations of selection in the *r*-environment.

of populations called *rK* and *r* × *rK* (see Mueller et al. 1991 for details of their derivation). Both sets of new populations also showed the evolution of trade-offs in population growth rates after twenty-five generations of further evolution (Mueller et al. 1991; figure 9.5).

The changes in population growth rates due to density-dependent natural selection can involve the evolution of several phenotypes. In accordance with the predictions of the *Drosophila* model (Mueller 1988a), larval competitive ability increases in populations kept under crowded larval conditions (Mueller 1988b). Egg-to-adult viability was also affected by the evolution of increased pupation height in the crowded cultures (Mueller and Sweet 1986; Joshi and Mueller 1993).

Crowding *Drosophila* larvae is expected to cause competition for food and space. It has also been observed that, over the span of one generation, crowded larval environments show a temporal decline in quality (Borash et al. 1998). Ammonia levels increase over time, while food and ethanol levels decrease. This complexity appears to be responsible for a genetic polymorphism in crowded populations. Very early-developing genotypes have high feeding rates but low tolerance to ammonia, while late-developing genotypes feed more slowly and can tolerate higher ammonia levels. There may be many natural environments that exhibit similar patterns of temporal decay (Borash et al. 1998).

In *Drosophila*, larval competitive ability is determined primarily by the larval feeding rate (Burnet et al. 1977; Joshi and Mueller 1988; Fellowes et al. 1998). Better competitors feed at a faster rate. However, it appears that feeding fast decreases the efficiency of food utilization (Mueller 1990; Joshi and Mueller 1996). Feeding rate also responds to selection
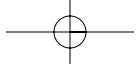
for several other larval stressors, including ammonia in the larval environment (Borash et al. 2000), urea in the larval food (Borash et al. 2000), parasitoids (Fellowes et al. 1999), and reduced time for larval development (Borash et al. 2000; Prasad et al. 2001). These seemingly disparate results indicate that the larval energy budget is sensitive to changes in larval feeding rates. Lowering feeding rates, which occurs in larvae adapted to urea, ammonia, and parasitoids, may increase efficiency and provide larvae with the energy needed to meet the demands of a stressful environment (Mueller et al. 2005).

Luckinbill (1978) studied density-dependent natural selection in *Escherichia coli* by creating cultures that underwent exponential growth to simulate low-density, or *r*-selection, conditions. High-density, or *K*-selection, conditions were created by letting populations grow exponentially followed by periods of maintenance at saturation density. Luckinbill observed that *K*-selected bacteria grew faster than *r*-selected bacteria at all test densities. Vasi et al. (1994) studied the evolution of *E. coli* in seasonal environments that were similar to Luckinbill's *K*-selected environment. Vasi et al. used their data to estimate the parameters for a model of bacterial population dynamics, and then showed that these populations had evolved traits that would be most important during the exponential growth phase of the environment, while parameters that would be most important during the periods of saturation density had not changed. Thus, Luckinbill's results may simply reflect differences in the intensity of selection during the exponential growth phase rather than differences in selection at high and low density.

The evolution of population dynamics is of great practical interest for conservation biology (see Saccheri and Hanski 2006). Genetic changes that may affect either the equilibrium population size or the ability of a population to grow at low densities may in turn have an impact on the persistence of a population over time. Examples of genetic variation in natural populations that affects their dynamics are hard to come by, but they do in fact exist. For instance, a genetic polymorphism for horn shape in Soay sheep appears to affect density-dependent rates of population growth (Moorcroft et al. 1996). More recently, *Pgi* polymorphisms in the butterfly *Melitaea cinxia* have been implicated in their population growth (Hanski and Saccheri 2006).

EVOLUTION OF POPULATION STABILITY

If density-dependent rates of population growth evolve, then it makes sense that population stability might in turn evolve, since both ultimately depend on nonlinear responses to density. The first test of this idea came from Mueller et al. (Mueller and Joshi 2000; Mueller et al. 2000). These tests placed populations with different selection histories in environments that caused population cycling. Although there was clear evidence of evolution in both population carrying capacity and larval feeding rates, there was no discernible change in the stability properties of any populations. Thus, if stability does evolve, it does so much more slowly than other phenotypes that are affected by population crowding.
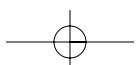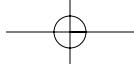
In designing their experiments, Mueller et al. (2000) chose techniques that would minimize the effects of inbreeding, because it is known that this will cause a decline in female fecundity, which in theory could secondarily increase population stability. A result of this kind was in fact obtained by Prasad et al. (2001). They selected populations for rapid development and early reproduction. These populations showed a reduction in female fecundity relative to controls. Prasad et al. observed increased stability in the rapidly developing populations compared to their controls, as predicted by the simple theories discussed earlier (Mueller and Huynh 1994; Mueller et al. 2000). This same phenomenon may have been responsible for the evolution of increased stability in Nicholson's blowfly experiments (Stokes et al. 1988).

More complicated predator-prey systems also demonstrate the impact of evolution on population dynamics. Fussman et al. (2003, 2005) studied a rotifer-algal system in chemostats. They found that the rotifers evolved lower rates of sexual reproduction, and the algae evolved higher rates of herbivore resistance. However, herbivore resistance was accompanied by reduced growth rates.

DISCUSSION

A major goal of experimental evolution is to simplify the conditions under which evolution occurs in order to effectively study how evolution operates. Much of the research outlined here has shown that, even in apparently simple laboratory settings, understanding the ecological and evolutionary forces at work can be quite tricky. Without doubt, the real world is usually even more complicated. The work of Fussman et al. reveals the complication of interacting species that are both capable of evolving. The effects on population dynamics of such coevolution could be substantial.

An area of research that is still understudied is the dynamics of populations with age structure. Although the experimental systems of *Tribolium* and blowflies have age-structured populations, the extant models of these systems have assumed that all adults are equivalent. While that assumption might be adequate for these particular experimental systems, it does not show that age structure is generally unimportant. Of course, severe practical problems make the study of age structure with model systems technically difficult, although not impossible (see Mueller and Joshi 2000, chapter 6).

The study of adaptation in different laboratory environments and studies of demography could benefit from fitness estimates using the hemiclone technology developed for *Drosophila*. One difficult problem with demography is that measurements of age-specific survival cannot be made on single individuals. However, hemiclones could be used to estimate demographic parameters for individual genotypes. This could permit direct estimates of genetic and environmental variation in demographic parameters that are crucial components for demographic theories of late life (Mueller et al. 2003).

Overall, few of the simple models and empirical predictions that were first developed concerning relationships among fitness, age structure, and density dependence have
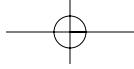
survived the scrutiny that laboratory experiments have afforded. The study of the joint action of population dynamics and natural selection has thus made salutary progress, at least in this respect.

A recurring theme in experimental population biology has been the wealth of unanticipated effects that are detected. This collective history argues strongly for the pursuit of research with experimental systems since the prospect of revealing, defining, and understanding these complications in uncontrolled natural conditions is low.

SUMMARY

Experimental laboratory systems that combine elements of population biology have contributed to our understanding of many basic problems in ecological and evolutionary biology, particularly with respect to their interface. Some of these problems include the partitioning of fitness into components, epistatic interactions affecting fitness, ecological factors that determine population stability, phenotypic evolution due to density-dependent natural selection, and the role of evolution in molding population stability. Laboratory systems are designed to be simple, but the factors that affect the evolution and ecology of these systems can still be quite complicated. Consequently, experimental research in ecology and evolutionary biology will continue to make important contributions to our understanding of the basic principles of these fields.

ACKNOWLEDGMENTS

REFERENCES

Anderson, W. W. 1971. Genetic equilibrium and population growth under density-regulation selection. *American Naturalist* 105:489–498.

Ayala, F. J. 1965. Relative fitness of populations of *Drosophila serrata* and *Drosophila birchii*. *Genetics* 51:527–544.

Bakker, K. 1961. An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. *Archives Néerlandaises de Zoologie*. 14:200–281.

Barton, N. H., and L. Partridge. 2000. Measuring fitness by means of balancer chromosomes. *Genetical Research, Cambridge* 75:297–313.

Benton, T. G., S. J. Plaistow, and T. N. Coulson. 2006. Complex population dynamics and complex causation: Devils, details and demography. *Proceedings of the Royal Society of London B, Biological Sciences* 273:1173–1181.

Bohannan, B. J., and R. E. Lenski. 2000. Linking genetic change to community evolution: Insights from studies of bacteria and bacteriophage. *Ecology Letters* 3:362–377.

Borash, D. J., A. G. Gibbs, A. Joshi, and L. D. Mueller 1998. A genetic polymorphism maintained by natural selection in a temporally varying environment. *American Naturalist* 151:148–156.
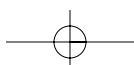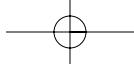
Borash, D. J., Teotónio, H., M. R. Rose, and L. D. Mueller. 2000. Density-dependent natural selection in *Drosophila*: Correlations between feeding rate, development time, and viability. *Journal of Evolutionary Biology* 13:181–187.

Britnacher, J. G. 1981. Genetic variation and genetic load due to the male reproductive component of fitness in *Drosophila*. *Genetics* 97:719–730.

Bundgaard, J., and F. B. Christiansen. 1980. Dynamics of polymorphisms. I. Selection components in an experimental population of *Drosophila melanogaster*. *Genetics* 71:439–460.

Burnet, B., D. Sewell, and M. Bos. 1977. Genetic analysis of larval feeding behavior in *Drosophila melanogaster*. II. Growth relations and competition between selected lines. *Genetical Research* 30:149–161.

Carson, H. L. 1961a. Heterosis and fitness in experimental populations of *Drosophila melanogaster*. *Evolution* 15:496–509.

Carson, H. L. 1961b. Relative fitness of genetically open and closed populations of *Drosophila robusta*. *Genetics* 46:553–567.

Charlesworth, B. 1971. Selection in density-regulated populations. *Ecology* 52:469–474.

Chippindale, A. K., J. R. Gibson, and W. R. Rice. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proceedings of the National Academy of Sciences of the USA* 98:1671–1675.

Christiansen, F. B. 1980. Studies on selection components in natural populations using population samples of mother-offspring combinations. *Hereditas* 92:199–203.

Christiansen, F. B., and O. Frydenberg. 1973. Selection component analysis of natural polymorphisms using population samples including mother-offspring combinations. *Theoretical Population Biology* 4:425–445.

Christiansen, F. B., O. Frydenberg, and V. Simonsen. 1973. Genetics of *Zoarces* populations. IV. Selection component analysis of an esterase polymorphism using samples including mother-offspring combinations. *Hereditas* 73:291–304.

———. 1978. Genetics of *Zoarces* populations. X. Selection component analysis of the *Est*III polymorphism using samples of successive cohorts. *Hereditas* 87:129–150.

Clark, A. G., M. W. Feldman, and F. B. Christiansen. 1981. The estimation of epistasis in components of fitness in experimental populations of *Drosophila melanogaster*. I. A two-stage maximum likelihood model. *Heredity* 46:321–346.

Clarke, B. 1972. Density-dependent selection. *American Naturalist* 106:1–13.

Costantino, R. F., J. M. Cushing, B. Dennis, and R. A. Desharnais. 1995. Experimentally induced transitions in the dynamic behaviour of insect populations. *Nature* 375:227–230.

Costantino, R. F., R. A. Desharnais, J. M. Cushing, and B. Dennis. 1997. Chaotic dynamics in an insect population. *Science* 275:389–391.

de Jong, G. 1976. A model of competition for food. I Frequency dependent viabilities. *American Naturalist* 110:1013–1027.

Dennis, B., R. A. Desharnais, J. M. Cushing, and R. F. Costantino. 1995. Nonlinear demographic dynamics: Mathematical models, statistical methods, and biological experiments. *Ecological Monographs* 65:261–281.

Dobzhansky, T., B. Spassky, and T. Tidwell. 1963. Genetics of natural populations. XXXII. Inbreeding and mutational loads in natural populations of *Drosophila pseudoobscura*. *Genetics* 48:361–373.
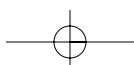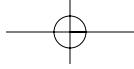
Fellowes, M. D. E., A. R. Kraaijeveld, and H. C. J. Godfray. 1998. Trade-off associated with selection for increased ability to resist parasitoid attack in *Drosophila melanogaster*. *Proceedings of the Royal Society of London B, Biological Sciences* 265:1553–1558.

———. 1999. Association between feeding rate and parasitoid resistance in *Drosophila melanogaster*. *Evolution* 53:1302–1305.

Fisher, R. A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. *Transactions of the Royal Society of Edinburgh* 52:399–433.

Fowler, K., C. Semple, N. H. Barton, and L. Partridge. 1997. Genetic variation for total fitness in *Drosophila melanogaster*. *Proceedings of the Royal Society of London B, Biological Sciences* 264:191–199.

Fussmann, G. F., S. P. Ellner, and N. G. Hairston. 2003. Evolution as a critical component of plankton dynamics. *Proceedings of the Royal Society of London B, Biological Sciences* 270:1015–1022.

Fussmann, G. F., S. P. Ellner, N. G. Hairston, L. E. Jones, K. W. Shertzer, and T. Yoshida. 2005. Ecological and evolutionary dynamics of experimental plankton communities. Pages 221–243 *in* R. A. Desharnais, ed. *Advances in Ecological Research*, vol. 37. Amsterdam: Elsevier.

Fussmann, G. F., S. P. Ellner, K. W. Shertzer, and N. G. Hairston. 2000. Crossing the Hopf bifurcation in a live predator-prey system. *Science* 290:1358–1360.

Gardner, M., K. Fowler, L. Partridge, and N. H. Barton. 2001. Genetic variation for preadult viability in *Drosophila melanogaster*. *Evolution* 55:1609–1620.

———. 2005. Genetic variation for total fitness in *Drosophila melanogaster*: Complex yet replicable patterns. *Genetics* 169:1553–1571.

Haldane, J. B. S. 1927a. A mathematical theory of natural and artificial selection. Part IV. *Proceedings of the Cambridge Philosophical Society* 23:607–615.

———. 1927b. A mathematical theory of natural and artificial selection. Part V. Selection and mutation. *Proceedings of the Cambridge Philosophical Society* 23:838–844.

Hanski, I., and I. J. Saccheri. 2006. Molecular-level variation affects population growth in a butterfly metapopulation. *PLoS Biology* 4:e129.

Hastings, A., Serradilla, J. M., and Ayala, F. J. 1981. Boundary-layer model for the population dynamics of single species. *Proceedings of the National Academy of Sciences of the USA* 78:1972–1975.

Haymer, D. S., and D. L. Hartl. 1983. The experimental assessment of fitness in *Drosophila*. II. Comparison of competitive and noncompetitive measures. *Genetics* 104:343–352.

Houle, D., K. A. Hughes, D. K. Hoffmaster, J. Ihara, S. Assimacopolous, D. Canada, and B. Charlesworth. 1994. The effects of spontaneous mutation on quantitative traits. I. Variance and covariance of life history traits. *Genetics* 138:773–785.

Joshi, A., and L. D. Mueller, 1988. Evolution of higher feeding rate in *Drosophila* due to density-dependent natural selection. *Evolution* 42:1090–1093.

———. 1993. Directional and stabilizing density-dependent natural selection for pupation height in *Drosophila melanogaster*. *Evolution* 47:176–184.

———. 1996. Density-dependent natural selection in *Drosophila*: Trade-offs between larval food acquisition and utilization. *Evolutionary Ecology* 10:463–474.

King, C. E., and P. S. Dawson. 1972. Population biology and the *Tribolium* model. *Evolutionary Biology* 5:1789–1805.
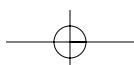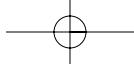
Lenski, R. E., and B. R. Levin. 1985. Constraints on the coevolution of bacteria and virulent phage: A model, some experiments, and predictions for natural communities. *American Naturalist* 125:585–602.

Luckinbill, L. S. 1978. *r*- and *K*-selection in experimental populations of *Escherichia coli*. *Science* 202:1201–1203.

MacArthur, R. H. 1962. Some generalized theorems of natural selection. *Proceedings of the National Academy of Sciences of the USA* 48:1893–1897.

Marinkovic, D. 1967. Genetic loads affecting fecundity in natural populations of *Drosophila pseudoobscura*. *Genetics* 56:61–71.

Moorcroft, P. R., S. D. Albon, J. M. Pemberton, I. R. Stevenson, and T. H. Clutton-Brock. 1996. Density-dependent selection in a fluctuating ungulate population. *Proceedings of the Royal Society of London B, Biological Sciences* 263:31–38.

Mueller, L. D. 1988a. Density-dependent population growth and natural selection in food limited environments: The *Drosophila* model. *American Naturalist* 132:786–809.

———. 1988b. Evolution of competitive ability in *Drosophila* due to density-dependent natural selection. *Proceedings of the National Academy of Sciences of the USA* 85:4383–4386.

———. 1990. Density-dependent natural selection does not increase efficiency. *Evolutionary Ecology* 4:290–297.

Mueller, L. D., and F. J. Ayala. 1981a. Dynamics of single species population growth: Experimental and statistical analysis. *Theoretical Population Biology* 20:101–117.

———. 1981b. Fitness and density dependent population growth in *Drosophila melanogaster*. *Genetics* 97:667–677.

———. 1981c. Trade-off between *r*-selection and *K*-selection in *Drosophila* populations. *Proceedings of the National Academy of Sciences of the USA* 78:1303–1305.

Mueller, L. D., M. D. Drapeau, C. S. Adams, C. W. Hammerle, K. M. Doyal, A. J. Jazayeri, T. Ly, S. A. Beguwala, A. R. Mamidi, and M. R. Rose. 2003. Statistical tests of demographic heterogeneity theories. *Experimental Gerontology* 38:373–386.

Mueller, L. D., D. G. Folk, N. Nguyen, P. Nguyen, P. Lam, M. R. Rose, and T. Bradley. 2005. Evolution of larval foraging behaviour in *Drosophila* and its effects on growth and metabolic rate. *Physiological Entomology* 30:262–269.

Mueller, L. D., P. Z. Guo, and F. J. Ayala. 1991. Density-dependent natural selection and trade-offs in life history traits. *Science* 253:433–435.

Mueller, L. D., and P. T. Huynh. 1994. Ecological determinants of stability in model populations. *Ecology* 75:430–437.

Mueller, L. D., and A. Joshi. 2000. *Stability in Model Populations*. Princeton, NJ: Princeton University Press.

Mueller, L. D., A. Joshi, and D. J. Borash. 2000. Does population stability evolve? *Ecology* 81:1273–1285.

Mueller, L. D., and V. F. Sweet. 1986. Density-dependent natural selection in *Drosophila*: Evolution of pupation height. *Evolution* 40:1354–1356.

Nicholson, A. J. 1954a. Compensatory reactions of populations to stresses, and their evolutionary significance. *Australian Journal of Zoology* 2:1–8.

———. 1954b. An outline of the dynamics of animal populations. *Australian Journal of Zoology* 2:9–65.
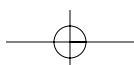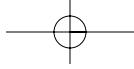
———. 1957. The self adjustment of populations to change. *Cold Spring Harbor Symposium on Quantitative Biology* 22:153–173.

Norton, H. T. J. 1928. Natural selection and Mendelian variation. *Proceedings of the London Mathematical Society* 28:1–45.

Nunney L. 1983. Sex differences in larval competition in *Drosophila melanogaster*: The testing of a competition model and its relevance to frequency dependent selection. *American Naturalist* 121:67–93.

Pearl, R. 1927. The growth of populations. *Quarterly Review of Biology* 2:532–548.

Pearl, R., J. R. Miner, and S. L. Parker. 1927. Experimental studies of life. XI. Density of population and life duration in *Drosophila*. *American Naturalist* 61:289–317.

Pischedda, A., and A. K. Chippindale. 2006. Intralocus sexual conflict diminishes the benefits of sexual selection. *PLoS Biology* 4:e356.

Polivanov, S. 1964. Selection in experimental populations of *Drosophila melanogaster* with different genetic backgrounds. *Genetics* 50:81–100.

Prasad, N. G., M. Shakarad, D. Anitha, M. Rajamani, and A. Joshi. 2001. Correlated responses to selection for faster development and early reproduction in *Drosophila*: The evolution of larval traits. *Evolution* 55:1363–1372.

Prout, T. 1965. The estimation of fitness from genotypic frequencies. *Evolution* 19:546–551.

———. 1971a. The relation between fitness components and population prediction in *Drosophila*. I. The estimation of fitness components. *Genetics* 68:127–149.

———. 1971b. The relation between fitness components and population prediction in *Drosophila*. II. Population prediction. *Genetics* 68:151–167.

———. 1980. Some relationships between density-independent selection and density-dependent population growth. *Evolutionary Biology* 13:1–68.

Roughgarden, J. 1971. Density-dependent natural selection. *Ecology* 52:453–468.

Saccheri, I. J., and I. Hanski. 2006. Natural selection and population dynamics. *Trends in Ecology & Evolution* 21:341–347.

Sang, J. H. 1949. Population growth in *Drosophila* cultures. *Biological Review* 25:188–219.

Seager, R. D., F. J. Ayala, and R. W. Marks. 1982. Chromosome interactions in *Drosophila melanogaster*. II. Total fitness. *Genetics* 102:485–502.

Stokes, T. K., W. S. C. Gurney, R. M. Nisbet, and S. P. Blythe. 1988. Parameter evolution in a laboratory insect population. *Theoretical Population Biology* 34:248–265.

Sved, J. A. 1971. An estimate of heterosis in *Drosophila melanogaster*. *Genetical Research* 18:97–105.

Sved, J. A., and F. J. Ayala. 1970. A population cage test for heterosis in *Drosophila pseudoobscura*. *Genetics* 66:97–113.

Tracey, M. L., and F. J. Ayala. 1974. Genetic load in natural populations: Is it compatible with the hypothesis that many polymorphisms are maintained by natural selection? *Genetics* 77:569–589.

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. *American Naturalist* 144:432–456.