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EVOLUTION OF AGING AND LATE LIFE

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THE IMPACT OF EXPERIMENTAL EVOLUTION ON AGING RESEARCH

Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments, edited by Theodore Garland, Jr., and Michael R. Rose.

Aging, like all biological characters, evolves. However, unlike many other biological characters, the evolution of aging is not primarily shaped by powerful natural selection balanced against such mitigating factors as clonal interference, linked deleterious alleles, directional mutation, and the like. Instead, aging is evolutionarily distinctive because it arises from the progressive fall in Hamilton's forces of natural selection acting on the survival and reproduction of the somata of ovigerous species (Hamilton 1966; Rose et al. 2007). Therefore, the evolutionary mechanisms underlying aging are different from those underlying most other phenotypes. That is, aging does not have a function or purpose, nor is it adaptive.

Thus, aging must be studied from a different theoretical and experimental perspective than other biological characteristics. From an evolutionary point of view, aging can be defined as a sustained age-specific decline of fitness-related characteristics, such as survival probability and fertility, that is not due to external environmental factors like disease, predation, or climate. Indeed, experimental evolutionary approaches to the problem of aging have mainly focused on manipulating and measuring age-specific survival probability and age-specific fecundity.

Experimental evolution has played an important role in aging research over the last few decades. However, the success of the experimental evolution approach in aging research is in large part due to the availability of a well-developed body of evolutionary theory that concerns aging (see also Mueller this volume). This strong theoretical foundation has provided experimental evolutionary biologists with relatively straightforward predictions to test. Nonetheless, the evolutionary biology of aging would not have progressed as it has over the last few decades without the relatively clear results obtained from some remarkably repetitious, if not dreary, experimental work.

In this chapter, we discuss the evolutionary theories of aging and the experimental studies that have tested and, in a few cases, challenged these theories. We also discuss the postaging phase of life, referred to here as "late life," and the experimental and theoretical work surrounding this phenomenon. The discovery, explanation, and manipulation of late life have served to put the evolution of aging into a strikingly different context. However, we will also discuss a variety of dissenting views, ranging from biomedically motivated research with large-effect mutants to anti-Hamiltonian research by demographers. As we will explain in detail, we regard much of this work as positively misleading with respect to how and why aging evolves.

EVOLUTIONARY THEORIES OF AGING

Although the evolution of aging was discussed in the nineteenth century by Alfred Russel Wallace and August Weismann, it was not until the advent of theoretical population genetics in the twentieth century that the evolutionary analysis of aging was made relatively coherent (Rose 1991). In early discussions of aging, a few evolutionary biologists erroneously proposed that aging evolved to eliminate older individuals to make way

for the young (e.g., Weismann 1891). This is an idea that is still repeated to this day, at least by some authors without training in evolutionary theory. The key mistake common to all these proposals is that they presume that, without aging, there would be an abundance of frail older organisms in natural populations. However, for the vast majority of species in the wild, this is not likely to be the case because disease, starvation, predation, accident, and bad weather normally remove most individuals from wild populations before significant aging has occurred (Medawar 1952), the most important exceptions being species that have benefited from human interventions, including humans themselves, our pets, zoo animals, laboratory model organisms, and species kept in ecological preserves from which their normal predators and other hazards have been wholly or largely removed.

By the middle of the twentieth century, R. A. Fisher (1930), J. B. S. Haldane (1941), P. B. Medawar (1946, 1952), and G. C. Williams (1957) had developed a different kind of evolutionary theory of aging. Their proposals were based on the idea that natural selection should operate with less effectiveness on later fitness components because these characters would be expressed less often, per lifetime, than early fitness components, simply because the probability of survival from birth to any age falls with age. Haldane (1941) first intuited the fall in the force of natural selection acting on mortality when he proposed that the human genetic syndrome of Huntington's disease was common relative to many other genetic diseases, despite its lethality and allelic dominance, because it was not expressed until middle age, after most of its carriers would have reproduced. Verbal hypotheses like these set the stage for the development of the formal evolutionary theory of aging, which was to follow in the last third of the twentieth century.

HAMILTON'S FORCES OF NATURAL SELECTION

The basic elements of the mathematical population genetics of aging were developed primarily by Hamilton (1966) and Charlesworth (e.g., 1980, 1994). Hamilton (1966) derived the result that the force of natural selection acting on mortality is given by $s(x)/T$, where x is chronological age and T is a measure of generation length. The function s at age x is given by

$$s(x) = \sum_{y=x+1} e^{-ry} l(y) m(y),$$

where r is the Malthusian parameter, or the growth rate of the population, associated with the specified $l(y)$ survivorship and $m(y)$ fecundity functions. The $s(x)$ function represents the relative fitness impact of an individual's future reproduction, after age x. Note that, before the first age of reproduction, s is always equal to 1 once reproduction has ended, s is equal to 0 forever after and during the reproductive period, $s(x)$ progressively falls (figure 18.1). This result shows that the *force of natural selection acting on survival falls with adult age*, at least when the assumptions of this analysis are met.

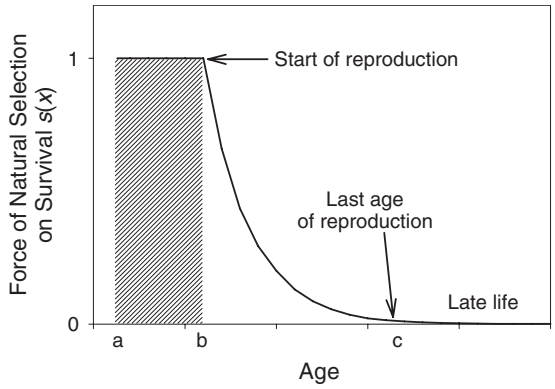

FIGURE 18.1

The age-specific force of natural selection acting on survival, as a percentage of its full force, from Hamilton's (1966) equations. Natural selection is strong at earlier ages (a) until the start of reproduction (b), after which it starts to decline until the last age of reproduction (c) in a population's evolutionary history. Note that the force of natural selection becomes weaker with age until it reaches, and remains at, zero after the last age of reproduction (c). The onset of late life also occurs after this point (c), when the force of natural selection is negligible.

Recently, the generality of Hamilton's results have been challenged (Baudisch 2005, 2008). The function $s(x)$ was derived by Hamilton by implicitly differentiating the Lotka equation

$$\sum_{y=0}^{\infty} e^{-ry} l(y) m(y) = 1$$

and finding the first partial differential

$$\frac{dr}{d \ln p_a},$$

where p_a is the survival from age a to age $a + 1$. A new and different approach to the problem of aging was suggested by Baudisch (2008:22), who proposed that "equally reasonable, alternative forms would have been dr/dp_a, dr/dq_a, $dr/d\ln q_a$ or $dr/d\ln \overline{u}_a$," where $q_a = 1\text{-}p_a$, and $\ln \overline{u}_a = -\ln p_a$. If by "reasonable" Baudisch means "easy to compute," then her suggestion might be acceptable. However, there is more known about the role of $s(x)$ in the evolution of age-structured populations today than there was when Hamilton first wrote his paper. Explicit population genetic models for survival show that the fate of alleles at a single locus are dependent on the genotypic equivalent of $s(x)$ (Charlesworth 1980:207–208). We do not have equivalent results for the other proposed measures of Baudisch, and therefore, her proposed fitness measures do not have equal standing with Hamilton's original measure. Put another way, in terms of explicit population genetics, they have no well-founded basis, unlike Hamilton's measures, which repeatedly crop up as terms in the equations of theoretical population genetics (e.g., Rose 1985).

A similar analysis for effects on age-specific fecundity gives a comparable "scaling equation," as follows:

$$s'(x) = e^{-rx}l(x).$$

All variables in this equation have the same definitions as those in the equation for the force of natural selection acting on survival. In this case, if population growth is not negative, then the force of natural selection once again declines with age. But if population growth is strongly negative, then the force of natural selection acting on age-specific fecundity may increase with age for a time. This arises when a population is declining rapidly, so that two offspring produced when the population size is six hundred are "worth" less to natural selection than two offspring produced some time later when the population size is sixty. In the latter instance, the offspring are a larger proportion of the population. However, these cases will normally be rare, since populations that are declining rapidly will often become extinct, rendering them unobservable. The typical pattern will remain one in which the force of natural selection acting on fecundity will decline with age and, most important, will converge on zero after the last age of survival in the population's evolutionary history.

The application of Hamilton's (1966) forces of natural selection to the evolution of aging has some tricky features when population genetics are introduced theoretically (for examples, see Charlesworth and Williamson 1975; Charlesworth 1980; Rose 1985). But the effect of the pronounced decline in these functions is so marked that Hamilton's (1966) original verbal interpretation of these functions remains salient to this day (Rose et al. 2007), with one striking exception that we will discuss in detail later.

From these theoretical results, evolutionary biologists have been able to explain the evolution of declining age-specific fitness components among adults, or aging, in very general terms. In particular, the evolutionary theory of aging does not require or predict the action of any particular mechanism of physiological deterioration. Instead, aging is expected to be a pervasive failure of adaptation across most, if not all, of the physiological mechanisms that sustain survival and reproduction among young individuals. For this reason, evolutionary biologists have generally been skeptical of proposals that attribute "the cause of aging" to any one physiological mechanism or gene for aging or programmed death. Although common genetic pathways might be identified that contribute to aging among a variety of organisms (cf. Guarante and Kenyon 2000), a possibility that the Hamiltonian theory of aging does not preclude, the cause of aging in any one species is expected to involve a diversity of physiological mechanisms and genes, with additional diversity of mechanisms among different species (Rose 1991). Leaving aside specific physiological mechanisms, the forces of natural selection acting on mortality and fecundity are quantitatively similar in their effects at later ages, and they thus will shape both age-specific mortality rates and fecundity within populations in a comparable manner. Specifically, age-specific mortality rates should tend to increase with organismal age, while fecundity should decrease with age, whenever Hamilton's forces of natural selection decline.

The Hamiltonian theory of aging predicts that all species that exhibit a well-defined separation of germ line from soma will age, while all species with strictly symmetrical fissile reproduction will be free of aging (Rose 1991; Rose et al. 2007). A significant complication, and a source of some misunderstanding, is that many species exhibit neither a well-defined soma nor fissile reproduction. Still other species combine fissile with sexual reproduction.

Aging is expected to be universal in very large phylogenetic groups that meet the requirements of Hamiltonian theory. The most common cases are to be found in the ovigerous metazoa that lack the sort of vegetative reproduction that is found among coelenterates and many plants. In obligately ovigerous animals, an egg develops into an immature animal and ultimately into a reproductively mature animal. Both insects and vertebrates exhibit this type of life cycle.

Insects and vertebrates are two of the most intensively studied groups of species. Actuarial and physiological aging are hard to detect in most wild populations of these organisms. For example, several fish species appear to attain great age in the wild without signs of physiological aging, with prominent examples being sturgeon and rockfish. However, when cohorts of fish are maintained in the laboratory free of contagious disease until all die, they show demographic aging (see Comfort 1979). For mammals, the evidence for the ubiquity of aging is best, with respect to both the numbers of species studied and the quality of their care. In general, in insects and vertebrates, there are no well-attested laboratory refutations of the Hamiltonian prediction that all these species must age (Comfort 1979; Rose 1991). This is a notable comparative finding, particularly in view of the prediction of some non-Hamiltonian demographers (e.g., Baudisch 2008) that nonaging species can evolve under such conditions. However, as noted previously, these theoretical predictions of nonaging come from models that use indicators of evolution that are different than those that naturally follow from population genetic models.

At the other end of the spectrum with respect to the expectations of the Hamiltonian theory of aging are the symmetrically fissile species. It is important to understand that the concept of fissile here does not mean merely asexual reproduction or budding. Obviously asymmetrical fission in organisms like budding yeast, the asexual protozoan *Tokophyra*, and the bacterium *Caulobacter crescentus* are all known to show observable demographic aging in laboratory cohorts (Rose 1991; Ackermann et al. 2003). What has been surprising recently is that less obviously asymmetrical fissile species, such as *Schizosaccharomyces pombe* and even the humble *Escherichia coli*, also, at least sometimes, show both asymmetrical fission and demographically measurable aging (Barker and Walmsley 1999; Stewart et al. 2005).

The crux of Hamiltonian expectations for the evolution of aging is whether or not asexual reproduction involves a kind of "adult" producing "juvenile" offspring, in which the juvenile is produced relatively intact, while the adult accumulates damage (cf. Stewart

et al. 2005; Ackermann et al. 2007). When this occurs in a way that can give rise to differential genetic effects on such "adults" and "juveniles," then aging is expected to evolve in Hamiltonian theory. The cases where aging cannot, according to Hamiltonian theory, evolve are those with strict symmetry between the products of fission. In these cases, aging would extinguish all the descendant lineages, wiping out any such lineage.

SPECIFIC POPULATION GENETIC HYPOTHESES FOR AGING

Subordinate to basic Hamiltonian theory for aging are alternative population genetic hypotheses for the evolution of aging. These hypotheses are not incompatible with one another; they could be simultaneously valid. At present, there are two population genetic mechanisms of primary interest: antagonistic pleiotropy and mutation accumulation.

ANTAGONISTIC PLEIOTROPY

Antagonistic pleiotropy arises when alleles that have beneficial effects on one set of fitness components also have deleterious effects on other fitness components, a long-standing concept in evolutionary theory (Rose 1982). Both Medawar (1952) and Williams (1957) verbally argued for the importance of this population genetic mechanism in the evolution of aging during the 1950s. The underlying concept is one of trade-offs, such that alleles with early beneficial effects in some way produce bad side effects later in life. Genes having such actions remain in populations because the forces of natural selection are typically high at earlier ages when these genes have beneficial effects and low at later ages when these same genes have detrimental effects, and thus are not selected against.

Charlesworth (1980) and Rose (1985) analyzed the action of antagonistic pleiotropy mathematically using explicit population genetics and showed that the declining force of natural selection would lead to a tendency for selection to fix alleles that have early beneficial effects but later deleterious effects. It is a noteworthy result of these analyses that Hamilton's forces of natural selection reappear as basic terms in the equations determining the outcome of natural selection when the Malthusian parameter is fitness, contrary to some of the speculations of Baudisch (2005, 2008). In addition, antagonistic pleiotropy may lead to the maintenance of genetic variability for aging and related characters, which is of great experimental significance. It should be noted that antagonistic pleiotropy does not always maintain genetic variability within populations, as it is possible to have genes with antagonistic pleiotropic effects that are fixed in the population but that still contribute to aging.

Other theories similar to the antagonistic pleiotropy theory have independently been proposed to explain the evolution of aging. One such theory is the "disposable soma" theory, which more specifically assumes trade-offs between somatic maintenance and reproduction (Kirkwood 1977; Kirkwood and Holliday 1979). According to this theory, investment in reproduction or somatic maintenance at earlier ages should result in a

decrease in an individual's ability to maintain somatic tissue at later ages. This theory is more specific in its form than the antagonistic pleiotropy theory, which allows for gene actions that involve trade-offs between different ages with respect to fecundity alone, among other possibilities (Kirkwood and Rose 1991).

MUTATION ACCUMULATION

The other cogent population genetic mechanism for the evolution of aging is mutation accumulation. Mutation accumulation arises when the force of natural selection has declined to a point where it has little impact on recurrent deleterious mutations with effects confined to late-life. Medawar (1946, 1952) was the main advocate of the importance of this mechanism in the evolution of aging. Charlesworth (e.g., 1980, 1994, 2001) analyzed the population genetics of mutation accumulation mathematically, showing that the frequency of deleterious mutations can rise with adult age because of the declining force of natural selection at late ages, although Baudisch (2005, 2008) has strongly argued against the importance of mutation accumulation in the evolution of aging. That is, alleles that differ only with respect to detrimental effects expressed only at later ages will tend to remain and accumulate within a population over time because the force of natural selection is too weak to eliminate them. This population genetic mechanism also can maintain genetic variability for aging, like antagonistic pleiotropy.

LARGE-EFFECT MUTANTS AND THE GENETICS OF AGING

One approach that has become increasingly common in the characterization of the genetics of aging is to isolate aging mutants, usually from mutagenesis experiments, and then to determine the mechanistic basis for the unusual life span in the mutants. This approach has led to the discovery of genes that can enhance (e.g., Maynard Smith 1958; Lin et al. 1988; reviewed in Guarente and Kenyon 2000, Kim 2007) or reduce life span (e.g., Pearl and Parker 1922). Most of the large-effect mutants affecting aging decrease longevity or fecundity, but it has been possible to use fairly ingenious protocols to find mutants that increase longevity, particularly in *Drosophila* (e.g., Lin et al. 1988), nematodes (e.g., Lin et al. 2001), and yeast (e.g., Kennedy and Guarente 1996). The obvious value of this approach has been the direct confirmation of the genetic control of aging.

Unfortunately, it only provides identification of genes that have demonstrable effect as major mutants. As such, it is a relatively indirect approach that requires tremendous effort to determine the evolutionary biology involved (Toivonen et al. 2007; Giannakou et al. 2008). Of greater concern, however, is the difficulty of reproducing the beneficial effects on life span of some of these mutations (e.g., Khazaeli et al. 2005). Among other problems, genotype-by-environment interactions arise for such mutants, such that they survive well under the conditions of specific, sometimes highly artificial, laboratory screening protocols, but not otherwise (Van Voorhies et al. 2006). Within the context of experimental evolutionary research, particularly in its applications to the problem of

aging, inbreeding and genotype-by-environment interactions have long been of concern, both in general (e.g., Rose 1991) and in specific hypothesis-testing experiments (e.g., Service and Rose 1985). Studying the genetics of aging using inbred stocks that have not been given sufficient opportunity to adapt to laboratory conditions (cf. Simões et al. 2007, 2008) is expected to generate a variety of artifacts. This makes the failures of replication that have arisen with mutant stocks (e.g., Khazaeli et al. 2005) unsurprising.

This criticism does not necessarily imply that there is never any value in such genetic studies. For one thing, there is little doubt that the long tradition of large-effect "longevity" mutants shows that such mutants predictably, if not universally, entail fitness costs that will be either obvious (e.g., Maynard Smith 1958) or somewhat recondite (Van Voorhies et al. 2006). In particular, it is at least plausible that many of the "longevity mutants" supply interesting worked-out examples of antagonistic pleiotropy, often with much physiological detail.

Unfortunately, it is very difficult to proceed from the pleiotropic effects of alleles that have probably never risen to high frequency in natural populations to the evolution of aging in general. For example, it would be an inappropriate conclusion from such work, though one that is common enough (e.g., Baudisch 2008), to conclude that such genetic research constitutes support for the importance of antagonistic pleiotropy, relative to mutation accumulation, in the evolution of aging. Yet this is not to deny that the loci that are revealed by such mutants may indeed have other, moderately hypomorphic alleles that do indeed play a role in the evolution of aging. They might. But, as we will now show, there are much better methods for studying the evolution of aging than research using "longevity mutants."

THE ROLE OF EXPERIMENTAL EVOLUTION IN TESTING HAMILTONIAN THEORIES OF AGING

LABORATORY EVOLUTION OF AGING

Unlike many theories in biology, the Hamiltonian analysis of aging provides fairly obvious strategies for experimentally testing its validity. Two straightforward predictions can be extracted from theoretical work on the evolution of aging: (1) natural selection should accelerate aging in populations with relatively earlier ages of reproduction, and (2) natural selection should slow aging in populations with relatively later ages of reproduction. One of the most elegant experimental approaches in gerontology is the manipulation of the force of natural selection to shape the evolution of aging patterns. This experimental strategy was first proposed by Edney and Gill (1968). The design of these experiments depends on the manipulation of the age of reproduction.

For example, normal laboratory fruit fly culture involves reproduction at fourteen days of age, when cultured at 25°C, with just a few hours for egg laying allowed. This focuses the force of natural selection on that, relatively early, age. Genetic effects expressed at later ages, much after fourteen days, are subject to negligible natural selection. This type

of experimental regime allows the evolution of accelerated aging. Sokal (1970) in *Tribolium castaneum*, Mueller (1987) using *Drosophila melanogaster*, and Passananti et al. (2004) also using *D. melanogaster* all found more rapid aging after selection for early fertility.

An alternative experimental regime is to keep adult flies alive for some time before they are allowed to contribute offspring to the next generation. This is done by discarding any eggs that they lay until they have reached the age allowed for reproduction, which can be as late as ten weeks from emergence of the larva. Note that this procedure does not require that the fruit flies be kept virgin; mating is allowed, just not successful reproduction. This regime is expected to lead to the evolution of relatively later aging. Wattiaux (1968) and Rose and Charlesworth (1980, 1981b) found evidence of enhanced later-age fertility and longevity, with depressed early fertility, when looking at *Drosophila* populations selected for later ages of reproduction without replication.

Properly replicated experiments using this second experimental approach were not performed until the 1980s, particularly by Rose (1984) and Luckinbill et al. (1984). Rose (1984) analyzed longevity and fecundity differences between three populations selected for earlier reproduction and three populations selected for increasingly later reproduction. These early and late reproducing populations were derived from the same outbred laboratory population of *D. melanogaster*, but they had been separated and selected for their relative ages of reproduction for more than fifteen generations at the time of their first assays. Significant differences were observed in mean longevity between the early and late reproducers, with the late reproducers having an increased mean longevity (figure 18.2). Earlier fecundity was also found to be higher in the earlier-reproducing populations at earlier ages compared to the later-reproducing populations, which provides support for the predictions made by the antagonistic pleiotropy theory of aging. Luckinbill et al. (1984) found essentially the same results, further demonstrating that selection on age of reproduction can alter longevity in ways consistent with the antagonistic pleiotropy mechanism for the evolution of aging.

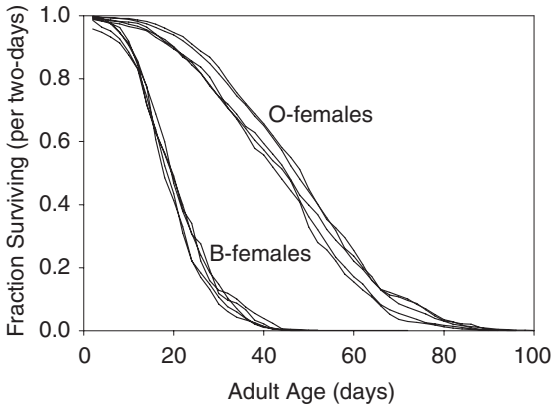

FIGURE 18.2
Fraction of females surviving during adulthood in the B and O populations of Rose (1984). Data are taken from Rose et al. (2002), but newly plotted.

Experiments using one or the other of these basic designs are now routine, often using fruit fly species of the genus *Drosophila,* but sometimes other species are used (see, e.g., Nagai et al. 1995; Reed and Bryant 2000).

One concern that has been raised about this experimental strategy involves the nature of the starting populations (e.g., Linnen et al. 2001). In this respect, the field of experimental evolution as a whole is fortunate that the Matos laboratory (see Simões et al. this volume) has devoted some years to sorting out the pattern and reproducibility of initial laboratory adaptation. Without rehearsing the findings of Matos and colleagues at length here, it is clear that *Drosophila* samples taken from nature, if they are even moderately outbred during laboratory culture, then rapidly adapt to laboratory conditions, with significant changes in life-history characters, particularly early fecundity. Thus, in the case of the much-studied populations of Rose (1984; Rose et al. 2004), it is relevant that these populations were maintained for more than one hundred generations in the laboratory prior to the start of experimental evolution work in which the age of first reproduction was progressively delayed. The findings of Simões et al. (this volume; see also Simões et al. 2007, 2008) show that these populations had probably largely adapted to laboratory conditions by that time. This in turn raises the question as to whether or not populations like this are "representative" of populations in nature. The answer is obvious. They *are not* representative of the populations from which they were derived. It is pellucidly clear that aging and other life-history characters rapidly evolve in laboratory populations of reasonable size, even without deliberate selection, as decades of work in the Rose (Rose et al. 2004) and Matos (Simões et al. this volume) laboratories have illustrated. Instead, such laboratory populations are like particles accelerated to great speeds in the experiments of high-energy physics: particular contrived cases in which evolutionary mechanisms that might act in nature are deliberately exaggerated to extreme levels. This is a general principle that affects all of experimental evolution (see also Futuyma and Bennett this volume; Huey and Rosenzweig this volume); there is nothing special about experimental evolution research on aging in this regard.

The results of changing the ages at which selection is strong have been striking for aging research with *Drosophila*. Mean and maximum life span dramatically increase when the force of natural selection is experimentally strengthened at later ages, as the evolutionary theory predicts. In addition, female fecundity, male virility, flight endurance, locomotion, stress resistance, among a variety of functional characters, are also enhanced (Rose et al. 2004). These results show that aging is not a by-product, accidental or otherwise, of an unmodifiable biochemical process. Rather, aging is an easily modified product of evolution. Rose and colleagues have further gone on to spend some years working out the physiological particulars that underlie this evolutionary transformation in the case of *Drosophila*, much of this work being compiled in Rose et al. (2004), but such research is not the particular concern of this article. (This type of research is considered in more detail in Harshman and Zera this volume.) However, it might at least be argued that such evolutionary physiology research is a preferable alternative to

the longevity mutant research, given all the problems for evolutionary interpretation that attend the latter (Van Voorhies et al. 2006).

EXPERIMENTAL POPULATION GENETICS OF AGING

Given the preeminence of the strength of natural selection in determining patterns of aging, the next major experimental question is, What are the population genetic mechanisms that underlie the evolution of aging? As described earlier, there are two main hypotheses: antagonistic pleiotropy and mutation accumulation. The experimental evidence that has been brought to bear on these hypotheses has been of two main kinds: (1) correlation between relatives and (2) indirect responses to selection. More recently, researchers have also investigated the effects of changing extrinsic mortality rates both in the lab and in nature (e.g., Stearns et al. 2000). In addition, there is the issue of the number of genetic loci involved in the evolution of aging. Several different methods have been used to address this last question.

CORRELATION BETWEEN RELATIVES

One of the classic techniques in quantitative genetics is the study of the correlations between relatives for characters that are, in part, inherited. Correlated inheritance of two characters indicates the pattern of pleiotropy, with positive correlations indicating that alleles affect two characters in the same direction, and negative correlations indicating that alleles affect two characters in opposite directions, on average. Linkage disequilibrium can, in principle, also generate genetic correlations, but the likelihood that it will systematically generate strong genetic correlations of one sign or another, when many segregating loci affect a quantitative character, is generally discounted. The antagonistic pleiotropy mechanism for the evolution of aging requires that some early and late characters exhibit negative genetic correlations with respect to each other in populations with abundant segregating genetic variation. This pattern has been found in a few cases, notably between early fecundity and longevity in fruit flies (Rose and Charlesworth 1981a). However, a positive correlation has been found in many more (reviewed in Rose et al. 2005, 2007). Artifacts may be responsible for some of these results, particularly inbreeding and novel environment effects, both of which are expected on theoretical grounds to bias genetic correlations toward positive values (Rose 1991) and which have been experimentally demonstrated (e.g., Service and Rose 1985), as already discussed.

The mutation accumulation hypothesis for the evolution of sensecence requires that genetic correlations between early and late characters be approximately zero. The problem with this hypothesis is that these genetic correlations tend to be strongly positive among newly occurring mutations, and sometimes negative in populations with established genetic variation. Mutation accumulation also requires that heritable genetic variation increase with age, but this has not been shown to occur in some experiments

(Rose and Charlesworth 1980; Promislow et al. 1996; Shaw et al. 1999), although other experiments have found this effect (Hughes and Charlesworth 1994; Shaw et al. 1999). Shaw et al. (1999) went to considerable trouble to unravel the many difficulties with experiments of this kind, in particular and in general. One basic conclusion that has been offered is that evidence relating age-specific genetic variance to the evolution of aging is arduous to collect and ambiguous in net import (Rose et al. 2007). In particular, since both antagonistic pleiotropy and mutation accumulation can generate changes in age-specific genetic variance, and their joint action would allow almost any pattern of age dependence in the quantitative genetics of age-specific mortality and fecundity, it is doubtful that experiments of this kind are worthwhile from the standpoint of general scientific inference.

The antagonistic pleiotropy and mutation accumulation hypotheses have both received weak and inconsistent experimental support with regard to genetic correlations (Rose et al. 2005, 2007). An important factor to note, however, is that both these population genetic mechanisms may act simultaneously and, in so doing, cancel out the predicted effects of *both* mechanisms on correlations between relatives when they are acting jointly. This is like the difficulties facing experiments that study age dependence of genetic variances. Despite the years of work, some of it our own, that have gone into measuring these quantitative-genetic parameters, it is doubtful that their estimation is the most efficient way to address the population genetics that underlie the evolution of aging.

INDIRECT RESPONSES TO SELECTION

To some extent, selection experiments are better able to detect the simultaneous action of antagonistic pleiotropy and mutation accumulation. When, for example, postponed aging has evolved as a result of later reproduction in fruit flies or as a result of direct selection on longevity (e.g., Zwaan et al. 1995), it is often inferred that antagonistic pleiotropy causes a reduction in early fecundity (Rose and Charlesworth 1980, 1981b; Rose 1984; Clare and Luckinbill 1985; Luckinbill et al. 1987; Partridge et al. 1999). Sometimes this reduction in early fecundity is coupled with enhanced later fecundity, sometimes with a decrease in subsequent mortality, and sometimes with both enhanced later fecundity and decreased mortality. With antagonistic pleiotropy, it is expected that some early, functional characters, if not fecundity, will be depressed by the laboratory evolution of postponed aging (see Partridge and Fowler 1992). The observation of this pattern in a number of instances indicates that antagonistic pleiotropy is, in some cases, an important genetic mechanism in the evolution of aging.

The effects of mutation accumulation can also be observed in laboratory evolution experiments. For example, when later reproductive opportunities are denied, it is expected that after many generations later fecundity should be reduced, due to the accumulation of alleles with late-acting deleterious effects, while hybrids should show recovery of such reduced fecundity. This result was obtained by Mueller (1987) in fruit flies

denied any opportunity for later reproduction for more than one hundred generations. Similarly, Borash et al. (2007) found evidence of greater mutation accumulation at later adult ages in crosses of long-established experimental evolution lines of *Drosophila* tested for male virility. Mutation accumulation evidently can act over hundreds of generations to undermine functional characters, when the force of selection is reduced, making it an important mechanism for the evolution of aging, particularly in smaller populations.

Although it is ultimately difficult to determine the relative contribution that mutation accumulation makes to overall aging compared to antagonistic pleiotropy, we do not agree with Baudisch's (2008) strong dismissal of mutation accumulation. For instance, Baudisch (2008) relies heavily on the results of Pletcher et al. (1998) in formulating her arguments against the importance of mutation accumulation. This study, like that of Yampolsky et al. (2001), relies on lines of *Drosophila* that were allowed to accumulate mutations. The experiments are then designed to investigate the properties of these new mutants. An experimental difficulty with these types of experiments as tests of the population genetic mechanisms underlying aging are the number of new mutants one can reasonable expect to accumulate. In the Yampolsky et al (2001) study, there were originally two hundred parents; and in each generation, two hundred new parents were chosen—thus four hundred chromosomes chosen each generation. They used techniques to make N_e as large as possible and to avoid inbreeding effects. Now if we assume a mutation rate of 10^{-6} at loci that affect age-specific survival at later ages, and perhaps one thousand such loci, then over thirty generations the total number of newly arising mutants would be $(400) \times (30) \times (10^{-6}) \times (1{,}000) = 12$. (Note that these hypothetical assumptions are biased so as to favor the use of this experimental design to test for mutation accumulation.) At best, these new mutants are neutral—given the culture techniques—and therefore, if they arise as a single copy, there is a 25 percent chance of the newly arisen mutant being lost in the next generation. So at best we might be expecting around three new mutants in the course of this entire experiment. For these reasons, we believe it will be difficult to use these types of studies as a means of gaining detailed understanding of the role of mutation accumulation in the evolution of aging.

If mutation accumulation is contributing to aging in existing populations of *Drosophila*, then there ought to be many such deleterious alleles at selection-mutation equilibrium in existing populations. Consequently, experiments designed to uncover these existing deleterious alleles provide direct evidence for the importance of mutation accumulation to aging. Two studies not cited by Baudisch have done exactly this (Mueller 1987; Borash et al. 2007), as already mentioned. These studies show that, over hundreds of generations, existing deleterious late-acting alleles can rise to high frequency once they are made effectively neutral. In addition, other evidence supporting the contribution of mutation accumulation to *Drosophila* aging exist that are also not cited by Baudisch (e.g., Kosuda 1985; Hughes et al. 2002). In conclusion, we feel the existing evidence does in fact support the conclusion that mutation accumulation contributes to aging in *Drosophila* and probably other organisms.

VARYING EXTRINSIC MORTALITY RATES

Inherent within the evolutionary theories of aging is the idea that an increase in extrinsic mortality rates could, over many generations, lead to increased intrinsic mortality rates, decreased life span, increased development time, and increased earlier reproduction in a population, depending on patterns of pleiotropy and the presence of sufficient genetic variation. This is because with higher extrinsic mortality rates, the force of natural selection will decline more rapidly. Researchers have investigated the evolutionary effects of changing extrinsic mortality rates both in the lab and in nature. Stearns et al. (2000) created two types of *Drosophila* lines in the lab by applying either high or low extrinsic mortality on the populations, using artificially imposed mortality schedules. Over time, they measured various life-history characters and found that lines that had been subjected to higher extrinsic mortality rates evolved as expected.

Reznick and colleagues have similarly manipulated extrinsic mortality rates in populations of guppies in nature by exposing these populations to different predation levels (Reznick et al. 1990, 1997). They have found that development time and early fecundity patterns have increased with increased extrinsic mortality. However, these predicted changes in early-age life-history characters are not coupled with the expected earlier onset of aging in either mortality or fecundity in the same populations (Reznick et al. 2004, 2006). Ackermann et al. (2007) found a similarly paradoxical result for the evolution of aging in asymmetrically fissile bacteria. The latter case is particularly instructive, in that Ackermann et al. were able to resolve some of the evolutionary mechanisms involved in their overall failure to get the result expected from a simple application of Hamiltonian principles, particularly the complexity of the two-morph life cycle of the bacterium that they studied. In addition, Ackermann et al. did observe one case in which Hamiltonian expectations were met, the invasion of a mutant that increased the rate of aging in one of their bacterial lines that had been reproduced predominantly with younger bacterial "mothers." Such examples illustrate the importance of knowing enough about the biology of the experimental evolution system that you are using, in that a complex set of selection mechanisms may be at work, some of which may artifactually undermine Hamiltonian force of natural selection expectations.

These examples serve to illustrate the general point that the more complex designs of experiments that manipulate the level of imposed mortality rates, unlike the simpler procedure of altering the first age of reproduction in a laboratory population, may in turn make these experiments systematically more difficult to interpret. Futuyma and Bennett (this volume) also discuss the merits of simple experimental manipulations.

THE NUMBER OF GENES AFFECTING AGING

Early evolutionary discussions of aging, such as those by Williams (1957) and Maynard Smith (1966), characteristically concluded that a large number of loci are likely to affect aging. This gave rise to some pessimism among evolutionary biologists concerning the

feasibility of postponing aging. However, the success of laboratory evolution experiments in producing organisms with genetically postponed aging forced a reexamination of earlier assumptions concerning the number of loci affecting aging. Various experimental techniques have been used to answer this question, including segregation analysis (Hutchinson and Rose 1990), 2D protein electrophoresis (Fleming et al. 1993), and gene expression microarrays (Pletcher et al. 2002). Given the ambiguities and limitations of large-effect mutant studies of aging, discussed earlier, those publications do not provide very useful evidence with respect to the question of the number of loci that affect aging. At present, the best answer to the question of the number of genes controlling aging is many (Rose and Long 2002), in keeping with the original expectations of evolutionary biologists.

However, studies of the genetics of the experimental evolution of aging are now amenable to the application of genomic methods. Among the possibilities that are likely to transform research in this area are global comparisons of gene expression microarrays, whole-genome SNP association studies, and even large-scale genomic resequencing between populations that have evolved different rates of aging. With results like these in hand, we should be in a much better position to clarify the extent, and possibly the nature, of the genes involved in the evolution of aging and related characters. What will be of particular interest as this research unfolds is the relationships between the loci revealed by such whole-genome methods and the physiological mechanisms that underlie the evolution of life-history (see Harhsman and Zera this volume). Some hints of such relationships are provided by the tentative findings from protein electrophoretic studies of populations in which physiological mechanisms have been partially uncovered. For example, phosphoglucomutase is an enzyme that plays a key role in energetic catabolism; the Rose populations that live longer have both altered energetic metabolism and corresponding changes in the frequencies of alleles at this locus (Deckeret-Cruz et al. 1997; Rose et al. 2004). However, such research was carried out using very primitive technology compared to what is now available. Research over the next decade or so should reveal many points of interest concerning the physiological genetics that underlie the evolution of aging across entire genomes.

EVOLUTIONARY BIOLOGY OF LATE LIFE

THE DISCOVERY OF LATE LIFE

Aging has most often been numerically characterized in terms of the following equation for age-specific mortality rates, based on an idea originally proposed by Benjamin Gompertz early in the nineteenth century:

$$\mu(x) = Ae^{\alpha x}, \tag{1}$$

where x is age, $u(x)$ is the age-specific mortality rate, A an age-independent parameter that gives the baseline mortality rate, and α an age-dependent parameter, or the rate of

aging. Although this equation works quite well when fitting mortality data obtained from small cohorts, it has long been known to fail when it came to late-age mortality data from very large human cohorts (e.g., Greenwood and Irwin 1939; Comfort 1964; Gavrilov and Gavrilova 1991), which show a slowing or stabilization of age-specific mortality rates at very late ages. However, this quantitative aging pattern in humans was generally ignored until it was also observed in the 1990s in large cohorts from two dipteran species (Carey et al. 1992; Curtsinger et al. 1992; reviewed in Charlesworth and Partridge 1997). In these studies, late ages were characterized by an apparent cessation of age-related deterioration in age-specific survival probabilities (see figure 18.3). That is, mortality rates increased exponentially during midlife in these populations, as expected in aging organisms, but stopped increasing rapidly and crudely "plateaued" at later ages.

At first, the cessation of aging at late ages seemed paradoxical to most biologists who studied aging, because the widely presumed progressive accumulation of molecular and cellular damage with age was expected to increase until mortality rates reached 100 percent, especially given the Gompertz equation. However, since the definitive discovery of this postaging period of life, or "late life," data from a variety of labs has suggested that late life occurs generally, though not universally, among aging organisms (Fukui et al. 1993; Tatar et al. 1993; Brooks et al. 1994; Kannisto et al. 1994; Charlesworth and Partridge 1997; Vaupel et al. 1998; Carey 2003). Without question, mortality patterns in very old organisms from sufficiently large cohorts do not always follow the Gompertz

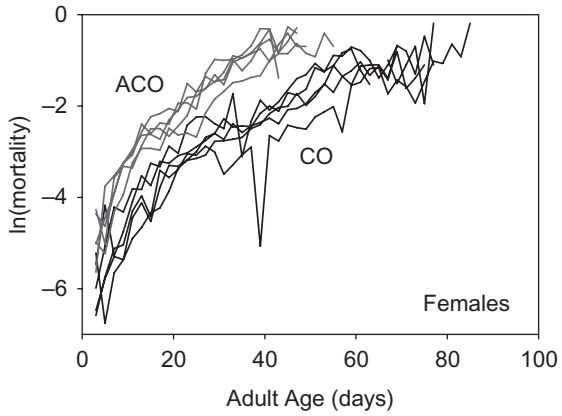

FIGURE 18.3
Two-day mortality rates in females for five replicated populations selected for early reproduction (ACO, gray lines) and five replicated populations selected for later reproduction (CO, black lines). Age-specific mortality rates increase with age until later ages when the rate of increase slows and mortality rates "plateau." Note that the slowing in mortality rate increase occurs at later ages in populations selected for later reproduction (CO) compared to populations selected for early reproduction (ACO). This finding supports the predictions of the evolutionary theory of aging and late life based on the force of natural selection. Data from Rose et al. (2002).

pattern. Late-life mortality rates vary widely among species, but a reliable attribute of late life is the switch from accelerating mortality to a relatively stable age-specific mortality rate, on average.

Mortality levels during the late-life period vary widely. The late-life age-specific mortality rates of some animals, humans being one example, are sometimes very high, relative to the baseline mortality rate, *A*. In other species, such as the medfly, the late-life mortality rate is not as high relative to *A* (Carey et al. 1992). In such species, it is conceivable that many adult organisms will achieve late life in laboratory cohorts and other protected settings. In any case, regardless of its observability in small cohorts, the distinctive feature of late life is the transition from rapidly accelerating mortality rates to a rough "plateau" of mortality. This is one of the most important findings of aging-related research since 1990, as it indicates that there can be an end to aging among some sufficiently old organisms.

Late life is characterized not only by a deceleration in age-specific mortality rates but, more recently, also by a cessation in the age-specific decline in reproduction (Rauser et al. 2003, 2006b). Together these unexpected observations of mortality and fecundity patterns strongly suggest that late life is as distinct a phase of life history as either development or aging. From an evolutionary standpoint, the onset of late life can be defined as the age at which age-specific fitness components stop deteriorating, on average. Since the first laboratory observations of late life, significant experimental and theoretical attention has been focused on explaining this phenomenon.

EXPLAINING LATE LIFE WITH HAMILTONIAN THEORY

PLATEAUS IN THE FORCES OF NATURAL SELECTION

Recall that the Hamiltonian theory for the evolution of aging is based on the a priori analysis of Hamilton (1966), as later refined by Charlesworth (e.g., 1980, 1994). A key feature of this theory is that the force of natural selection acting on survival falls to, and remains at, zero once reproduction has ended (see figure 18.1). In other words, the force of natural selection "plateaus" at zero at very late ages. This suggests that this same theory can explain late-life plateaus in mortality rates, providing only that the decline in age-specific fitness components observed during aging is due to the parallel decline in Hamilton's forces of natural selection, with the eventual plateau in these forces generating the fitness component plateaus of late life. The key to this is that s and s' must both equal zero for all ages after reproduction and survival ceased in the evolutionary history of a population. These plateaus in the forces of natural selection imply that natural selection did not discriminate among genetic effects that act at ages so late that they have no impact on fitness during the evolutionary history of a population.

This intuition was confirmed in explicit numerical simulations of population genetic scenarios for the evolution of age-specific mortality rates, scenarios that included antagonistic pleiotropy, mutation accumulation, or combinations of the two mechanisms

(Mueller and Rose 1996). Charlesworth (2001) supplied analytical solutions of this kind for special cases restricted to mutation accumulation. In both of these studies, it turned out that aging was generated by falling forces of natural selection, with the eventual plateau in these forces leading to a marked deceleration in the deterioration of aging. There have been several critiques of these evolutionary theories of late-life mortality plateaus (Charlesworth and Partridge 1997; Pletcher and Curtsinger 1998; Wachter 1999), and additional theoretical research on these models would no doubt improve them as purely theoretical constructs. Of greater interest here, however, is the success of these theories in tests using experimental evolution.

As with the force of natural selection acting on survival probabilities, Rauser et al. (2003, 2006b) noted that the force of natural selection acting on fecundity, as described earlier, asymptotically declines to zero at late ages. This plateau in the force of natural selection acting on fecundity was also shown to translate into late-life plateaus in fecundity by Rauser et al. (2006b) using computer simulations. The age at which s' declines to zero is dependent on the last age of survival in the environment in which evolution has occurred, rather than the last age of reproduction as with survival. Therefore, age-specific fecundity should stop declining and roughly "plateau" at late ages like the plateau in age-specific mortality rates, because the force of natural selection acting on fecundity is so low at late ages that it cannot distinguish fitness differences in fecundity.

Although it is possible that these late-life plateaus will be at the zero-survival or zero-fecundity levels in some species (cf. Pletcher and Curtsinger 1998), when there are enough alleles that have sufficiently age-independent beneficial effects, it is possible to have positive-valued average survival and average fecundity values (see Charlesworth 2001; Reynolds et al. 2007). Any beneficial effect that is not overly age-dependent will continue to benefit individuals who remain alive after the force of natural selection has converged on zero. And after that age, the intensity of natural selection will no longer depend on chronological age. If there are any age-independent genetic benefits, they will be favored by natural selection acting at early ages, with a pleiotropic echo benefiting later ages.

The expansion of the evolutionary theory of aging to include late life has been significant for the understanding of both aging and late life. Although a more formal mathematical derivation of the late-life theory is necessary, this theory yields testable predictions regarding age-specific fitness components of late life. Now evolutionary biologists must not only be concerned with why aging happens but why, when, and how aging ceases at late ages.

EXPLAINING LATE LIFE WITH NONEVOLUTIONARY THEORIES

Hamiltonian theory is not the only idea that has been proposed to explain the leveling of mortality rates at late ages. Another set of theories that have attempted to do so are demographic in nature and are based on the hypothesis of lifelong differences in individual

robustness within a population. We will call these theories collectively the "lifelong heterogeneity" theories of late life. The common assumption among these theories is that there are significant differences in robustness between individuals that make up a population and that these differences are not age-specific, but are maintained for the entire duration of an individual's life. That is, an individual that is born more robust remains robust until the day it dies, and conversely for those individuals that are less robust. Lifelong heterogeneity can produce rough late-life mortality rate plateaus because the less robust individuals will die off at earlier ages, leaving only the more robust individuals at late ages to define the population's mortality rate pattern.

Vaupel et al. (1979) developed the first lifelong heterogeneity theory that could conceivably explain late-life mortality patterns. Their theoretical analysis assumed that a population was made up of subgroups that were each characterized by unique Gompertz functions. Thus, one subgroup could have a low baseline mortality rate, A in equation (1), compared to other subgroups, but still have the same rate of aging. This would reduce this subgroup's age-specific mortality rate throughout life. Another formulation of this idea allows for variation among subgroups for the aging parameter, α, of equation (1) (Pletcher and Curtsinger 2000).

There are no lifelong heterogeneity theories that have been proposed to explain the fecundity plateaus that have been observed at late ages, and that are readily explicable using Hamiltonian theory. However, post hoc explanations could be concocted. For late-life fecundity plateaus, it is conceivable that there are lifelong differences in individual female fecundity, with some females laying a lot of eggs per day and some females laying only a few eggs per day. For example, high-rate egg layers might lay a lot of eggs at early ages resulting in their early death, leaving only the low-rate egg layers at later ages contributing to the population's late-life fecundity plateau. Note that this explanation assumes that there is a trade-off between fecundity and mortality. That is, high-rate egg layers also have high mortality, and vice versa for low-rate egg layers, a natural "reproductive effort" trade-off concept. A number of variations on this same idea are imaginable. However, it should be noted that, to the extent to which such variant life histories are affected by segregating alleles, Hamiltonian theory suggests that there should be selection against slower egg laying in most natural populations, all other things being equal.

EXPERIMENTALLY TESTING HAMILTONIAN THEORIES OF LATE LIFE

BASIC PREDICTIONS OF THE HAMILTONIAN THEORY OF LATE LIFE

Because the Hamiltonian late-life theory is an evolutionary theory, late life should evolve in ways predictable from the forces of natural selection, given enough genetic variation and an ample number of generations. Rose et al. (2002) used computer simulations of life-history evolution in which late-life mortality plateaus evolved in response to changes in the timing of reproduction. Recall that the force of natural selection acting on mortality

converges on zero sometime after the last age of reproduction. Therefore, Rose et al. (2002) predicted that late-life mortality plateaus should evolve accordingly in the experimental evolution of life history. Their simulation results explicitly demonstrated that the start of mortality rate plateaus should be positively correlated with the last age of reproduction, when Hamilton's forces of natural selection control the evolution of age-specific life-history characters. Similarly, Rauser et al. (2006a) found from numerical simulations that the last age of survival in the evolutionary history of a population should determine the start of late-life plateaus in fecundity. The evolutionary theory of late life based on the force of natural selection thus predicts that late life should evolve according to the timing of the convergence of the forces of natural selection on zero.

Experimental evolution has been used to test both these predictions. Both mortality rate and fecundity patterns in late life have been measured using multiple populations of *D. melanogaster* that have been maintained for many generations with different ages at which the forces of natural selection decline to zero. Thus far, all such experimental studies have strongly corroborated the predictions inferred from evolutionary theory. These studies will now be discussed in some detail.

MORTALITY

Rose et al. (2002) first tested the Hamiltonian analysis of late life using different types of replicated populations of *D. melanogaster* long selected for different ages of reproduction. They experimentally demonstrated that the start of late-life mortality plateaus evolves according to the last age of reproduction as predicted by the Hamiltonian theory for late life (figure 18.3). This study involved three different independent experimental tests, using a total of twenty-five evolutionary distinct cohorts of fruit flies. Two of these tests compared replicated populations long selected for different ages of reproduction. These tests featured either a fifty-five-day or a twenty-day contrast in last ages of reproduction. Age-specific mortality rates were measured for all populations, and the ages at which mortality rates plateaued were determined and compared between the different groups of populations. They found that late life started later in populations with *later* last ages of reproduction compared to populations that had *earlier* last ages of reproduction.

FECUNDITY

The Hamiltonian predictions for the evolution of late-life fecundity were experimentally tested by Rauser et al. (2006b)—specifically, whether late-life fecundity evolves as predicted using a comparison analogous to the comparison tests used by Rose et al. (2002) for mortality, except that Rauser et al. measured mid- to late-life fecundity from large cohorts derived from ten populations. Like the study of Rose et al. (2002), Rauser et al. (2006b) found that the start of late-life fecundity plateaus was earlier in the populations that had an earlier last age of survival in their evolutionary history, compared to the populations allowed to survive for longer periods, as predicted by Hamiltonian theory (figure 18.4).

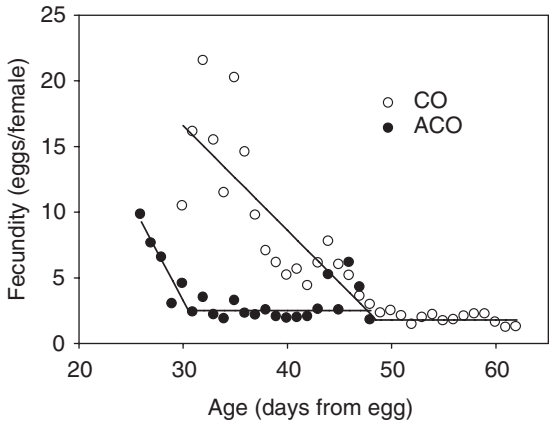

FIGURE 18.4

Mean age-specific fecundity for a later-reproducing CO population (open circles) and an early-reproducing ACO population (solid circles) for mid and late-life ages. This figure represents one of five pairwise comparisons between the CO and ACO populations from Rauser et al. (2006b) and demonstrates, after statistical analyses, that fecundity declines during midlife and stops declining and "plateaus" at late ages. Note that the slowing in the decline in fecundity occurs at later ages in the population selected for later reproduction (CO) compared to the population selected for early reproduction (ACO). This finding was consistent among all five pairwise comparisons and supports the predictions of the evolutionary theory of aging and late life based on the force of natural selection.

POPULATION GENETIC MECHANISMS OF LATE-LIFE EVOLUTION

Both theoretical and experimental analyses have revealed that late-life patterns of survival and reproduction are greatly affected by plateaus in Hamilton's forces of natural selection. The next question is whether the population genetic mechanisms that underlie the evolution of aging also underlie the evolution of late life.

With antagonistic pleiotropy, genes having a positive effect on early-age fitness components also have a detrimental effect at later ages. Therefore, with antagonistic pleiotropy involving late life, a shift to earlier *last* ages of reproduction and survival should result in a rapid shift in the age at which fitness components stabilize at late ages. Rose et al. (2002) and Rauser et al. (2006b) found just such rapid shifts in the onset of both late-life mortality and fecundity, respectively, in *Drosophila* populations. Rose et al. (2002) used an experimental design that started with replicated populations of fruit flies long selected for late reproduction. They then changed the last age of reproduction to a much earlier age, fifty-six days earlier, for only twenty-four generations, and then they compared the age of mortality plateau onset of the newly derived early reproduced populations to the original late-reproduced populations. The onset of late-life mortality plateaus from populations long having late last ages of reproduction responded quickly to selection for an earlier cessation of reproduction, implicating antagonistic pleiotropy

as a genetic mechanism shaping late-life mortality patterns (Rose et al. 2002), since the response was too rapid to be explained by mutation accumulation, given the limits on the quantitative effects of mutation accumulation over a short number of generations, as discussed earlier.

Rauser et al. (2006b) used the same experimental design to test whether antagonistic pleiotropy also works to shape late-life fecundity patterns, except that they started with replicated populations that last reproduced in midlife. Thus, the difference in age of reproduction between the original and newly derived populations with earlier termination of reproduction was only eighteen days. After making mid- and late-life fecundity comparisons and statistically determining the age of onset of late-life plateaus in fecundity, Rauser et al. (2006b) found that late-life fecundity also responds quickly to selection for earlier reproduction, with an earlier last age of survival at which reproduction can occur. Thus, antagonistic pleiotropy is also implicated as a genetic mechanism shaping late-life fecundity patterns.

Rose et al. (2002) tested whether mutation accumulation was contributing to late-life mortality patterns by using five genetically independent populations, long selected for the same age of reproduction, and performing all possible crosses between these populations to create new "hybrid" populations. The age of onset of late-life mortality plateaus was measured in all such hybrid populations and compared to the original populations from which they were derived. However, Rose et al. (2002) did not observe differences between crossed and uncrossed populations in late-life survival probabilities, failing to support the hypothesis that mutation accumulation acts in shaping late-life mortality, unlike the results obtained by Mueller (1987) or Borash et al. (2007) for reproductive characters during aging. Although this experiment from Rose et al. (2002) did not support mutation accumulation as a genetic mechanism in the evolution of late life, it did not refute it. While hybrid superiority supports the involvement of mutation accumulation, failure to detect it does not necessarily show that it isn't involved in the evolution of a life-history character.

Reynolds et al. (2007) used chromosomes extracted from isofemale lines of *D. melanogaster* to test for age specificity in allelic effects on age specificity. Their goal was to determine whether or not such allelic effects were too age-specific for Charlesworth's (2001) explanation for the evolution of late-life plateaus with nonzero values of life-history characters. They found enough width of age specificity to support Charlesworth's hypothesis, in their opinion. However, their experimental analysis suffers from uncertainty about the degree to which chromosomes extracted from isofemale lines are adequately representative of the effects of the genetic variation that predominates among outbred populations, given the artifacts discussed at some length earlier, both inbreeding effects and genotype-by-environment interaction. Nonetheless, their results certainly constitute an example in which a conscientious attempt to critically test Hamiltonian theory for late life failed to refute that theory.

EXPERIMENTALLY TESTING NONEVOLUTIONARY THEORIES OF LATE LIFE

Although the lifelong heterogeneity theories of late life are heavily emphasized by some scientists (e.g., Vaupel et al. 1979), these theories have not withstood experimental testing. This is unlike the experimental work that has been done to test the alternative Hamiltonian theories of late life.

MORTALITY

Inbreeding Genetic variation could drive the heterogeneity models. But extensive experimental work has shown that, after removing genetic variation by extensive inbreeding, well-defined plateaus continue to be observed (Curtsinger et al. 1992; Fukui et al. 1993, 1996). In the absence of genetic variation, all variation must be environmental in origin. Detailed studies with *Drosophila* have shown that environmental changes that affect longevity, like dietary restriction, do so by changing the age-independent parameter of the Gompertz equation not the age-dependent parameter (Nusbaum et al. 1995). However, empirical estimates of the levels of variation in the age-independent parameter that would be required to produce plateaus suggest these requirements are far greater than observed levels of variation (Mueller et al. 2003). Consequently, standard lifelong heterogeneity models fail to explain the absence of an effect of inbreeding on the occurrence of late-life mortality plateaus.

Experimental Manipulation of Environmental Variation The experiments establishing the existence of late-life mortality rate plateaus were all carried out under laboratory conditions where environmental variation is purposely minimized, although it is technically impossible to eliminate all environmental variation. Khazaeli et al. (1998) reduced the environmental variation experienced by the preadult, developmental stages of *Drosophila* by collecting subsamples of eggs and pupae that had more similar environmental histories. The mortality of adults from these subsamples was compared to adults that experienced the full range of environments. If the Gompertz demographic parameters are affected by these environmental effects then plateaus should be less prominent or nonexistent in the subsampled populations. There was no difference found in the timing of mortality deceleration, as a result of this heterogeneity-reducing procedure, suggesting that these preadult environments contribute little to the creation of the lifelong heterogeneity in demographic parameters required by anti-Hamiltonian demographers.

Age-Specific Variance Service (2000, 2004) showed that the natural log of age-specific mortality rates should show a unimodal distribution if there is sufficiently large variation in A and α among genetically different populations. We have examined this variance across highly differentiated populations. Despite the fact that these populations had been isolated and had undergone independent evolution for one hundred to five hundred generations,

the pattern predicted by Service was not seen (Mueller et al. 2003). Such negative outcomes from experimental tests of lifelong heterogeneity theory do not preclude the possibility that purposeful methods of creating genetic differentiation between populations, like selection or inbreeding, might result in the patterns adduced by Service as a result of lifelong heterogeneity.

Extreme Age Distribution Several studies have examined models that assume there is population variation for α in the Gompertz equation (Pletcher and Curtsinger 2000; Service 2000; Mueller et al. 2003). When Service varied α in order to produce late-life mortality rate plateaus, he generated simulated populations with average longevities of 50 days, which is reasonable for *Drosophila*, but simulated maximum life spans of 365 days, which are unknown for this species. Mueller et al. (2003) followed up on this result by attempting to make parameter estimates of a model with variation in α that was based on observed mortality data from many populations of *Drosophila*. These maximum-likelihood estimates also lead to predictions of more very old individuals than were observed in these populations, if one starts from the assumption that lifelong heterogeneity produces late-life mortality rate plateaus.

The conclusions of Mueller et al. (2003) have been criticized by de Grey (2003). A response to those criticisms have also been published (Mueller and Rose 2004). Without rehashing the details of that response, there are two specific problems with de Grey's (2003) analysis.

The first is that de Grey expresses concerns about the consequences of using the maximum-likelihood techniques and then goes on to develop his own method for estimating model parameters. The scientific onus is on de Grey to show that his technique provides superior estimators of model parameters relative to the maximum-likelihood estimators. This must be done in terms of specific properties of estimators, such as their bias and variance (e.g., see Mueller et al. 1995).

Second, the heterogeneity versions of the Gompertz equation that are at issue are continuous time models with instantaneous mortality functions that we will label $f(t)$. Therefore, in an experiment where observations are made at discrete times, the observed mortality, Δm, between times t_1 and t_2 should be equal to $1 - (p(t_2)/p(t_1))$, where $p(t)$ is

$$\exp\left\{-\int_0^t f(x)dx\right\}.$$

de Grey's parameter estimation scheme equates Δm to $f(t)$. Parameters estimated in this fashion have no logical connection to the original model.

Robust Flies Theories that explain late-life mortality deceleration on the basis of a conjectured abundance of lifelong differences in robustness must imply differences in the properties of late life when selection radically improves robustness at early ages. In particular, populations that are much more robust should proceed to late-life mortality stabilization at later ages. Using populations of *Drosophila* successfully selected for much

greater starvation resistance and their controls, Drapeau et al. (2000) found no such late-life differences between more and less robust populations. But a reanalysis of these data led Steinsaltz (2005) to different conclusions. However, in a major methodological departure from normal practice in these experiments, Steinsaltz chose to remove from his analysis the mortality data observed in early life. It is hardly surprising that under these conditions the results might differ between the studies of Drapeau et al. (2000) and their reanalysis by Steinsaltz (2005). The process of removing data is always fraught with danger since it is by and large a subjective procedure often guided by a priori expectations that are in fact part of the hypotheses being tested.

Density Treatments Carey has argued (Carey et al. 1995; Carey 2003) that if mortality is increased by increasing the population density, then the age at which a mortality plateau occurs should decline. This follows reasonably enough because, at high density, the less robust groups are being eliminated faster, and thus the age at which only the most robust groups are left, and the age at which pronounced late-life deceleration in mortality occurs, should come sooner. However, in experiments with Mediterranean fruit flies, changing adult density had no detectable effect on the age at which mortality rates leveled off (Carey et al. 1995; Carey 2003). Carey (2003) concludes that "leveling off of mortality is not an artifact of changes in cohort composition."

Natural Selection and Heterogeneity As evolutionary theory predicts late-life plateaus in underlying propensities to die or reproduce, proponents of heterogeneity theories also need to supply a model as to why this type of evolution will not happen. Admittedly, creationists have such a model, but there are some important details left out of creationist theory, such as how creation is supposed to work. A lot of explanatory work is required to justify why evolution does not shape characters like mortality and fecundity, which are integral to the population genetic theory of selection and adaptation.

If environmental variation affects age-specific survival probabilities or age-specific fertility, then it affects fitness. There is significant mathematical theory that shows that under these conditions, natural selection will favor the evolution of biological mechanisms that reduce the environmental variation in components of fitness (Gillespie 1973). Such evolution would again narrow the conditions under which the lifelong heterogeneity theory of late-life plateaus works.

FECUNDITY

Although the lifelong heterogeneity theories have not been extended to include fecundity, as previously mentioned, there are several post hoc explanations based on the lifelong heterogeneity hypothesis that can be contrived so as to explain the observed late-life fecundity plateau data (Rauser et al. 2003; Rauser et al. 2006b). The scientifically inspiring thing about robustness in fecundity is that it can be measured over the lifetime of an

individual, unlike the case of the hypothetical robustness underlying mortality in lifelong heterogeneity theories. Thus, the observation of late-life fecundity plateaus provides a platform for more direct tests of the lifelong heterogeneity theories for late life. One way of testing for a connection between individual "robustness" and fecundity is to experimentally measure daily fecundity throughout adult life for all individuals in a *Drosophila* population, and compare the fecundity of those individuals that live to lay eggs in late life with those that do not.

Rauser et al. (2005) undertook the task of measuring the daily fecundity and time of death in three large cohorts of *D. melanogaster* in order to experimentally test lifelong heterogeneity theories of late life. With either of the lifelong heterogeneity theories described earlier for fecundity, early-life fecundity of an individual should indicate the fecundity of that individual in late life: the high-rate egg layers at early ages should either die before late life or be the individuals contributing to late-life fecundity patterns, depending on the lifelong heterogeneity theory you are adhering to. Rauser et al. (2005) could easily test both versions of the lifelong heterogeneity theory for fecundity: the results showed that knowledge of female fecundity in early life does not predict survival to late life. This was yet another refutation of the lifelong heterogeneity theory of late life.

ARE WE NEEDLESSLY CRUEL?

Were it not for the vast amounts of data required to test lifelong heterogeneity theories for late life properly, it would be an amusing recreation to refute them over and over again. Furthermore, as evolutionary biologists have a perfectly usable, and already corroborated, Hamiltonian alternative, it seems unreasonable for us to continue pummeling this theory. However, we have late-life physiological data forthcoming that will do just that, though we will spare our present readers such gratuitous intellectual bloodshed. For now.

THE IMPACT OF EXPERIMENTAL EVOLUTION ON AGING RESEARCH

Experimental evolution has had a major impact on aging research over the last few decades. It has corroborated much of the evolutionary theory derived by Hamilton (1966) and Charlesworth (e.g., 1980, 1994) and has plausibly explained why aging occurs. Experimental evolution has also revealed the need for more theoretical work, especially in the newly discovered area of late life.

Although late life was first established as a scientific phenomenon by biologists who were interested in demographic theories appropriate to the "oldest old" (see Vaupel et al. 1998), evidence is accumulating that the phenomenon is not a mere "sampling effect" arising within cohorts. Rather, we contend that late life is an evolutionarily distinct phase of life history, evolving according to strictures very different from those that mold both

early life and aging. Late life arises after the strength of natural selection acting on mortality and fecundity have approached zero. From work on both mortality and fecundity in late life, there is at least some pleiotropic connection with early life. When the last age of reproduction is abruptly changed, the timings of the mortality rate and fecundity plateaus shift evolutionarily (Rose et al. 2002; Rauser et al. 2006b). Mortality rate plateaus change by almost a day for each generation of laboratory evolution. This is a remarkable speed of evolution that is only explicable in terms of late-life pleiotropic effects of genetic change arising from strong selection on alleles that have effects early in life.

We have only begun to characterize the evolution of late life. But it is already quite clear that it is as different from aging as aging is from development. These phases of life are connected by pleiotropic gene action, but each phase evolves according to very different rules. Given that late life apparently evolves at least as rapidly as aging itself does, it is apparent that this is a fertile area for the use of experimental evolution, just as aging is.

SUMMARY

Evolutionary biologists have supplied a formal theory for the evolution of aging, a theory that depends critically on Hamilton's forces of natural selection. This "Hamiltonian" theory has been tested repeatedly using the techniques of both genetics and experimental evolution, particularly using the genus *Drosophila*. Of the two approaches, experimental evolution has given much clearer, readily reproducible, and generally supportive results. However, Hamiltonian evolutionary theory seemed to come under challenge with the discovery of a rough plateauing in age-specific mortality rates during the later ages of very large cohorts of insects. This period during which aging ostensibly slows, or even stops, has been called "late life," particularly in the evolutionary literature. It turned out that, rather than being undermined by the existence of late life, Hamiltonian theory naturally implied the possibility of a late-life deceleration of aging. Furthermore, experimental evolution has corroborated this extension of the original theory, in particular by showing that changing the ages at which Hamilton's forces of natural selection plateau leads to corresponding changes in the ages at which late life starts, as predicted by evolutionary theory. Thus, the case of aging and late life is one of the better illustrations of the value of experimental evolution as a tool for testing and refining evolutionary theories.

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We wish to apologize to any readers who have reached this point in our monstrously long chapter. It was no intention of ours to write a chapter of such length, and indeed our original submission was nothing so elephantine. We would like to give credit where it is due and acknowledge that it was only the diligence of our corresponding editor, T. Garland, that forced us to expatiate so tediously. We are also grateful for the comments and suggestions from one of our referees. Parts of the experimental work discussed in this chapter were

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