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EXPERIMENTAL STUDIES OF PLEIOTROPY AND EPISTASIS IN *ESCHERICHIA COLI*. II. COMPENSATION FOR MALADAPTIVE EFFECTS ASSOCIATED WITH RESISTANCE TO VIRUS T4

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Abstract.—Mutations in *Escherichia coli* that confer resistance to virus T4 also have maladaptive effects that reduce competitive fitness. After resistant populations had evolved for 400 generations in the absence of T4, their fitness approached that of sensitive populations allowed to evolve under identical conditions. However, the resistant populations had not reverted to sensitivity. Instead, this convergence in fitness resulted from genetic changes that compensated for maladaptive pleiotropic effects of the resistance mutations. An allele selected in an evolving resistant population reduced the competitive disadvantage associated with resistance by almost half. Interestingly, this allele was also beneficial in sensitive populations, although its fitness advantage was only about one-fifth as great as it was in the resistant population. These results run counter to a commonly held view that trade-offs between components of fitness should become more pronounced as populations approach their "selective equilibria." If a trade-off derives from some limiting energetic or material currency, then it is likely to become more pronounced as a population becomes more finely adapted. If a trade-off derives from the disruption of genetic integration, then it is likely to be diminished with further adaptation.

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Sewall Wright (1977 [and references therein]) has presented a general theoretical framework for understanding evolutionary processes that involve many loci when many alleles have pleiotropic effects. Wright's "adaptive landscape" portrays mean fitness in a population as a multidimensional function of allelic composition over all loci. Accordingly, selection acting on any given allele cannot be understood in isolation but depends on the genetic background in which it is found.

Natural selection pushes populations to local peaks in this adaptive landscape but opposes shifts to other peaks, even those that are higher, because intermediate states have lower fitness. Each peak may be viewed as a "coadapted gene pool" (Dobzhansky, 1955; Mayr, 1963; Carson and Templeton, 1984). Shifts between adaptive peaks are assumed to be important in differentiation of populations and species, because they can generate selection for barriers to gene flow (Endler, 1977; Barton and Rouhani, 1987).

Wright's "shifting balance" theory allows for several mechanisms whereby movement between adaptive peaks can occur. Many evolutionary biologists have emphasized the role of stochastic processes, particularly founder effects and the resulting genetic drift, in permitting an adaptive shift (Mayr, 1963; Templeton, 1980; Barton and Charlesworth, 1984; Carson and Templeton, 1984; Lande, 1985; Barton and Rouhani, 1987; Rouhani and Barton, 1987), but the likelihood of shifts depends on a number of parameters that are difficult to measure. There exist other mechanisms for producing adaptive shifts that do not require stochastic effects arising in small populations (Dodson and Hallam, 1977; Kirkpatrick, 1982). A transient environmental change may produce a new adaptive landscape, so that selection favors an alternative genetic composition. Although the environment subsequently resumes its original state, selection may favor changes other than those that simply restore the population's original genetic composition, because of intervening adaptation to the transient environment (Fig. 1). Wright (1977) has termed this mechanism for producing an adaptive shift "mass selection under changing conditions."

Mutations in *Escherichia coli* B that confer resistance to the virus T4 also impose

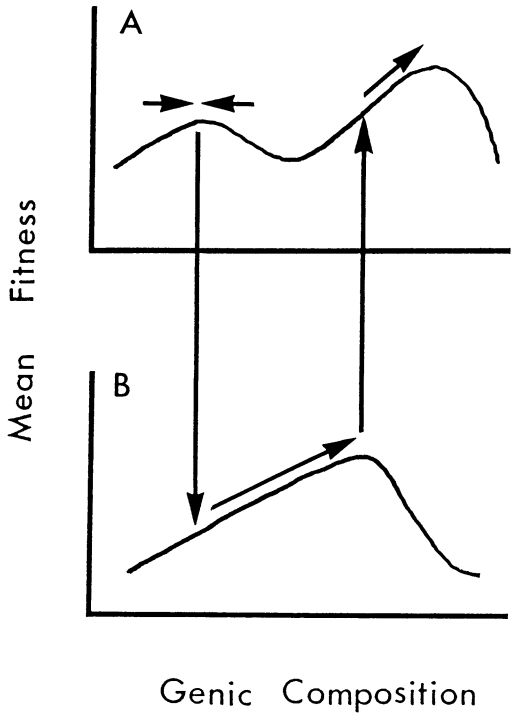

FIG. 1. Schematic illustration of a shift between two adaptive peaks precipitated by a transient environmental change. The vertical axis represents fitness, while the horizontal axis represents some critical dimension of genetic variation. In environment A, selection pushes a population to a local adaptive peak, but opposes a shift to another adaptive peak, which may be higher or lower than the first peak. In environment B, the adaptive topography has changed, causing selection to favor a new genetic composition. Although the environment subsequently reverts to its former state, the population is pushed to a novel adaptive peak. Wright (1977) calls this process "mass selection under changing conditions."

severe maladaptive pleiotropic effects on competitive fitness (Lenski, 1988). In this paper, I examine adaptation in populations with genetic backgrounds that differ in the presence or absence of these resistance alleles. Do resistant populations revert to sensitivity in the absence of the virus, as might be expected given the maladaptive pleiotropic effects? If the populations do not revert to sensitivity, are alleles that reduce the "cost" of resistance selected? If such alleles are selected, what effect do they have on competitive fitness in the sensitive genetic background? Are epistatic effects on fitness sufficient to precipitate a shift by resistant populations to a novel adaptive peak?

MATERIALS AND METHODS

Bacterial and Viral Strains.—All bacterial strains used in this study were derived from one strain of *Escherichia coli* B, and all possess several phenotypic markers that unambiguously distinguish mutants from possible outside contaminants (Lenski, 1988). The parental strain is sensitive to virus T4 and is unable to utilize the sugar arabinose, but T4-resistant mutants and Ara^+ revertants are readily selected (Lenski, 1988).

Genetic adaptation was monitored for 400 generations under culture conditions described below in six populations sensitive to the virus T4 and in five populations resistant to T4. Each population was initiated from a single colony, so that any mutations selected in these evolving populations must have arisen independently. Three each of the T4-sensitive and T4-resistant clones used to found the evolving populations were Ara^+, while the remaining clones were Ara^-. The arabinose phenotype has no detectable effect on fitness in the medium in which the populations were allowed to evolve and compete; the 95% confidence interval for the relative fitness of otherwise isogenic Ara^- and Ara^+ strains ranges from 0.99 to 1.01 (Lenski, 1988). This neutral marker did, however, serve two important purposes in this study. First, the marker provided protection against cross-contamination of evolving populations, which could otherwise go undetected. No cross-contamination occurred, but a sixth resistant replicate was lost due to contamination from an outside source during the experiment. Second, competing strains could be readily differentiated by colony color when plated on tetrazolium arabinose indicator agar (Levin et al., 1977).

After 400 generations, each of the 11 evolving populations was sampled, and single colonies were chosen at random. Each of these "evolved" clones was immediately stored at −80°C to preclude further genetic changes, as were the strains used to initiate the 11 populations.

Culture Conditions.—10-ml cultures were maintained in 50-ml Erlenmeyer flasks held in a shaking incubator set at 37°C and 120 rpm. The culture medium consisted of a minimal-salts solution supplemented with

25 μg/ml of glucose (Lenski, 1988). Each day, 0.1 ml of each culture was transferred into 9.9 ml of fresh medium. The 100-fold daily increase corresponds to about 6.6 generations of binary fission; 60 transfers were necessary to complete the 400 generations of experimental evolution.

Measurements of Fitness and Resistance.—Relative fitness was measured by competition experiments involving two strains, one Ara^- and one Ara^+, for one day (6.6 generations) in the culture conditions described above. All competing strains were preconditioned, inoculated, and sampled as described previously (Lenski, 1988). Unless otherwise noted, one of the two competitors was an unevolved, T4-sensitive strain, and competitive fitness is expressed relative to this baseline. Relative fitness was calculated as the ratio of the number of doublings between initial and final samples for the two competitors. Resistance to viruses T4 and T7 was determined by titering concentrated lysates on bacterial lawns in soft agar.

Isolation of a "Fitness" Allele Selected in a Resistant Population on the Sensitive Genetic Background.—"Fitness" alleles have no known phenotypic effect other than the quantitative advantage they confer. Thus, it is exceedingly difficult to map fitness alleles obtained from evolving populations (Dykhuizen and Hartl, 1983; Chao and McBroom, 1985), because methods of formal genetic analysis require the ability to directly select qualitatively distinct phenotypes. For this same reason, fitness alleles cannot be reliably moved from one genetic background to another. Genetic manipulation is further complicated in this system, because alleles conferring T4-resistance (which can be directly scored) cannot be readily moved, since they engender cross-resistance to the transducing phage P1 (Tamaki and Matsuhashi, 1971; Wright et al., 1980).

These difficulties were circumvented as follows. It has been shown that mutations causing T4-resistance also cause increased sensitivity to the antibiotic novobiocin (Tamaki and Matsuhashi, 1971; Havekes et al., 1976; Nikaido, 1979; Lenski, 1988). Although it is difficult or impossible to select T4-sensitive revertants from most T4-resistant strains (Lenski, pers. observ.), I chose one Ara^- T4-resistant mutant that could be reverted to sensitivity using novobiocin selection. The mutation also conferred cross-resistance to T7, and it mapped to (or near) the *lpcB* locus (Lenski, 1988). I will refer to this particular resistance allele as R_{47}. It was necessary to demonstrate that reversions restored the competitive fitness of the original sensitive allele, designated *S*. To this end, I selected five independent revertants to T4-sensitivity and performed competition experiments with each against the baseline Ara^+ strain.

It is often not clear whether an evolved strain contains one or more than one fitness mutation. In order to increase the likelihood that only a single genetic substitution had occurred in a given evolved line, I employed the neutral arabinose marker as follows (see also Luckinbill [1984] for a similar selection procedure using a histidine marker). A population was initiated from a mixture of Ara^- and Ara^+ clones of the revertible T4-resistant mutant described above, and this population was allowed to evolve under the standard culture conditions. After eight days (53 generations), there was a sudden upward shift in the ratio of Ara^- to Ara^+ cells, which indicated that a favored mutant had swept through the Ara^- subpopulation. The clone carrying the mutant allele retained its resistance to T4 but also remained revertible to T4-sensitivity using the novobiocin selection procedure. Five independent T4-sensitive revertants of this evolved clone were selected, and each was tested in competition experiments against the baseline Ara^+ competitor. F_{R47} will be used to indicate the fitness allele selected on the resistant background, while the wild-type allele at this undetermined locus will be designated +. Possible epistatic effects on competitive fitness were thus examined using strains expressing either the sensitive (*S*) or the resistance (R_{47}) allele at one locus, and possessing either the wild-type (+) or the fitness allele isolated on the resistant background (F_{R47}) at another.

RESULTS

Changes in Fitness over 400 Generations in Populations of Sensitive and Resistant Bacteria.—The relative fitnesses estimated for all initial and final clonal isolates are

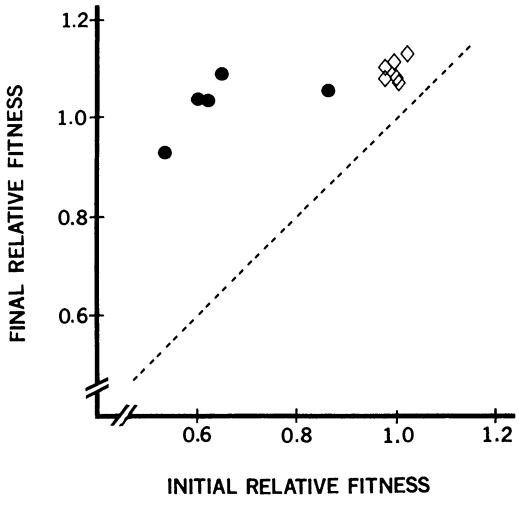

FIG. 2. Changes in relative fitness for six sensitive populations (open diamonds) and five resistant populations (closed circles) evolving in parallel environments. The abscissa gives the average of two fitness estimates for each clone isolated at the start of the experiment; the ordinate gives the average of two fitness estimates for each clone isolated after 400 generations. All fitnesses are expressed relative to baseline sensitive strains. None of the resistant populations reverted to sensitivity, but there was significant compensation for maladaptive effects associated with resistance. See text for details.

shown in Figure 2. Fitnesses are expressed relative to the Ara^- and Ara^+ baseline (unevolved, T4-sensitive) strains. Each point corresponds to one population, with the abscissa providing the mean of two replicate fitness estimates for the initial isolate (unevolved), and the ordinate the mean of two estimates for the final isolate (evolved for 400 generations).

The average fitness estimated for the six sensitive populations at the start of this experiment was very nearly equal to 1.00, as expected because there had been no opportunity for adaptation. The average fitness for the sensitive populations after 400 generations had increased to 1.10. The average fitness of the resistant populations at the start of the experiment was only 0.66. After 400 generations, the average fitness for the five resistant populations had increased to 1.03. All five populations retained complete resistance to virus T4, even while they attained fitnesses roughly comparable to the baseline sensitive strains. The increased fitnesses of the resistant populations indicate adaptation to the environment in which they were cultured, compensation for the maladaptive pleiotropic effects of resistance, or both. One must compare the increases in fitness that were observed for the resistant populations to those observed for the sensitive populations, which evolved in parallel under identical environmental conditions, in order to distinguish among these alternatives.

A measure of the increase in fitness for each sensitive and resistant population can be visualized as the displacement of each point above the diagonal line in Figure 2. The mean fitness increase in the sensitive populations was 0.10, while the mean fitness increase in the resistant populations was 0.38. A two-tailed Mann-Whitney test indicates that the fitness increases observed in the resistant populations were significantly greater than those observed in the sensitive populations ($P < 0.01$). These results therefore demonstrate substantial compensation for the maladaptive pleiotropic effects of T4-resistance.

Cross-Resistance to T7.—Four of the five T4-resistant mutants used to initiate the evolving populations were cross-resistant to T7. T4-resistant mutants that are cross-resistant to virus T7 have more severe maladaptive pleiotropic effects than T4-resistant mutants that are sensitive to T7 (Lenski, 1988). After 400 generations, all four populations initially cross-resistant to T7 retained plating efficiencies for that virus that were several orders of magnitude below the one population that was initially sensitive to T7. Therefore, the genetic substitutions that led to increased fitnesses of T4-resistant populations did not involve the same genetic differences responsible for the variation in pleiotropic effects among independent T4-resistant mutants.

Effects of Allele F_{R47} *on Fitness in Resistant and Sensitive Genetic Backgrounds.*—Fitnesses of T4-resistant strains with and without the "fitness" allele F_{R47} were measured relative to the baseline, T4-sensitive strain (possessing the alternate arabinose marker) and relative to the same T4-resistant strain lacking F_{R47} (but also possessing the alternate arabinose marker). Each treatment comprised 25 replicate measurements of fitness. Nonparametric tests and confi-

dence intervals are presented below to avoid violating assumptions of normality and homoscedasticity; 95% confidence intervals for the median of each treatment were computed according to Mosteller and Rourke (1973) and are given parenthetically. A fitness of 0.57 (0.47–0.66) was observed for the resistant strain lacking allele F_{R47} relative to the sensitive strain. Relative fitness increased to 0.85 (0.84–0.88) when the resistant strain possessed allele F_{R47}. A Mann-Whitney test indicates that the difference in fitness estimates for the resistant strain with and without F_{R47} is highly significant ($P < 0.001$). The fitness of the Ara^- resistant strain relative to its isogenic Ara^+ counterpart was 1.07 (0.93–1.34); one cannot reject the null hypothesis that the arabinose marker is neutral on the resistant background ($0.1 < P < 0.25$). Finally, the fitness of the resistant strain with allele F_{R47} was 1.58 (1.50–1.88) relative to the resistant strain with the wild-type allele. A Mann-Whitney test indicates that this fitness differential is highly significant relative to the arabinose marker control ($P < 0.001$). Note that allele F_{R47} had similar effects on the fitness of the resistant strain whether it was measured relative to a sensitive ($0.85/0.57 = 1.49$) or resistant ($1.58/1 = 1.58$) competitor.

To determine the effect of allele F_{R47}, which was selected in a resistant strain, on the fitness of sensitive strains, I obtained five estimates of fitness for each of five independent sensitive revertants of the T4-resistant strain possessing F_{R47} and for each of five revertants of the same T4-resistant strain possessing the wild-type allele. Fitnesses were determined relative to a baseline sensitive strain with the alternate arabinose marker. The mean fitnesses estimated for the five revertants carrying the F_{R47} allele ranged from 1.05 to 1.15 (with a grand mean of 1.09), while the mean fitnesses estimated for the five revertants with the wild-type allele ranged from 0.98 to 1.01 (with a grand mean of 0.99). A two-tailed Mann-Whitney test indicates that the F_{R47} allele selected in the resistant strain was also beneficial on the sensitive genetic background ($P < 0.01$).

Equally important, however, is the fact that 24 of 25 measurements of the fitness of the resistant strain with allele F_{R47} relative to the resistant strain with the wild-type allele (as reported earlier) were greater than any of the 25 measurements of the fitness of the sensitive strains with F_{R47} relative to the sensitive strain with the wild-type allele (as just reported above). A two-tailed Mann-Whitney test indicates that this difference is highly significant ($P < 0.001$), so one must conclude that there is epistasis for competitive fitness. On the resistant background, the fitness allele F_{R47} confers a selective advantage of about 50%, whereas this allele confers an advantage of about 10% on the sensitive background.

Preliminary Data on the Similarity of Fitness Alleles Isolated from Sensitive and Resistant Strains.—Note that the selective advantage of the F_{R47} allele on the sensitive genetic background is similar to the advantage accrued by sensitive populations during 400 generations of evolution (see Fig. 2). This suggests that fitness-enhancing alleles isolated from the sensitive genetic background may be similar or identical to F_{R47}.

In order to test this hypothesis, I chose one of the sensitive strains that had evolved for 400 generations; I designate the fitness allele(s) in this strain as F_S. I selected from this evolved sensitive strain a mutant that confers resistance to T4 and cross-resistance to T7, and which maps at (or near) the *lpcB* locus, as does the R_{47} allele. I then obtained five replicate measurements of the fitness of this mutant relative to the baseline, T4-sensitive strain (possessing the alternate arabinose marker); these ranged from 0.76 to 0.83, with a mean of 0.80. This fitness is higher than that observed for any of 16 mutants resistant to T4 and cross-resistant to T7 that were isolated previously from the unevolved sensitive strain (Lenski, 1988 fig. 1). This is true even when the fitnesses of these 16 mutants are multiplied by 1.1, which reflects the expectation under a multiplicative model of the effects of the resistance and fitness alleles. This result demonstrates that F_S compensates for maladaptive effects associated with resistance, as does F_{R47}, supporting the hypothesized similarity of fitness alleles isolated from the sensitive and resistant genetic backgrounds. However, the F_S and F_{R47} alleles are not identical. The F_{R47} allele is neutral on the sensitive background when malt-

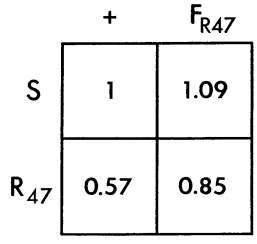

FIG. 3. Relative fitnesses of four haploid genotypes. S and R_{47} are alleles at one locus conferring sensitivity and resistance, respectively, to viruses T4 and T7. At another locus, + is the wild-type allele, whereas F_{R47} is an allele selected by virtue of its beneficial effect in a strain possessing the R_{47} allele. Varying the fitness of the S/F_{R47} genotype radically alters the adaptive topography. See text for further discussion.

ose is substituted for glucose in the experimental medium, whereas the F_S allele is actually disadvantageous under these same circumstances (Lenski and Travisano, unpubl.).

DISCUSSION

Figure 3 summarizes the epistatic effects on competitive fitness associated with one gene determining resistance to viruses T4 and T7 and another gene detected by virtue of its fitness-enhancing effect in a resistant strain. There are four haploid genotypes; I use S and R_{47} to indicate sensitive and resistant alleles at one locus and + and F_{R47} to indicate wild-type and "fitness" alleles at another. One can look at the effects on fitness associated with genetic substitutions in two ways. First, what is the effect of the fitness allele on the sensitive and resistant backgrounds? On the resistant background, this allele confers a 49% advantage (0.85/0.57), while on the sensitive background it confers an advantage of only 9% (1.09/1). Second, what is the effect of the resistance allele on backgrounds with the wild-type and fitness alleles? On the wild-type background, the resistance allele imposes a "cost" of 43% (1 − [0.57/1]), while on the background containing the fitness allele, the cost of resistance is only 22% (1 − [0.85/1.09]).

The concept of an adaptive landscape provides an appealing framework for discussing constraints on adaptation, taxonomic differentiation, and other issues in evolutionary biology that require consideration of multiple gene substitutions which may have epistatic effects on fitness. Nonetheless, it is difficult to use this framework to direct empirical research, because there is an implicit assumption that one has taken into account the effects of all relevant genetic variation. If not, there remains the very real possibility that two adaptive peaks apparently separated by maladaptive intermediate states are, in fact, connected by a "ridge" of well adapted intermediate states (Barton and Charlesworth, 1984; Barton and Rouhani, 1987). Thus, there is a problem in choosing a priori those dimensions that are sufficient for understanding the complex dynamics of natural selection (Lewontin, 1974).

In this study, I have attempted to determine whether sensitive and resistant populations evolving in parallel were approaching the same adaptive peak or whether they were approaching alternative peaks. As illustrated in Figure 1, even a transient environmental change (here, the introduction of a viral parasite) could have precipitated a shift from one adaptive peak to another. The resistant and sensitive populations allowed to evolve for 400 generations clearly attained different genetic states, because none of the resistant populations reverted to sensitivity. But this evidence alone does not imply that there exists an adaptive "valley" between the evolved sensitive and resistant states. In fact, quite the opposite was true, because it was demonstrated that there remained a selective advantage associated with reversion to sensitivity even after the substitution of the allele F_{R47}, which compensated for maladaptive effects of the resistance allele R_{47}. This can be seen clearly in Figure 3 as the fitness gain associated with the transition from the R_{47}/F_{R47} genotype to the S/F_{R47} genotype. Therefore, the failure of evolving resistant populations to revert to sensitivity must be attributed to the low rate of mutations causing reversion and not to the attainment of a novel adaptive peak by the resistant populations.

What would have constituted evidence for an adaptive shift triggered by transient selection for resistance? Imagine that the S/F_{R47} genotype had a fitness of 0.9. In that case, the F_{R47} allele would be beneficial on the resistant background, but deleterious on the sensitive background. At first glance, this

would seem to indicate the sort of tight coadaptation of the resistance and fitness genes necessary to separate alternative adaptive peaks. In fact, however, there would remain only a single adaptive peak in this modified landscape, that being the genotype $S/+$. For there to have been a second adaptive peak, the fitness of the S/F_{R47} genotype would have to be even lower (say, 0.7) than the fitness of the R_{47}/F_{R47} genotype. In that case, there would indeed exist two adaptive peaks, because the transition from the R_{47}/F_{R47} genotype to the more fit $S/+$ genotype entails two substitutions, each of which alone would reduce fitness.

Although these experiments did not indicate a shift between adaptive peaks, they have demonstrated that the "cost" of resistance was diminished by further genetic substitutions that compensated for maladaptive effects associated with mutations conferring resistance. Similar epistatic modifiers of maladaptive pleiotropic effects have been demonstrated to act on mutations conferring resistance to pesticides (McKenzie et al., 1982), and these have important consequences for the management of pest populations (Uyenoyama, 1986).

The diminished "cost" of resistance runs counter to a commonly held view in evolutionary biology that antagonistic pleiotropy should become more pronounced as an organism approaches its "selective equilibrium" (Falconer, 1981; Rose, 1982; Service and Rose, 1985). I believe that there is an implicit assumption in this view, which may often be incorrect. In particular, this view seems to depend upon the assumption that trade-offs derive from some limiting energetic or material currency, such that an increased "investment" in one fitness component necessarily reduces the pool of resources available for other components (Service et al., 1985). Although this must often be the case, it is certainly not the only reason to expect antagonistic pleiotropy (Dykhuizen, 1978).

Trade-offs may occur simply because the genomes of extant organisms are highly integrated. A change in some environmental condition (e.g., the introduction of a viral parasite) may favor a novel phenotype (e.g., resistance). Newly favored mutations are nonetheless random and, therefore, are more likely to disrupt than to improve other aspects of an organism's performance (Caspari, 1952; Wright, 1968). Prolonged selection favors genetic changes that modify the expression of maladaptive pleiotropic effects and thereby restore some degree of genetic integration (Caspari, 1952; Fisher, 1958; Uyenoyama, 1986). In such cases, the severity of a trade-off is likely to be diminished with time.

The relative importance of these two causes of antagonistic pleiotropy is not clear. On the one hand, the problem of allocating finite resources to competing physiological demands represents a "universal" constraint, which acts in all organisms (Maynard Smith et al., 1985). On the other hand, we have very little knowledge concerning the proximity of natural populations to their evolutionary equilibria (Lande, 1983; Carson and Templeton, 1984).

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