

VARIATION IN THE STRENGTH AND SOFTNESS OF SELECTION ON DELETERIOUS MUTATIONS

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Deleterious alleles constantly enter populations through mutation. Understanding the nature of selection against such alleles is required to assess their impact on populations. In a subdivided population, two distinct aspects of selection are important: the strength and softness of selection. Using *Drosophila melanogaster*, we estimated both aspects of selection for each of eight loci across two environments. These data allow us to test conflicting predictions about the factors affecting the softness of selection. First, we show that the softness of selection is not determined by ecological conditions alone. Second, we find that resource limitation makes selection stronger but does not make it softer. Third, we find that wild-type individuals tend to benefit more than mutants from being reared with competitors of low genetic quality. This means that selection is effectively “harder” on mutants than wild types. A model is presented showing that the sensitivities of mutants and wild types to local competitors differentially affect equilibrium mutation frequency and measures of load.

KEY WORDS: Deleterious mutations, hard and soft selection, mutation load, population structure.

Deleterious alleles exist in all populations despite their negative fitness effects because they are constantly introduced by mutation (Haldane 1937; Muller 1950; Lynch et al. 1999; Keightley and Lynch 2003). Biologists have hypothesized that such alleles may be important for a wide variety of evolutionary phenomena including the maintenance of genetic variation for fitness (Rowe and Houle 1996; Crow 1997; Charlesworth and Hughes 1999), the evolution of sex and recombination (Kondrashov 1982; Agrawal and Chasnov 2001; Keightley and Otto 2006), and the phylogenetic diversity in genome size and complexity (Lynch and Conery 2003). The frequency of deleterious alleles and their impact on a population depends on several aspects of selection. In addition to selection strength, the “softness” of selection is critical to the study of deleterious alleles in subdivided populations (Whitlock 2002; Agrawal 2010).

The softness of selection quantifies the extent to which an individual’s fitness depends on the genetic quality of its neighbors. In some respect, the softness of selection is more important than the strength of selection as only the former is predicted to

affect how much a mutation reduces mean fitness at equilibrium (Whitlock 2002). Despite its importance to theory, this aspect of selection is somewhat misunderstood, in part because of a lack of empirical data. Here, we present estimates of both the strength and softness of selection from multiple genes in each of two ecological contexts, providing insights into what makes selection soft. We note that our goals here are very different from studies of phenotypic selection in the wild that estimate the strength of selection under the alternative assumptions of either pure hard or pure soft selection (e.g., Juenger et al. 2000; Kelley et al. 2005).

Before discussing hard and soft selection, we first define the (absolute) fitness of an individual as the number of offspring it contributes to the next generation of the metapopulation. “Relative fitness” is an individual’s contribution to the metapopulation relative to the global average of contributions (i.e., absolute fitness) from all other individuals. The “genetic quality” of an individual is a latent variable that is positively correlated to fitness, though the precise relationship between the two depends on the hardness of selection (as discussed below).

Drawing on economic principles, Wallace introduced the terms “hard” and “soft” selection to describe alternative scenarios for how selection could operate in a subdivided population (Wallace 1968, 1975). Hard selection occurs when an individual’s fitness depends on its genetic quality and is independent of those in its local surroundings. As a consequence, subpopulations or demes containing higher quality genotypes contribute more to the next generation than those containing lower quality genotypes. In contrast, with soft selection, an individual’s fitness depends on its genetic quality relative to the quality of others in its patch and the contribution of a deme to the next generation is independent of the genetic quality of its inhabitants (Wallace 1975; Wade 1985; Whitlock 2002). In reality, selection may not be purely hard or purely soft. Instead, it is more useful to view this aspect of selection on a continuous scale and measure the “softness” of selection (Whitlock 2002). Theory shows that as selection becomes softer, selection becomes less efficient, resulting in an increased frequency of deleterious alleles, an elevated mutation load, and stronger inbreeding depression (Whitlock 2002; Glémin et al. 2003; Roze and Rousset 2004).

Historically, the idea of soft selection was motivated by ecological concepts such as competition and local density regulation (Wallace 1968, 1975). For example, if individuals compete within demes for a limiting resource, then an individual’s fitness may depend heavily on the genetic quality of those it is competing against. The productivity of the deme as a whole may be determined by the amount of resources and be independent of the genotypes competing for them. The importance of ecology has been so heavily emphasized that some authors have implied that ecological conditions alone determine whether selection is hard or soft (Wade 1985; van Tienderen 1997; Whitlock 2002; Roze and Rousset 2004), leading to the expectation that all genes should experience the same softness of selection. Others have pointed out that the specific phenotypic effects of an allele must also play a major role in determining the type of selection it experiences, thus predicting that genes will vary in their softness of selection (Holsinger and Pacala 1990; Ravigné et al. 2004; Agrawal 2010).

Even if phenotypic effects are important, there is no debate that competition and resource limitation are critical for soft selection (Wallace 1968, 1975). If an individual must compete with others in its deme for a limited resource, then the fitness of that individual is expected to depend on its genetic quality relative to that of its neighbors, that is, selection will be soft. If resources are abundant so that there is little competition, selective differences can occur because individuals may differ in their intrinsic survival or ability to process available resources. However, under this scenario of no local competition, an individual’s absolute fitness will be relatively independent of the quality of its neighbors, that is, selection will be hard. Thus, it is reasonable to expect that selection should be softer, on average, when resources are

more limiting. However, this prediction has never been tested and recent theory (Agrawal 2010) suggests that this may not be true.

Materials and Methods

OVERVIEW

We measured fitness, defined here as egg to adult survivorship, of wild type and mutant *Drosophila melanogaster* in artificial demes that varied in their genetic composition. Specifically, we created demes that consisted of (1) 100% wild type, (2) 75% wild type and 25% mutant, and (3) 50% wild type and 50% mutant. Using a likelihood-based approach, we estimated the parameters of the following model. Let the genetic quality of a wild-type individual be $\omega_+ = k$ and the genetic quality of a mutant be $\omega_- = k(1 - s)$. The absolute fitness of an individual of genotype i in deme j ($W_{i,j}$) is a function of its genetic quality (ω_i) and the average genetic quality of others in its deme ($\bar{\omega}_j$). This relationship is given by

$$W_{i,j} = \frac{\omega_i}{1 - \psi(\omega_+ - \bar{\omega}_j)/\omega_+}. \quad (1)$$

In this framework, the parameter s measures the strength of selection; s quantifies the extent to which a mutant has lower fitness than a wild-type individual when both are from the same deme. The parameter ψ represents the softness of selection. When $\psi = 0$, the model depicts pure hard selection in which an individual’s relative fitness is independent of the other genotypes in its deme. When $\psi = 1$, the model depicts pure soft selection. In this case, an individual’s fitness depends on its genetic quality relative to the others in its deme. Moreover, when $\psi = 1$, the average fitness of a deme is independent of its genetic composition ($\bar{W}_j = \omega_+$ for all j), which fits with traditional definitions of soft selection (Wallace 1975; Wade 1985; Whitlock 2002).

EXPERIMENTAL METHODS

Our experiment involved eight autosomal mutations in *D. melanogaster* obtained from the Bloomington *Drosophila* Stock Center. These mutations all have visible phenotypic effects in adult heterozygotes, affecting eyes (*Dr*, *R*), bristles (*Pin*, *Sb*), antennae (*Antp*), wings (*Adv*, *Ly*), and body color (*Frd*). Each of the eight mutations was backcrossed into our wild-type outbred stock population for a minimum of seven generations. The stock population was a large outbred population originally collected from Dahomey, Africa. It has been maintained in various laboratories at a large population size since 1970 and in the present laboratory since 2004.

For logistical reasons, the genes were studied in two sets (Gene Set 1 included the genes *Adv*, *Antp*, *Pin*, and *R* and Gene Set 2 included the genes *Dr*, *Frd*, *Ly*, and *Sb*). Each set was examined in two phases. In the first phase of the experiment for each gene

set, we tested demes containing only wild type (+/+) individuals and demes containing approximately 50% wild type (+/+) and 50% mutants (*M*/+). For each gene set at each density, there was ca. 160 replicates of pure wild-type demes and about 40 replicates of the 50% mutant demes for each gene. To create demes with 100% wild-type individuals, +/+ females were mass mated to +/+ males and allowed to lay eggs on grape-agar medium seeded with live yeast pellets (to induce egg laying). Demes containing approximately 50% mutants were created by collecting the appropriate number of eggs from virgin +/+ females that were mass mated to mutant (*M*/+) males.

In the second phase of the experiment for each gene set, we tested demes containing ca. 50% wild type (+/+) and 50% mutants (*M*/+) and demes containing ca. 75% wild type (+/+) and 25% mutants (*M*/+). For each gene, there were ca. 40 replicates of each deme type for each density. Demes containing ca. 50% mutants were constructed as described above. Demes containing approximately 25% mutants were created by collecting half of the required eggs for each vial from a cross between wild-type females and males and the other half from a cross between +/+ females and *M*/+ males.

For both phases, the experiments were conducted at two densities. For each “high” density replicate, 360 eggs were counted and transferred to a 13.5 dram (wide) vial containing 7 mL of a slight variant of standard yeast–sugar–agar food medium, seeded with live yeast. (For these experiments, we used food containing 75% of the standard concentration of yeast and sugar to intensify competition for resources, especially at the high density.) For the other density treatment, 90 eggs were placed into each vial. We refer to this as the “low” density treatment to emphasize the contrast with the high-density treatment. However, we note that 90 eggs per vial is not truly a low density as it is higher than the density that confers maximal survival.

For each vial, the number and genotype of surviving adults was assayed by checking vials on days 12 and 16 after the initial set-up. By checking the vials on multiple days, we were able to count essentially all emerging adults. In total, over 2500 replicate vials were examined, which involved counting over 560,000 eggs and scoring almost 270,000 offspring.

STATISTICAL ANALYSIS

In the context of this experiment, the fitness model given in equation (1) can be simplified to

$$W_{i,j} = \frac{\omega_i}{1 - \psi_x f_{x,j} s_x}, \quad (2)$$

where $f_{x,j}$ is the frequency of mutation x in deme j . A likelihood model was constructed to estimate the parameters of this equation—most importantly, the strength (s_x) and softness (ψ_x) of selection. The goal of the analysis was to find the parameter

values that were most likely to have produced the observed numbers of surviving wild type and mutant individuals from demes of varying genetic composition. Various levels of uncertainty were inherent to the experimental design and these were incorporated into the model as described below.

The framework for the likelihood model is based on the following logic. Let $e_{i,j}$ be the number of eggs of genotype i placed in deme j and $n_{i,j}$ be the number of surviving adults of this genotype from this deme. We calculated the likelihood of observing $n_{+,j}$ wild-type adults and $n_{x,j}$ mutant adults in deme j based on the binomial probability of observing $n_{+,j}$ successes in $e_{+,j}$ trials with a per-trial probability of success of $W_{+,j}$ and the binomial probability of observing $n_{x,j}$ successes in $e_{x,j}$ trials with a per-trial probability of success of $W_{x,j}$.

In our experiment, each deme is intended to have a specific number of (fertilized) eggs of each genotype, that is, $e_{+,j}$ and $e_{x,j}$. For example, for demes intended to have 50% *Adv* mutants in the high-density treatment, each vial would ideally contain 180 wild-type eggs and 180 *Adv*/+ eggs. In reality, the actual numbers of eggs will deviate from this expectation for two reasons. First, there may be some error in counting and transferring of eggs, thus the total number of eggs may not be exactly 360 as intended. Second, the eggs are obtained from a cross of +/+ females by *Adv*/+ males and so the actual frequency of mutants among the transferred eggs may differ from 50% because of sampling. Because we do not know $e_{+,j}$ and $e_{x,j}$ exactly, we would like to integrate over all possible values to calculate the likelihood of having observed a specific number of survivors in a given vial. To approximate this integration, we generated 200 possible values of $e_{+,j}$ and $e_{x,j}$ for each vial and then calculated the likelihood based on these 200 possible samples. To generate a single set of sample values for a single vial, the following procedure was used. We assumed that the number of eggs transferred to a vial was ϵ , which is a random variate, rounded to the nearest integer, from a normal distribution with mean equal to the intended number of eggs μ and a standard deviation of 0.05μ . (This value for the standard deviation was guesstimated based on our experience in counting and transferring eggs and informal repeatability measures.) When offspring were coming from a cross involving mutants (+/+ \times *M*/+), the number of zygotes with the mutant genotype was determined by choosing a random number ϵ_x from a binomial distribution with ϵ trials and a success rate of 0.5; the number of wild-type eggs was $\epsilon - \epsilon_x$. If the randomly generated numbers resulted in fewer eggs of either genotype than the corresponding number of observed adults, then that combination of random values was replaced with an alternative set of random values. This procedure was repeated until we had 200 possible values for the egg numbers in each vial. For simplicity, the value of $f_{x,j}$ used in the calculation of $W_{i,j}$ above always used the intended frequency of the mutant for each specified deme type.

Two additional sources of variance were formally included in the model. To account for random variation in vial quality that affects survivorship, we added a normally distributed error term to the logit of both genotype's expected survival value given by equation (2) and then back-transformed the resulting values to the original scale to give the realized survival probabilities. This error term had mean zero and standard deviation σ . A discretized approximation to the normal distribution was used in calculating the likelihood. Inclusion of σ in the likelihood model is necessary to account for among-vial variation that may otherwise be wrongly attributed to a specific factor such as density or gene. As mentioned above, the experiment for each gene set was carried out in two phases. To account for temporal differences between these phases in overall survival, the full likelihood model allowed the baseline level of survival, k , to differ between the two phases (i.e., an extra parameter Δ was included to estimate this difference).

The full likelihood model involved 21 parameters: k_H , Δ_H , $s_{H,1}$, $\psi_{H,1}$, $s_{H,2}$, $\psi_{H,2}$, $s_{H,3}$, $\psi_{H,3}$, $s_{H,4}$, $\psi_{H,4}$, k_L , Δ_L , $s_{L,1}$, $\psi_{L,1}$, $s_{L,2}$, $\psi_{L,2}$, $s_{L,3}$, $\psi_{L,3}$, $s_{L,4}$, $\psi_{L,4}$, and σ . The subscripts H and L refer to the high- and low-density treatments. To obtain the maximum likelihood estimate, we followed a several step procedure. By using this multistep procedure, we maximized our ability to find the global maximum likelihood (Brooks and Morgan 1995). We began by using a simulated-annealing based approach built in a custom program written in *C*. We repeated this 25 times, each time using a different set of random starting values. The majority of runs converged on a similar set of parameter values.

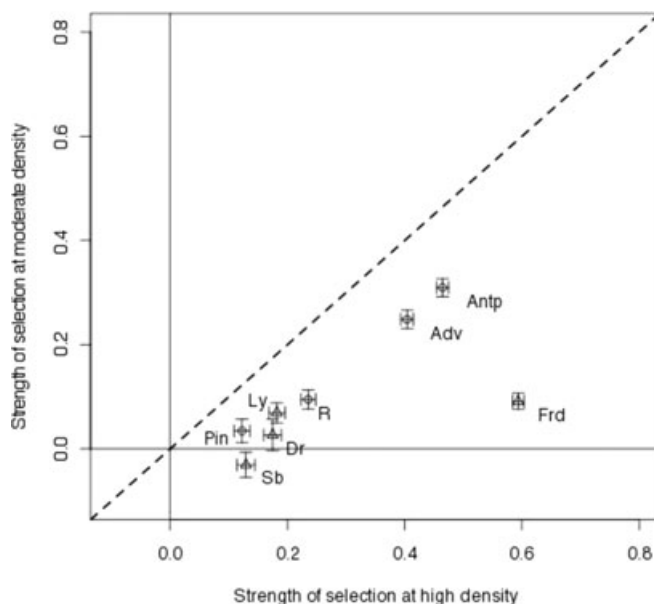


Figure 1. The strength of selection at moderate and high density. Dashed line is the one to one line, that is, equal selection across densities. Error bars represent approximate standard errors estimated from a quadratic approximation to the likelihood surface.

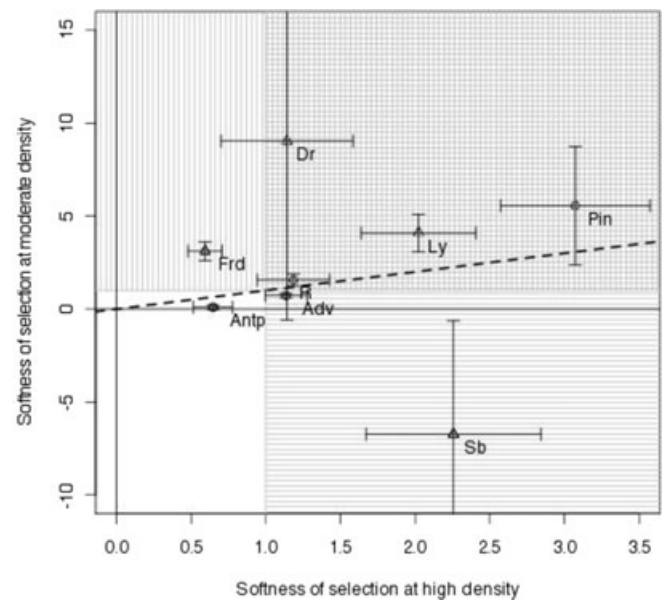


Figure 2. The softness of selection at moderate and high density. Dashed line is the one to one line, that is, equal softness across densities. The region shaded with gray vertical lines indicates super-soft selection in the moderate density treatment. The region shaded with gray horizontal lines indicates super-soft selection in the high density treatment. Error bars represent approximate standard errors estimated from a quadratic approximation to the likelihood surface.

The best set of values was then used as the starting parameter set for the optimization procedure *optim* in *R* (R Development Core Team 2008) using the Nelder–Mead optimization method. This was repeated until no further improvement was possible. Finally, this set of values was then used as the starting parameter set with the *mle* function in *R* using the BFGS optimization method. This *mle* function also provides standard errors of the estimated values based on a quadratic approximation of likelihood surface (technically, by using the inverse of the Hessian matrix (Bolker 2008)). The estimates and standard errors for the strength and softness of selection are shown in Figures 1 and 2. In addition to the full model described above, we examined various constrained models to evaluate the differences among genes and between density treatments with respect to the strength and softness of selection.

Results

As expected, the density treatment has a strong effect on survival. The viability of wild-type flies in pure wild-type demes declines from 0.83 (SE = 0.004) at the moderate density to 0.34 (0.003) at the high density. The strength of selection s on each of the genes across the two densities is shown in Figure 1. For all eight genes, selection against the mutant is significantly stronger at high density than at the lower density (Table 1). These results

Table 1. Examining the effects of density on the strength and softness of selection for individual genes. In each case, the full model (Model F, Table 2) was constrained with respect to either s or ψ for a single gene. P -values are calculated by a likelihood ratio test relative to the full model (chi-squared test with one degree of freedom). The negative log likelihood scores for the full model are 11,931.45 for Gene Set 1 and 10,855.7 for Gene Set 2.

Gene set	Gene	Constrained selection strength ($s_{H,x}=s_{L,x}$)		Constrained selection softness ($\psi_{H,x}=\psi_{L,x}$)	
		$-\ln(\text{Likelihood})$	P	$-\ln(\text{Likelihood})$	P
1	<i>Adv</i>	11,961.5	$<10^{-10}$	11,934.0	0.023
	<i>Antp</i>	11,961.3	$<10^{-10}$	11,936.3	1.9×10^{-3}
	<i>Pin</i>	11,938.5	1.7×10^{-4}	11,932.5	0.15
	<i>R</i>	11,950.9	4.2×10^{-10}	11,932.0	0.58
2	<i>Dr</i>	10,879.0	$<10^{-10}$	10,870.3	6.4×10^{-8}
	<i>Frd</i>	11,166.0	$<10^{-10}$	10,894.7	$<10^{-10}$
	<i>Ly</i>	10,867.0	2.1×10^{-6}	10,859.6	5.2×10^{-3}
	<i>Sb</i>	10,882.4	$<10^{-10}$	10,875.8	2.3×10^{-10}

are consistent with other studies showing that selection tends to become stronger when resources are more limited (Kondrashov and Houle 1994; Yang et al. 2001; Wang et al. 2009; Agrawal and Whitlock 2010).

Our primary interest is in the softness of selection. Our estimates of the softness of selection parameter ψ for each gene at each density are shown in Figure 2. If ecological conditions alone determined the softness of selection, then all genes would be expected to experience the same softness of selection as all genes were tested under the same conditions. To test whether genes vary significantly, we compared two simplified models. First, we examined a simplified model (Model B, Table 2) in which the softness of selection was the same for all genes and across both densities. We compared this with a model (Model C) in which the softness of selection was allowed to differ among genes but was unaffected by density. Likelihood ratio tests confirm that the model in which each gene has its own ψ -value fits the data significantly better than model in which all genes are forced to share the same ψ -value (Gene Set 1: $\chi^2 = 87.9$, $df = 3$, $P < 10^{-9}$, Gene Set 2: $\chi^2 = 33.6$, $df = 3$, $P < 10^{-6}$). Alternatively, one could compare the full model (Model F) to one in which the softness of selection is constrained to be the same for all genes, though it may differ between densities (Model D), that is, $\psi_{H,1} = \psi_{H,2} = \psi_{H,3} = \psi_{H,4} = \psi_H$ and $\psi_{L,1} = \psi_{L,2} = \psi_{L,3} = \psi_{L,4} = \psi_L$. As for the test reported above, this test also yields a highly significant effect (Gene Set 1: $\chi^2 = 92.5$, $df = 6$, $P < 10^{-10}$, Gene Set 2: $\chi^2 = 55.3$, $df = 6$, $P < 10^{-9}$). In other words, there is significant variation among genes in the softness of selection, indicating that one cannot characterize selection as soft or hard by ecology alone. This result is not unexpected as several authors (Holsinger and Pacala 1990; Ravigné et al. 2004; Agrawal 2010) have pointed out that mutations affecting phenotypes involved in competition are likely to experience softer selection than those that do not.

The density in the high-density treatment is fourfold greater than in the low-density treatment. Consequently, per capita resources are expected to be much more limiting and this is the most likely reason that survival in the high-density treatment is reduced by $\sim 60\%$ relative to the low density (as reported above). Because competition is expected to be much stronger at the high density, it is expected that selection would be softer at this density. However, the data do not support this prediction. To test whether density had an average effect on selection softness, we first calculated the maximum likelihood of a model in which each gene had its own value for the softness of selection but this value was unaffected by density (Model C, $\psi_{L,x} = \psi_{H,x}$ for all genes within each gene set). This was compared to the maximum likelihood of a model in which the density treatment altered the softness of selection by the same amount for all genes (Model E, $\psi_{L,x} = \psi_{H,x} + d$ for all genes within each gene set). Although statistical models in which the softness of selection was affected by density fit the data significantly better than models in which it did not (Gene Set 1: $\chi^2 = 13.6$, $df = 1$, $P < 10^{-3}$, Gene Set 2: $\chi^2 = 67.0$, $df = 1$, $P < 10^{-9}$), the direction of this effect differed between the two gene sets.

A more detailed analysis reveals that these results were driven by gene-specific effects of density (Fig. 2). When each gene is tested individually (Table 1), two genes (*Pin* and *R*) show no significant difference in the softness of selection experienced between the two densities. Of the remaining genes, three genes show significantly softer selection at the high density (*Adv*: $\chi^2 = 2.57$, $df = 1$, $P = 0.02$; *Antp*: $\chi^2 = 4.84$, $df = 1$, $P = 0.002$; *Sb*: $\chi^2 = 20.1$, $df = 1$, $P < 10^{-9}$) but the other three genes show the reverse pattern (*Dr*: $\chi^2 = 14.6$, $df = 1$, $P < 10^{-7}$; *Frd*: $\chi^2 = 39.0$, $df = 1$, $P < 10^{-9}$; *Ly*: $\chi^2 = 3.91$, $df = 1$, $P = 0.005$). (Note that the cross-environment comparison involving *Sb* should be regarded with caution because the point estimate of selection against the

Table 2. Likelihood models examined and their AIC scores.

Model description	Num. parameters	AIC	
		Gene Set 1	Gene Set 2
Model A. Pure hard selection: $\psi_{L,x} = \psi_{H,x} = 0$	13	24,010.0	21,899.8
Model B. Softness of selection same for all genes and across both densities: $\psi_{L,x} = \psi_{H,x} = \psi$	14	24,002.4	21,869.0
Model C. Softness of selection different for each gene but unaffected by density: $\psi_{L,x} = \psi_{H,x}$	17	23,920.6	21,841.4
Model D. Softness of selection same for all genes but value differs between densities: $\psi_{L,x} = \psi_L$ and $\psi_{H,x} = \psi_H$	15	23,985.4	21,796.8
Model E. Each gene has its own value for selection softness but all genes are affected the same way by the density treatment: $\psi_{L,x} = \psi_{H,x} + d$	18	23,909.0	21,776.4
Model F. Full model. Unique value of selection softness for each gene at each density.	21	23,904.9	21,753.4

mutant allele is negative at the lower density.) To our knowledge, this is the first study to test the seemingly intuitive prediction that selection should be softer when resources are more limiting.

Of the models discussed above, the full model (Model F), which allows for a unique value of selection softness for each gene at each density, has the best AIC score for both gene sets (Table 2). Parameter estimates for Model F are provided in Table 3.

Overall, the estimates of selection softness are quite high. As shown in Figure 2, most of the genes experienced selection that was softer than expected under pure soft selection ($\psi > 1$) in at least one of the two density treatments. This is called “super-soft” selection (Agrawal 2010). Super-soft selection can occur when demes containing mutants have higher average fitness than demes containing only wild types. Despite the considerable fitness disadvantages of some mutants, demes containing mutants

Table 3. Parameter estimates for Model F. Approximate standard errors (based on quadratic approximation to likelihood surface) are given in parentheses. For Gene Set 1, genes 1–4 are *Adv*, *Antp*, *Pin*, and *R*, respectively. For Gene Set 2, genes 1–4 are *Dr*, *Frd*, *Ly*, and *Sb*, respectively.

Parameter	GeneSet1	GeneSet2
k_H	0.327 (0.007)	0.333 (0.009)
$s_{H,1}$	0.404 (0.011)	0.175 (0.015)
$\psi_{H,1}$	1.14 (0.14)	1.14 (0.44)
$s_{H,2}$	0.465 (0.011)	0.594 (0.010)
$\psi_{H,2}$	0.646 (0.131)	0.593 (0.114)
$s_{H,3}$	0.122 (0.013)	0.182 (0.014)
$\psi_{H,3}$	3.07 (0.50)	2.02 (0.38)
$s_{H,4}$	0.235 (0.013)	0.129 (0.015)
$\psi_{H,4}$	1.18 (0.24)	2.26 (0.59)
k_L	0.767 (0.007)	0.783 (0.008)
$s_{L,1}$	0.249 (0.017)	0.027 (0.030)
$\psi_{L,1}$	0.722 (0.115)	9.03 (9.63)
$s_{L,2}$	0.310 (0.017)	0.091 (0.015)
$\psi_{L,2}$	0.096 (0.122)	3.11 (0.50)
$s_{L,3}$	0.035 (0.023)	0.069 (0.019)
$\psi_{L,3}$	5.55 (3.19)	4.09 (1.00)
$s_{L,4}$	0.095 (0.019)	−0.031 (0.024)
$\psi_{H,4}$	1.56 (0.32)	−6.74 (6.11)
Δ_H	0.135 (0.008)	0.000 (0.009)
Δ_L	0.032 (0.007)	0.043 (0.007)
σ	0.424 (0.012)	0.487 (0.013)

often do as well or better than demes containing pure wild types. This is exemplified by *Adv* and *Antp*, which experience similarly strong selection (Fig. 1). At the high density, pure wild-type demes produce more survivors (mean \pm SE = 122.1 \pm 2.0) than demes

with 50% *Antp* mutants (100.8 ± 2.9), as expected. However, demes with 50% *Adv* produce more survivors (128.9 ± 4.7) than pure wild-type demes despite the selection against *Adv*. Selection is inferred to be somewhat hard on *Antp* ($\psi < 1$) but super-soft on *Adv*.

In general, high estimates of ψ imply that fitness is very sensitive to local genetic quality. However, visual inspection of the raw data indicated that the patterns of survival did not always match what was predicted based on high ψ -values. Specifically, there were several cases in which wild-type fitness was sensitive to local genetic quality but mutant fitness was not.

Most models of soft selection, including the one depicted by equation (1) as well as Whitlock's (2002) model, assume that mutant and wild-type genotypes are equally affected by the average local quality of competitors. However, mutants may have a different level of sensitivity to the local average genetic quality than wild types. This idea can be incorporated into the model represented by equation (1) by replacing ψ with ψ_i , that is, by making ψ genotype specific. In the Appendix, we present a theoretical analysis of this slightly more complicated model. This shows that high values of ψ_{mutant} increase the equilibrium frequency of a deleterious alleles whereas high values of $\psi_{\text{wild-type}}$ have the opposite effect. The two parameters also have different effects with respect to mutation load. For these reasons, it is worth exploring whether mutants and wild-types differ with respect to ψ .

If we define $a = \psi_{\text{mutant}}/\psi_{\text{wild-type}}$ (i.e., $\psi_{\text{mutant}} = a \times \psi_{\text{wild-type}}$), then our main analysis (above) assumes $a = 1$ ($\psi_{\text{mutant}} = \psi_{\text{wild-type}} = \psi$). An alternative assumption that does not require any additional degrees of freedom is $a = 0$ (or $\psi_{\text{mutant}} = 0$), meaning that mutant fitness is insensitive to local genetic quality, even if wild-type fitness is. For each gene separately, we examine this alternative as well as a model in which a is a free parameter (Table 4). Of the four genes in Gene Set 1, two genes (*Adv* and *R*) have better AIC scores when $a = 0$ than when $a = 1$. When we allow a to be a free parameter, the point estimate of a is negative for these genes, indicating that increasing the frequency of mutants elevates the per capita survival of wild types but reduces the per capita survival of mutants. For the other two genes (*Antp* and *Pin*), the point estimate of a is positive but less than unity, indicating that both wild types and mutants benefit from being surrounded by a higher frequency of mutants, but wild types benefit most. For the genes in Gene Set 2, we appear to have less power to assess the sensitivity of mutants to local quality but models with $a = 1$ are more likely than models with $a = 0$.

Discussion

The concept of hard and soft selection has been important in numerous theoretical studies of protected polymorphisms (Levene 1953; Dempster 1955; Christiansen 1975), the evolution

of altruism (Wade 1985; Kelly 1994; Queller 1994), and, more recently, mutation load (Agrawal and Chasnov 2001; Whitlock 2002; Glémin et al. 2003; Roze and Rousset 2004; Theodorou and Couvet 2006; Haag and Roze 2007). However, gene-level empirical data on this dimension of selection have been lacking. Our goal here was to test several basic ideas about factors affecting the softness selection.

Dating back to Wallace's (1968, 1975) early descriptions of soft selection, it has been clear that the scale of density regulation is important for determining the softness of selection. However, the role of ecology is overemphasized if one assumes ecological conditions alone determine a single softness of selection that applies to all genes across the genome. This is the implication made in several studies of mutation load in subdivided populations, with different authors insinuating genome-wide hard selection (Agrawal and Chasnov 2001), soft selection (Roze and Rousset 2004; Haag and Roze 2007), or intermediate levels of softness (Whitlock 2002). Other authors have explicitly emphasized that the softness of selection will vary across the genome as the softness experienced by a gene will depend, in part, on the type of traits that it affects (Holsinger and Pacala 1990; Ravigné et al. 2004; Agrawal 2010). Indeed, in our study, we find that genes differ in the softness of selection they experience.

Competition for local resources is central to the concept of soft selection. Naïve intuition would lead to the expectation that selection should become softer when competition is more intense. However, our data did not support this idea as increasing density affected selection softness in opposing directions for different genes. As suggested below, the ecological details of competition and selective mortality can quickly complicate predictions regarding the softness of selection.

An unanticipated result was the overall level of softness and the high frequency of super-soft selection ($\psi > 1$). Recent theory (Agrawal 2010) suggests a simple mechanism, rooted in classic ecology (Nicholson 1954, 1957), by which super-soft selection may commonly occur. When a juvenile dies before reaching maturity, the resources it consumes prior to its death can be considered wasted because nobody benefits from these resources (Nicholson 1954, 1957). Juvenile mortality, when it happens, may occur at a younger age for mutants than wild-type individuals. If so, fewer resources are wasted on each dying juvenile mutant than on each dying juvenile wild type. As nonwasted resources benefit the remaining individuals, demes with a higher frequency of mutants can end up with higher productivity. Consistent with this hypothesis, the only one of our genes that did not experience super-soft selection at either density (*Antp*) has mutants that often appear to die at the pupal stage, after larval competition is complete (N. Sharp, pers. obs.).

The logic used above to explain super-soft selection may also explain why selection is not always softer at the higher density.

Table 4. Examining the sensitivity of mutants to local genetic quality. The main model (Model F, Table 2) assumes that wild-types and mutants are equally sensitive to local genetic quality, that is, $a=1$ where $a=\psi_{\text{mutant}}/\psi_{\text{wild-type}}$. The AIC score for this model is 23,904.9 and 21,753.4 for Gene Sets 1 and 2, respectively. Using this model as the base, we test two other models for each gene separately. First, we examine a model in which $a=0$ (or $\psi_{\text{mutant}}=0$) for the focal gene. Second, we examine a model in which a is a free parameter for the focal gene. The point estimate for a is given in parentheses. Within each row, the asterisk indicates which model best fits the data based on AIC scores (lowest score is best). If there is no asterisk in a row, then Model F ($a=1$) is the preferred model.

Gene Set	Gene	AIC score	
		Mutant fitness insensitive to local quality, $\psi_{\text{mutant}}=0$	Mutant fitness sensitivity to local quality differs from wild-type sensitivity, $\psi_{\text{mutant}}=a\psi_{\text{wild-type}}$
1 (AIC=23,904.9 for $a=1$)	<i>Adv</i>	23,848.6	23,830.9* ($a=-1.51$)
	<i>Antp</i>	23,905.2	24,304.2 ($a=0.61$)
	<i>Pin</i>	23,909.8	23,896.1* ($a=0.45$)
	<i>R</i>	23,888.0	23,887.2* ($a=-0.49$)
2 (AIC=21,753.4 for $a=1$)	<i>Dr</i>	21,793.4	21,755.4 ($a=1.05$)
	<i>Frd</i>	21,771.0	21,755.4 ($a=1.04$)
	<i>Ly</i>	21,781.8	21,756.0 ($a=1.12$)
	<i>Sb</i>	21,755.5	21,756.3 ($a=0.97$)

When juvenile mortality is explicitly modeled (Agrawal 2010), the softness of selection depends on the juvenile mortality rates of both wild types and mutants and the average amount of resources consumed by doomed wild-type and mutant juveniles. Although it is reasonable to assume that overall juvenile mortality will increase with density, it is difficult to predict how the juvenile mortality and consumption of mutants will change relative to that of wild types. Depending how these change with density, selection could become harder or softer.

Selection is soft whenever the fitness of a genotype depends on the average quality of its local competitors. The simplest assumption is that all genotypes are equally affected by local quality. However, we find several cases in which mutants benefit proportionally less from being in group of weak competitors than do wild types ($\psi_{\text{mutant}} < \psi_{\text{wild-type}}$). When we extend the mutation load theory to allow sensitivity to local competition to be genotype specific (Appendix), we find that ψ_{mutant} and $\psi_{\text{wild-type}}$ differentially affect the equilibrium allele frequency and the mutation load. If, as we observed for some genes, mutants are typically less sensitive to competitor quality than wild types, then local clustering of mutants through population structure will not shield them from selection. Thus, mutants will be less common at equilibrium than one would expect by assuming a common level of sensitivity across all genotypes. On the other hand, if mutation load as defined as the fitness advantage of wild types above the average, then load will remain high as long as wild types benefit from being surrounded by weak competitors even if mutants do not (see Appendix).

Broadly, our results confirm classic studies in *Drosophila* showing the fitness of a genotype depends on the genotypes

of its neighbors (Lewontin 1955; Bakker 1961; Bundgaard and Christiansen 1972; Mueller 1988). However, larger sample sizes and modern statistical techniques allowed us to separately estimate the strength and softness of selection on individual genes for the first time. Although the specific estimates are particular to the laboratory conditions in which they were measured, our primary goal was to examine untested predictions about factors affecting the softness of selection. We have shown that different genes can experience different softness of selection under the same ecological conditions and that manipulation of an ecological factor (density) has no consistent effect on selection softness. These results indicate that simplistic generalizations about the effect of ecological conditions on the softness of selection are often misleading. Rather, our results are consistent with theory (Agrawal 2010) showing that the phenotypic effects of specific mutations must be considered in relation to ecological processes (e.g., mutational effects on juvenile consumption and mortality rates affect both the number and genotype frequency of survivors). Because soft selection, and especially super-soft selection, can greatly increase the mutation load, it is important to understand what makes selection soft and how often these conditions arise in nature.

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Appendix

As in Whitlock (2002), we examine mutation load in a large metapopulation. The major difference here is that we allow genotypes to differ in the extent to which their fitness is affected by the genetic quality of local competitors. The analysis follows the approach used by Agrawal (2010) and details are only briefly described here.

Consider a single diallelic diploid locus. The genetic quality of the three genotypes are $\omega_{AA} = k$, $\omega_{Aa} = k(1 - hs)$, and

$\omega_{aa} = k(1 - s)$. The fitness of genotype i in deme j is

$$W_{i,j} = \frac{\omega_i}{1 - \psi_i(\omega_{AA} - \bar{\omega}_j)/\omega_{AA}}, \quad (A1)$$

where $\bar{\omega}_j$ is the average genetic quality of individuals in deme j . The parameter ψ_i measures the sensitivity of genotype i to the quality of local competitors. As described in the main text, when $\psi_i = \psi$ for all i , then $\psi = 0$ corresponds to pure hard selection and $\psi = 1$ corresponds to pure soft selection.

In the analysis, we assume that selection is weak and mutation is even weaker. We also assume population subdivision is weak. More formally, we assume s and F_{ST} are $O(\xi)$ and μ is $O(\xi^2)$ where $\xi \ll 1$.

The change in allele frequency due to selection is equal to the covariance of allele frequency with relative fitness (Price 1970). This covariance can be decomposed into within and among deme components (Price 1972; Wade 1985). These components are calculated using a third-order Taylor series approximation with respect to the distribution of allele frequency among demes. Following Whitlock (2002), we use the island model approximation for the skew in the allele frequency, that is, skew = $\bar{q}(1 - \bar{q})(1 - 2\bar{q})(2F_{ST}^2/(1 + F_{ST}))$ where \bar{q} is the average frequency of a across the metapopulation.

The change in allele frequency due to selection within demes is

$$\Delta q_{\text{within}} = -\bar{q}s(h + (F_{ST} + \bar{q}) \times (1 - h(3 + 2(\psi_{Aa} - \psi_{AA}))) + O(\xi^4)) \quad (A2)$$

and the change in allele frequency due to selection among demes is

$$\Delta q_{\text{among}} = -2\bar{q}hsF_{ST}(1 - \psi_{AA}) + O(\xi^4). \quad (A3)$$

The results above can be combined to obtain the total change due to selection. At equilibrium, this change due to selection is balanced by the input of new mutation. The equilibrium allele frequency is found to be

$$\bar{q}_{eq} = \frac{\mu}{hs} - \frac{\mu(F_{ST}(1 - h)hs + (1 - 2h)\mu)}{h^3s^2} + \frac{2\mu\psi_{Aa}F_{ST}hs}{h^2s^2} + \frac{2\mu^2(\psi_{Aa} - \psi_{AA})}{h^2s^2} + O(\xi^3). \quad (A4)$$

The first term is the classic result for panmictic populations (Haldane 1937). The second term results from the enhanced efficiency of selection due to the increased homozygosity, which is especially effective for partially recessive mutations (Crow and

Kimura 1970; Agrawal and Chasnov 2001; Whitlock 2002). The final two terms show that mutants persist at a higher frequency if mutants are sheltered from selection by competing against other low quality mutants ($\psi_{Aa} > 0$). This effect is reduced if wild-types exploit weakly competitive environments better than mutants ($\psi_{AA} > \psi_{Aa}$).

There are several reasonable ways to define load in a subdivided population. One measure of load is the selective disadvantage of the average individual in the actual metapopulation relative to a wild-type individual in that population:

$$L_1 = \frac{E[W_{\text{Wild-type_In_Actual}}] - E[W_{\text{Actual_In_Actual}}]}{E[W_{\text{Actual_In_Actual}}]}, \quad (A5)$$

where $E[W_{\text{Wild-type_In_Actual}}]$ is the expected fitness of a wild-type individual when placed randomly within the actual metapopulation. $E[W_{\text{Actual_In_Actual}}]$ is average fitness of an individual from the actual metapopulation. We find that

$$L_1 = 2\mu - \mu \frac{F_{ST}hs + \mu(1 - 2h)}{h^2s} + 4\mu\psi_{AA}F_{ST} + O(\xi^4). \quad (A6)$$

The last term illustrates that it is the sensitivity of wild types, rather than mutants, that affects the load under this definition.

An alternative definition of load that is analogous to that used by Whitlock (2002), is based on the average disadvantage of an individual from the actual metapopulation when placed in a metapopulation consisting only of wild types,

$$L_2 = \frac{E[W_{\text{Pure_Wildtype}}] - E[W_{\text{Actual_In_Wildtype}}]}{E[W_{\text{Pure_Wildtype}}]}, \quad (A7)$$

where $E[W_{\text{Pure_Wildtype}}]$ is the average fitness of a wild-type individual in metapopulation composed only of wild types. $E[W_{\text{Actual_In_Wildtype}}]$ is the expected fitness of a randomly chosen individual from the actual metapopulation when placed in a metapopulation composed only of wild types. We find that

$$L_2 = 2\mu - \mu \frac{F_{ST}hs + \mu(1 - 2h)}{h^2s} + 4\psi_{Aa}\mu F_{ST} + 4\frac{\mu^2}{hs}(\psi_{Aa} - \psi_{AA}) + O(\xi^4). \quad (A8)$$

By this definition of load, the sensitivity of mutants can be a more important factor than that of wild types in determining the load.

Together, these results illustrate that the equilibrium allele frequency and measures of load are affected differently by the extent to which mutant and wild-type fitness are sensitive to the quality of local competitors.