Selection, Epistasis, and Parent-of-Origin Effects on Deleterious Mutations across Environments in *Drosophila melanogaster*

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abstract: Understanding the nature of selection against deleterious alleles is central to determining how populations are affected by the constant influx of new mutations. Important progress has been made in estimating basic attributes of the distribution of selection coefficients and gene interaction effects (epistasis). Although most aspects of selection are likely to be context dependent, little is known about the effect of stress on selection and epistasis at the level of individual genes, especially in multicellular organisms. Using *Drosophila melanogaster*, we measure how selection on 20 mutant alleles is affected by direct and indirect genetic factors across two environments. We find that environmental stress increases selection against individual mutations but reduces selection against combinations of mutations (i.e., epistasis becomes more positive). In addition, we find a high incidence of indirect genetic effects whereby the strength of selection against the alleles carried by offspring is dependent on the genotypes of their parents.

Keywords: epistasis, deleterious mutations, *Drosophila melanogaster*, selection.

Introduction

Despite natural selection, all populations contain deleterious alleles because of the perpetual input of new mutations. The resulting load of mutations has been implicated in a variety of major evolutionary phenomena (Lynch et al. 1999), including population health and persistence (Kondrashov 1995; Lynch et al. 1995; Crow 1997), the maintenance of genetic variation (Haldane 1937; Kondrashov and Turelli 1992), and the evolution of sex and recombination (Kondrashov 1982; Charlesworth 1990; Otto and Feldman 1997; Agrawal and Chasnov 2001; Keightley and Otto 2006). While progress has been made in estimating basic attributes of mutations (Lynch et al. 1999; de Visser and Elena 2007; Eyre-Walker and Keightley 2007), several fundamental aspects of selection against deleterious mutations remain poorly understood empirically.

The strength of selection on deleterious mutations is a critical property that determines their equilibrium frequency in a population (Haldane 1937). A common assumption is that stressful environments tend to increase the strength of selection against deleterious mutations (Parsons 1987; Szafraniec et al. 2001; Kavanaugh and Shaw 2005; Jasnos et al. 2008; Roles and Conner 2008). This assumption is common because of the belief that an organism's ability to compensate for deleterious mutations is compromised under such conditions. However, empirical support has been mixed (Martin and Lenormand 2006*a*). Some studies show that stress can weaken the strength of selection (Chang and Shaw 2003; Kishony and Leibler 2003; Jasnos et al. 2008). A larger number of studies seem to indicate that selection is stronger under adverse conditions (Kondrashov and Houle 1994; Korona 1999; Vassilieva et al. 2000; Remold and Lenski 2001; Szafraniec et al. 2001; Yang et al. 2001; Cooper et al. 2005). However, many of these studies involve genotypes carrying unknown numbers of mutations (Kondrashov and Houle 1994; Korona 1999; Vassilieva et al. 2000; Szafraniec et al. 2001; Yang et al. 2001), which leaves open the alternative explanation that stress increases merely the number of mutations exposed to selection (Kondrashov and Houle 1994; Martin and Lenormand 2006*a*) rather than the strength of selection against each mutation. Unfortunately, there are few data on the effect of stress on selection against individual mutations, especially in multicellular organisms.

Recent theoretical work that uses a fitness-landscape model has resulted in several more-detailed predictions with respect to how selection will be affected by environmental factors (Martin and Lenormand 2006*a*, 2006*b*). First, for unconditionally deleterious mutations, selection should increase under stressful conditions. Second, some proportion of deleterious alleles may become beneficial in the adverse environment, such that selection switches in sign. Finally, as a result of the first two outcomes, stressful

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conditions will ultimately lead to greater variance in selection among mutations.

Of course, alleles are not selected in isolation. They can interact to diminish or enhance each other's detrimental effect (positive and negative epistasis, respectively). Although recent empirical studies have shown that epistasis can be sensitive to the environment (Remold and Lenski 2004; Harrison et al. 2007), little is known about how stress affects epistasis on average (Jasnos et al. 2008). Several authors have predicted that buffering is reduced under environmental stress so that epistasis becomes more negative (or less positive) among deleterious mutations (You and Yin 2002; Kishony and Leibler 2003). Recent work in yeast supports this prediction (Jasnos et al. 2008), but empirical evidence is absent for multicellular organisms.

Selection on an allele can also depend on the genetic background from which it is inherited, that is, parent-of-origin effects. Although such effects can have a variety of interesting evolutionary consequences (Wade 1998), the prevalence of parent-of-origin effects for fitness is unknown. As with the other properties, the strength of parent-of-origin effects on selection may also be influenced by the environment, but this has never been studied.

We measured selection and epistasis on deleterious mutations in two different environments. Low- and high-quality environments were generated by manipulating the food media, such that the low-quality environment was more stressful on larval survival. To perform our assays, we used 20 mutations with dominant phenotypic effects in *Drosophila melanogaster*. As in all other studies examining selection on individual mutations (Whitlock and Bourguet 2000; Remold and Lenski 2004; Harrison et al. 2007; Jasnos et al. 2008), the alleles used here might experience stronger selection than the average random mutation. The focus of studies that use large-effect alleles is not on the selection estimates per se but rather on how selection changes with context (e.g., environment, genetic background, parent of origin).

We performed reciprocal crosses between paired heterozygous mutants (fig. 1) that produce four genotypes in equal frequencies: wild-type, two different single mutants, and the double mutant (e.g., $A+ \times +B \rightarrow 1/4\ ++$, $1/4\ A+$, $1/4\ +B$, and $1/4\ AB$). Selection and epistasis were inferred from the deviation of observed genotype frequencies among surviving adults from those expected under neutrality. Because we performed reciprocal crosses ($A+ \times +B$ vs. $+B \times A+$), we were able to determine whether parent-of-origin effects influenced selection. Overall, 10 gene pairs were assayed, and a total of ~650,000 offspring were processed.

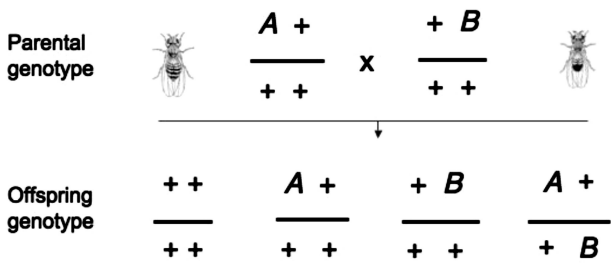

Figure 1: Schematic of the crossing design employed for fitness assays used to measure selection and epistasis on pairs of deleterious mutations. Two types of mutants, each heterozygous for a different dominant deleterious mutation, were crossed to each other, and their offspring were collected to be raised in the two different environmental settings (low vs. high quality). The cross was expected to produce four offspring genotypes in equal frequency: $1/4\ ++$, $1/4\ A+$, $1/4\ +B$, and $1/4\ AB$. Reciprocal crosses were performed simultaneously for each pairing, such that one cross was between males carrying the *A* mutation and females carrying the *B* mutation and the other cross was between females carrying the *A* mutation and males carrying the *B* mutation.

Material and Methods

Mutations

Our experiment involved 20 known dominant mutations in *Drosophila melanogaster* obtained from the Bloomington *Drosophila* Stock Center. These mutant alleles were from separate autosomal loci and were located on either the second (*Pin*, *Gla*, *L*, nw^B, *Bkd*, bw^D, *Frd*, *U*, *Adv*) or the third (*Sb*, *Dr*, *Ly*, *R*, k^D, *Antp*, *Gl*, *Pr*, *Bsb*, *Ki*, *W*) chromosome. All mutations used have visible phenotypic effects in adult flies, affecting the eyes (*Gla*, bw^D, k^D, *R*, *Gl*, *Ly*, *Dr*, *L*), wings (*W*, *U*, *Adv*, nw^B), bristles (*Pin*, *Ki*, *Bsb*, *Pr*, *Sb*), body color (*Bkd*, *Frd*), and antennae (*Antp*). These mutations were introgressed into our large outbred laboratory population through a minimum of 10 generations of serial backcrossing. The outbred population, originally collected in 1970 from Dahomey (now Benin), West Africa, has been maintained at a large population size in various labs since then and in our lab for the past 4 years. Each generation of backcrossing involved ~100 randomly sampled individuals from the outbred population. The resulting mutant lineages were expected to share randomized genetic backgrounds derived from the Dahomey population. All stocks were cultured using standard *Drosophila* protocol at 25°C on a 12L : 12D cycle, with 70% relative humidity (Ashburner et al. 2004).

Low- versus High-Quality Environments

The two different environments used in our experiment were created through manipulation of nutritional levels in the larval food media. The low-quality environment con-

tained 50% of the yeast and sugar concentrations of the high-quality environment (standard yeast-sugar medium). Offspring survival was reduced by 42% on average in the low-quality environment compared with that in the high-quality environment (see "Results").

Fitness Assays

The 20 mutations were paired more or less at random, under the constraint that double mutants were able to be phenotypically distinguished from both single mutants. To assay fitness (measured here as viability), we crossed parents that were heterozygous for different mutations (fig. 1). To create these heterozygous individuals to serve as parents, we crossed mutant males with females from the outbred population (~500 randomly sampled individuals for each cross) in groups of three to five males and five to eight females. All heterozygous mutants used as parents for the fitness assays were raised on standard yeast-sugar medium in 10-dram vials at moderate density and were collected as virgins. Flies were housed in same-sex, same-genotype vials containing standard media seeded with live yeast, at a density of 20–25 flies per vial for 2–5 days before being mated. Reciprocal crosses were performed simultaneously for each mutant pairing, such that one cross was between males carrying the *A* mutation and females carrying the *B* mutation, and the other cross was between females carrying the *A* mutation and males carrying the *B* mutation. Approximately 1,000–1,700 parents of each sex were used in each reciprocal cross. Matings were conducted en masse in cages, and eggs were collected on agar lay plates. From each reciprocal cross, groups of 100 eggs per replicate were transferred into either the low- or the high-quality environment (day 0), and approximately 160 replicates were set up for each of the two environments over a total of 5 days. Offspring were then allowed to develop in the different larval rearing environments (low vs. high quality) until they emerged as adults. Replicate vials from both environments and both reciprocal crosses were mixed together in trays and randomized daily during this period. The proportion of surviving offspring of each genotype (egg-to-adult viability) was determined by scoring phenotypes on day 11 and then again on day 15. The offspring of each cross were of four expected genotypes: wild-type, two different single mutants, and the double mutant (e.g., $A+ \times +B \rightarrow 1/4\ ++$, $1/4\ A+$, $1/4\ +B$, and $1/4\ AB$). In the absence of selection and epistasis, we expected the proportion of offspring of each genotype to be 25%. Deviations from the expected genotypic frequencies could then be used to calculate larval fitness to estimate selection and epistasis, as detailed below.

Statistical Analyses

For each gene pair in each environment, a linear model was fitted for the number of individuals of each genotype surviving to adulthood as a function of the state of its **A** locus (wild-type or mutant), the state of its **B** locus (wild-type or mutant), the interaction between the two genotypic states, the cross direction, and the vial identity nested within the cross direction. Vial identity was a random factor, and all others were fixed factors. The coefficients of this linear model were estimated with the assumption of a Poisson error structure for count data, with use of the generalized mixed model function "lmer" in R (R Development Core Team 2008).

We are interested in understanding selection and epistasis on each gene pair within each environment. Accordingly, the number of surviving individuals of each of the four genotypes in a given environment can be expressed as $W_{++} = k$, $W_{A+} = k(1 - s_A)$, $W_{+B} = k(1 - s_B)$, and $W_{AB} = k(1 - s_A - s_B + \epsilon)$, where a plus sign indicates a wild-type allele and *A* and *B* represent the dominant mutations at the first and second locus, respectively. We estimated selection (s_A, s_B) and epistasis (ϵ) for each gene pair in each environment, using the parameters from the linear model.

In the model described above, epistasis is defined as a deviation from additivity rather than as a deviation from multiplicativity (Wade et al. 2001). Epistasis is measured as the deviation from the additive effects of individual mutations because this is the measure that is most relevant to determining the immediate consequence for mean fitness of rearranging gene combinations (e.g., breaking down linkage disequilibrium; Barton 1995). In some population genetic models (Charlesworth 1990; Otto and Feldman 1997; Lenormand and Otto 2000), the multiplicative definition has been used because this form of epistasis determines the sign of linkage disequilibrium in the absence of evolutionary forces other than selection (Feldman et al. 1980). However, recent theoretical work indicates that epistatic selection is unlikely to determine the sign of linkage disequilibrium between deleterious alleles in real populations (Pylkov et al. 1998; Lenormand and Otto 2000; Otto and Barton 2001; Keightley and Otto 2006). It thus seems more useful to focus on the role of epistasis in determining the fitness consequences of breaking down linkage disequilibrium (for which the additive definition is most appropriate) rather than to focus on the role of epistasis in shaping linkage disequilibrium. Consequently, we focus our analyses on the additive model, though we also present values from a multiplicative model (where $W_{AB} = k(1 - s_A)(1 - s_B) + \epsilon$) for the sake of comparison with previous studies.

In the absence of sampling error and when averaged

over the two reciprocal crosses, the population genetic parameters are related to the coefficients of the regression model by the following equations:

$$k = \exp\left(\beta_0 + \frac{1}{2}\beta_{cd}\right), \quad (1)$$

$$s_A = 1 - \exp(\beta_A), \quad (2)$$

$$s_B = 1 - \exp(\beta_B), \quad (3)$$

$$\varepsilon = 1 + \exp(\beta_A + \beta_B + \beta_{A\times B}) - \exp(\beta_A) - \exp(\beta_B). \quad (4)$$

The terms β_0, β_{cd}, β_A, β_B, and $\beta_{A\times B}$ are the coefficients from the regression model for the intercept, the effect of cross direction, the effect of **A**-locus state, the effect of **B**-locus state, and the interaction, respectively. (For the multiplicative model, epistasis is measured as $\epsilon_m = -\exp(\beta_A + \beta_B) \times [1 - \exp(\beta_{A\times B})]$). These relationships were obtained by setting the fitness equations (W) equal to the corresponding equation from the regression model for each genotype and solving for the parameters.

To account for sampling error in the estimates of these coefficients, selection and epistasis and their sampling (co)variances were estimated by applying the delta method (Lynch and Walsh 1998) to the equations above. Paired *t*-tests were used to compare selection and epistasis estimates between environments. An *F*-test was used to compare the variance in selection estimates between environments.

Because significant effects of cross direction were frequently detected in the analysis described above, we performed a separate series of analyses to look for parent-of-origin effects on the strength of selection against the mutations. As a first step, the data from all gene pairs and treatments were analyzed simultaneously by MANOVA with the frequencies of the *A* and *B* alleles as the dependent variables. Gene pair, environment, cross direction nested within gene pair, and number of surviving wild-type offspring were included as explanatory variables. All terms in the model were highly significant ($P < .0001$ by Wilks's λ for all terms). Each gene was then analyzed individually. With the data from both environments for a given gene pair, a model for the frequency of *A* per vial was fitted with the following factors: environment, cross direction, their interaction, and number of surviving wild-type individuals. The last term was included in the model to help account for density differences that might result from differences in the overall hatchability of eggs from the two alternative types of mothers ($A+$ and $+B$). The same type of model was also run for the frequency of *B*. To obtain the selection estimates used for figure 4, we used the least squares mean estimates for allele frequency from each of the two cross directions from these gene-specific models (e.g., f_1 and f_2 for the allele frequencies in cross directions 1 and 2, respectively). Selection in cross directions 1 and 2 were estimated as $S_1 = (1 - 2f_1)/(1 - f_1)$ and $S_2 = (1 - 2f_2)/(1 - f_2)$. These relationships were obtained by rearranging the standard population genetic equation for allele frequency after selection—that is, $f_{after} = f_{before}(1 - S)/\overline{W}$—and noting that the initial expected frequency of each mutant allele was $f_{before} = 1/2$ and $\overline{W} = 1 - f_{before}S$. Note that this measure of selection represents the total selection experienced by a mutation and thus depends on both s and ϵ.

Results

Manipulation of larval diet quality had the intended effect on offspring survival. Averaged across gene pairs, the number of surviving wild-type flies in the low-quality environment is 42% lower than the number in the high-quality environment ($t = 8.7$, df $= 9$, $P < 10^{-4}$). The environment also affects selection against mutant alleles (fig. 2; table 1) in a pattern consistent with the predictions of the fitness-landscape model (Martin and Lenormand 2006*a*, 2006*b*), although our power to test two of the three predictions is limited. The variance in selection among genes in the low-quality environment is 1.77 times larger than the variance in the high-quality environment, but this is not statistically significant ($F_{19,19} = 0.565$, $P = .11$). Also, more mutations appear to be beneficial in the low-quality environment, but this is based on limited data (three vs. two mutations, from point estimates of selection). When all mutations are taken into account (including beneficial mutations), mean selection is not significantly different across the two environments ($t = -1.64$, df $= 19$, $P = .12$), and there is a strong relationship between the effects of selection across environments ($r^2 = 0.64$). However, for the 16 mutations that are deleterious in both environments, selection in the low-quality environment is significantly stronger than that in the high-quality environment ($t = -2.55$, df $= 15$, $P = .02$).

Not only does the environment affect how selection acts on mutations in isolation, but it also affects how selection acts on combinations of mutations (epistasis). Epistasis, defined as representing the nonadditive effect of having two mutant alleles together, is significantly more positive in the low-quality environment ($t = -3.02$, df $= 9$, $P = .01$). As shown in figure 3, almost all points lie above the line of equal effects (*dashed line*), which indicates that epistasis values are more positive in the low-quality environment. More often, epistasis is thought of as measuring a nonadditive effect of having two deleterious alleles together. Because most mutations are deleterious, these definitions are usually synonymous. However, in four of

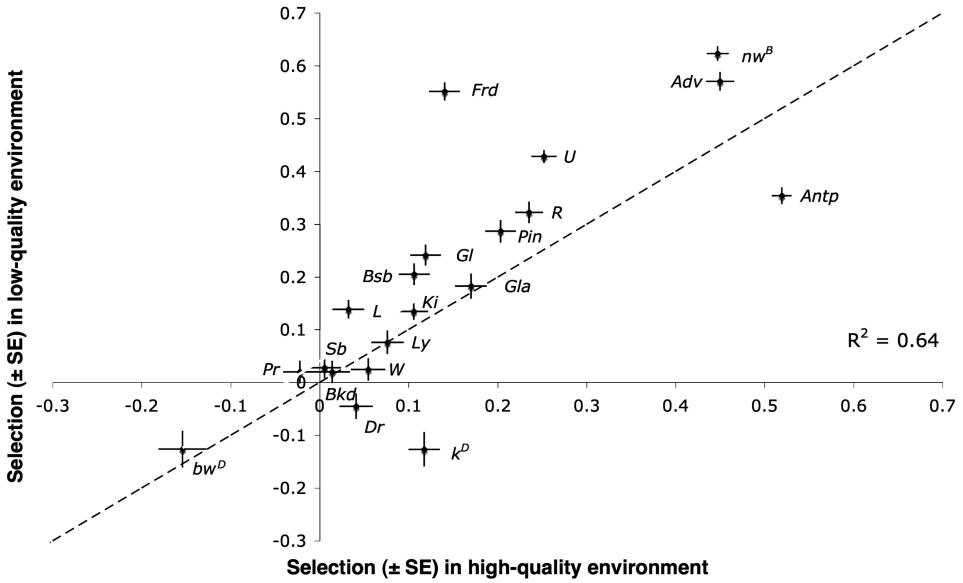

Figure 2: Selection estimates for each of the 20 mutations compared across the two different environments (low vs. high quality). Points in the upper right quadrant represent mutations that are deleterious in both environments. Points in the bottom left quadrant represent mutations that are beneficial in both environments. Points that lie in either of the other two quadrants represent mutations for which the selection estimates change in sign across environments (conditionally deleterious mutations). The dashed line represents the case in which the selection estimates are constant across the two environments.

the 10 gene pairs we tested, one mutation is beneficial in at least one environment. If epistasis is measured with respect to deleterious alleles rather than mutations, the result remains qualitatively similar: epistasis is more positive in the low-quality environment than in the high-quality environment ($t = -2.75$, df $= 9$, $P = .02$).

In our analyses of the number of survivors (see "Material and Methods"), we frequently observed an effect of cross direction (i.e., $A+ \times +B$ vs. $+B \times A+$) indicative of parent-of-origin effects. To test whether parent-of-origin effects affect selection rather than just overall survival, we used MANOVA with the frequencies of the *A* and *B* alleles from all gene pairs as the dependent variables. Because the cross-direction effect is highly significant ($P < .0001$ by Wilks's λ), we analyzed gene-specific models of mutant-allele frequency (see "Material and Methods"). The main effect of cross direction is significant for 14 of the 20 genes (table 2). Of the remaining six genes, two have significant cross direction × environment interaction effects. Thus, 80% (16/20) of the genes examined here show some evidence of parent-of-origin effects. Furthermore, parent-of-origin effects for seven of the mutations differ significantly between environments (table 2).

The gene-specific models described above were performed on all 20 mutations. This potentially creates a multiple-tests problem, though this risk is small, given the significant effects from the overall MANOVA model that evaluates data from all gene pairs simultaneously. Nonetheless, we considered the multiple-testing issue. With the assumption that the tests for the cross-direction effect on each individual gene are independent, only one false positive is expected in 20 tests if no mutations truly experienced parent-of-origin effects; the probability of observing 14 positives is 1.8×10^{-14} (when only main effects are considered). This indicates that it is exceedingly unlikely that there are no true parent-of-origin effects for any of the genes, but it does not tell us how many of our positive results are likely to be true.

Although we did not perform enough tests to properly employ modern false-discovery-rate algorithms (Storey and Tibshirani 2003), the following logic can be used to evaluate the evidence of parent-of-origin effects. With the assumption that the tests are independent, it is possible to employ a maximum likelihood procedure to estimate how many of the 20 mutations truly experience parent-of-origin effects. For a mutation that is truly unaffected by parent-of-origin effects, the probability of falsely observing a significant cross-direction effect is $\alpha = 0.05$. For a mutation that is truly affected by parent-of-origin effects, the probability of correctly observing a significant cross-direction effect is $1 - \beta$; this is the power to detect true positives. With use of binomial probability distributions with $p_{\text{false}} = \alpha$ and $p_{\text{true}} = 1 - \beta$, it is straightforward to calculate the likelihood of the observed outcomes if *x* of

Table 1: Estimates of selection and epistasis

Gene pair	s_A		s_B		ϵ		ϵ_m	
(*A*, *B*)	High	Low	High	Low	High	Low	High	Low
W, *Bkd*	.054 (.020)	.025 (.021)	.014 (.020)	.020 (.021)	.075 (.028)	.131 (.030)	.074 (.027)	.130 (.029)
R, nw^B	.236 (.016)	.322 (.021)	.448 (.013)	.623 (.014)	.179 (.021)	.332 (.027)	.073 (.015)	.131 (.016)
Pin, *Gla*	.203 (.017)	.286 (.022)	.170 (.018)	.183 (.024)	.010 (.026)	.142 (.033)	−.025 (.022)	.089 (.026)
Adv, bw^D	.450 (.016)	.570 (.018)	−.154 (.027)	−.126 (.035)	−.013 (.032)	.036 (.040)	.056 (.025)	.108 (.028)
k^D, *Frd*	.118 (.018)	−.127 (.033)	.140 (.017)	.551 (.017)	−.009 (.026)	.062 (.037)	−.026 (.022)	.132 (.026)
Ki, *U*	.106 (.016)	.134 (.016)	.252 (.014)	.428 (.012)	.128 (.022)	.227 (.021)	.102 (.018)	.169 (.015)
Pr, *Antp*	−.022 (.019)	.020 (.021)	.519 (.011)	.354 (.016)	.050 (.022)	.032 (.027)	.061 (.016)	.025 (.022)
Gl, *Bsb*	.119 (.017)	.241 (.020)	.106 (.017)	.205 (.020)	−.118 (.026)	−.086 (.030)	−.131 (.023)	−.136 (.024)
Ly, *Dr*	.076 (.018)	.076 (.022)	.041 (.019)	−.045 (.024)	−.022 (.027)	−.068 (.033)	−.025 (.025)	−.065 (.032)
Sb, *L*	.006 (.018)	.028 (.019)	.032 (.018)	.139 (.018)	−.046 (.026)	.009 (.027)	−.046 (.025)	.005 (.024)

Note: For each gene pair, values are given for both the high-quality environment and the low-quality environment. Standard errors are given in parentheses. The term ϵ represents epistasis from the additive model, whereas ϵ_m represents epistasis from the multiplicative model (see "Material and Methods").

the genes truly have no parent-of-origin effects and $20 - x$ of the genes do. To do these calculations, a value for β must be chosen, but the interpretation of the outcome is not very sensitive to this choice. With the assumption that we have very high power to detect true positives ($1 - \beta = 0.95$), the maximum likelihood number of mutations experiencing parent-of-origin effects is 14 ($2\ln$(likelihood), with support interval 13–16). If we assume a lower power to detect false positives, the maximum likelihood estimate is even higher (e.g., with $1 - \beta = 0.8$, the maximum likelihood estimate is 17, with support interval 14–20). It should be noted that this procedure assumes that the 20 tests are independent, but this is not strictly true since the genes were examined in pairs. However, the MANOVA accounts for this lack of independence between alleles in a gene pair, and this analysis estimates that the correlation in the frequencies of the *A* and *B* alleles is low ($\rho = -0.15$).

It is hard to escape the conclusion that parent-of-origin effects are common, even after multiple testing is considered. Furthermore, these parent-of-origin effects are reasonably large in magnitude. As a heuristic to evaluate the relative effect of cross direction, we calculated the standardized difference in selection between cross directions by calculating the difference in selection between the cross directions relative to their average (fig. 4; table 2). The mean change in selection due to cross direction is 86%, with only a minority of genes (four of 20) changing by less than 10%.

Discussion

Environmental conditions change over space and time. Here we have examined how patterns of selection change between a benign and a stressful environment. These results can be compared to predictions from recent fitness-landscape models (Martin and Lenormand 2006*a*, 2006*b*), though a subtle distinction should be acknowledged between our estimates of selection and those used in the model. In the model, selection on a mutation is measured relative to a single reference genotype. Our estimates of selection measure the fitness effects of a mutation averaged across a genetically variable background; this is a more appropriate measure of selection on mutations occurring in a genetically variable population. These alternative estimates of selection are the same under the model's assumptions (weak selection and Gaussian fitness) and will be similar in general, unless there is both a large amount of background variation and strong curvature in the fitness landscape. Moreover, the model's qualitative predictions with respect to the effects of environmental stress should apply to our estimates.

The fitness-landscape model (Martin and Lenormand 2006*a*, 2006*b*) predicts that in stressful environments (i) unconditionally deleterious mutations will be more strongly selected, (ii) a larger fraction of mutations will be beneficial, and (iii) there will be greater variance in selection among mutations. Our results are consistent with all three predictions. Our data most strongly support the prediction that stress increases the strength of selection against unconditionally deleterious mutations. This result is also in line with previous indirect evidence in flies (Kondrashov and Houle 1994; Yang et al. 2001). The only other study, to our knowledge, that reports direct estimates of selection against individual deleterious mutations across environments was done using *Escherichia coli* (Kishony and Leibler 2003). In that study, the incidence of cases in which stress alleviated selection was found to be much higher than that found in our study. A pattern similar to that reported for *E. coli* also appears in a recent study on yeast (Jasnos et al. 2008). It is unclear why such differences exist. One possibility is that the types of stresses and fitness

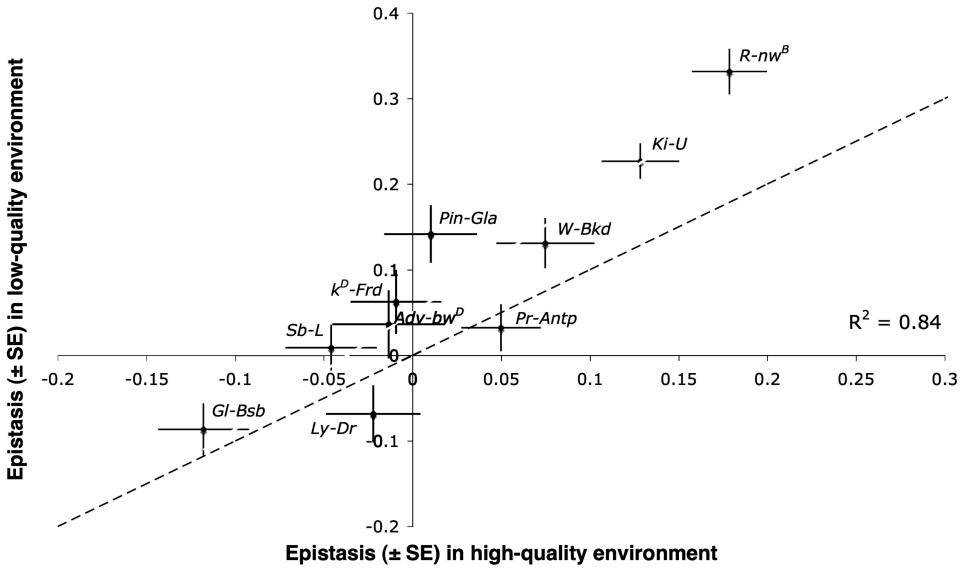

Figure 3: Epistasis estimates for each of the 10 mutation pairs compared across the two different environments (low vs. high quality). Points in the upper right quadrant represent mutations for which the epistasis is positive in both environments (i.e., the double mutant is more fit in both environments than is expected on the basis of the individual mutation effects). Points that lie in the bottom left quadrant represent mutations for which the epistasis estimates are negative in both environments (i.e., the double mutant is less fit in both environments than is expected on the basis of the individual mutation effects). Points that lie in either of the other two quadrants represent mutations for which epistasis changes in sign across environments. The dashed line represents the case in which epistasis is constant across environments.

measures used in flies differ considerably from those used in unicellular organisms. In addition to the obvious differences, studies on microorganisms typically measure selection against individual mutations in a single isogenic background, whereas we measured selection against individual mutations averaged across many randomized genetic backgrounds. However, it is difficult to see how this difference in methodology could cause the observed difference in results. A more intriguing explanation is that robustness to perturbation, both genetic and environmental, differs between unicellular and multicellular organisms. Previous authors have predicted that more complex organisms should be more robust than simpler organisms (Lenski et al. 1999; Sanjuán and Elena 2006; Sanjuán and Nebot 2008), but our results suggest the opposite.

Epistasis affects the efficiency of selection and has featured prominently in discussions of mutation load and the evolution of sex and recombination (Kimura and Maruyama 1966; Kondrashov 1982; Barton 1995; Otto and Feldman 1997; Roze and Lenormand 2005). Studies of gene interactions affecting fitness have shown that mean epistasis is close to 0 but that there is large variance around this mean (de Visser and Elena 2007; Martin et al. 2007). Most of these data come from unicellular organisms, so our ability to generalize to higher eukaryotes or to examine the relationship between epistasis and organismal complexity (Sanjuán and Elena 2006) has been limited. Consequently, there have been several recent calls for studies of epistasis in multicellular organisms (Fry 2004; Martin et al. 2007; Jasnos et al. 2008).

Consistent with previous studies (de Visser and Elena 2007), we find cases of both positive and negative epistasis (table 1). Our point estimate of average epistasis ($\bar{\varepsilon}_m \pm$ SE) was close to 0 and slightly positive: 0.011 ± 0.023 in the high-quality environment and 0.059 ± 0.031 in the low-quality environment. (Note that the values reported here refer to multiplicative epistasis to be consistent with previous authors.) Some authors have argued that negative epistastic interactions may be more likely between genes in the same pathway (Szathmary 1993; Rice 1998). Because of the need to phenotypically distinguish both single mutants from the double mutant, we paired mutations affecting different phenotypes. Consequently, our gene pairs may be biased against showing negative epistasis. However, since most randomly chosen pairs of genes will tend to come from different pathways, this bias should be small. The only other study to measure epistasis between individual mutations in *Drosophila melanogaster* (Whitlock and Bourguet 2000) reported cases of both positive and negative epistasis but with a tendency toward negative interactions (these authors also used mutations affecting dif-

Table 2: Evidence of parent-of-origin effects on selection against mutations

Gene pair (*A*, *B*)	Mutation *A*			Mutation *B*		
	Cross direction	Cross direction × environment	$\lvert\Delta S\rvert / \lvert\bar{S}\rvert$	Cross direction	Cross direction × environment	$\lvert\Delta S\rvert / \lvert\bar{S}\rvert$
W, *Bkd*	N	N*	1.09	Y	N	1.15
R, nw^B	Y	N	.84	Y	Y	.20
Pin, *Gla*	Y	N	.26	N	N	.15
Adv, bw^D	N	Y	.02	N	N	.26
k^D, *Frd*	Y	Y	2.39	Y	Y	.26
Ki, *U*	Y	Y	1.68	N	N	.00
Pr, *Antp*	Y	N	1.32	Y	N	.08
Gl, *Bsb*	Y	N	.21	N	Y	.09
Ly, *Dr*	Y	N*	.54	Y	Y	4.36
Sb, *L*	Y	N*	1.96	Y	N	.36

Note: The table indicates the significance of the cross-direction term and of the cross direction × environment interaction term from linear models of the frequency of each mutation. Y indicates $P < .05$; N indicates the opposite. An asterisk indicates $.05 < P < .1$. The standardized difference in selection, measured as $\lvert\Delta S\rvert / \lvert\bar{S}\rvert$, is a measure of the proportional change in selection between the alternative cross directions (see fig. 4 for details). The environment term in the linear model of mutation frequency (not shown) was highly significant ($P < .0001$) for all mutations except *Antp* ($P = .31$).

ferent phenotypes). The negative epistasis observed by Whitlock and Bourguet contributed to the observation of a negative correlation between complexity and epistasis (Sanjuán and Elena 2006). Our observation of a tendency toward positive epistasis would reduce the strength of this correlation.

Classic deterministic theory for the evolution of recombination predicts that recombination is favored if epistatic interactions are weak and negative (Barton 1995). However, it is insufficient for epistasis to be weak and negative on average; the variance in epistasis among gene pairs must also be low (Otto and Feldman 1997). The observed variation in epistasis in our study and previous studies is not conducive to the evolution of recombination under constant conditions.

Alternatively, theory predicts that recombination is favored if selection is temporally varying, such that the sign of epistasis between particular pairs of genes fluctuates (Charlesworth 1976; Maynard Smith 1978; Barton 1995; Gandon and Otto 2007). In real systems, environments are constantly changing across generations, but little is known about the extent to which epistasis is environmentally sensitive. Although rarely measured, plasticity in epistasis has been found in the few cases in which it has been studied (Remold and Lenski 2004; Harrison et al. 2007; Jasnos et al. 2008). To our knowledge, ours is the first experiment to examine plasticity in epistasis in a multicellular organism. We also find that epistasis changes across environments but that changes in the sign of epistasis are infrequent. Thus, we have no evidence that fluctuations in abiotic conditions are likely to cause fluctuations in the sign of epistasis. It has been argued that changes in biotic conditions (e.g., parasite-mediated selection) may be more likely to cause the appropriate fluctuations in epistasis (Bell and Maynard Smith 1987; Peters and Lively 1999).

The recombination models discussed above assume no genetic drift. Recent theory suggests that an interaction between drift and directional selection, known as the Hill-Robertson effect (Hill and Robertson 1966), can be more important than epistasis in determining linkage disequilibrium (Otto and Barton 2001), especially for deleterious mutations (Keightley and Otto 2006). Hill-Robertson effects are expected to cause negative linkage disequilibrium (i.e., an excess of $A+$ and $+B$ haplotypes). Recombination is then favored because it dissipates negative disequilibria, thereby increasing the variance in fitness and creating a "long-term" advantage to recombination. If Hill-Robertson effects cause negative disequilibria even when epistasis is positive, there is also a "short-term" advantage to recombination because recombinant types will be more fit, on average, than nonrecombinants (i.e., $W_{++} + W_{AB} > W_{A+} + W_{+B}$; see Agrawal 2006 for a discussion of short- and long-term effects). Thus, the positive epistasis that we observe should be particularly conducive to the evolution of recombination in finite populations.

We observe that, on average, epistasis becomes more strongly positive under stressful conditions, in contrast with the idea that stress exacerbates deleterious gene interactions (Peck and Waxman 2000; You and Yin 2002; Kishony and Leibler 2003). However, given that the magnitude of selection increases under stress, our results do match the prediction that epistasis should be more positive when the effects of individual mutations are larger (Wagner et al. 1998; Wilke and Adami 2001; You and Yin 2002). Jasnos et al. (2008) also measured epistasis under multiple

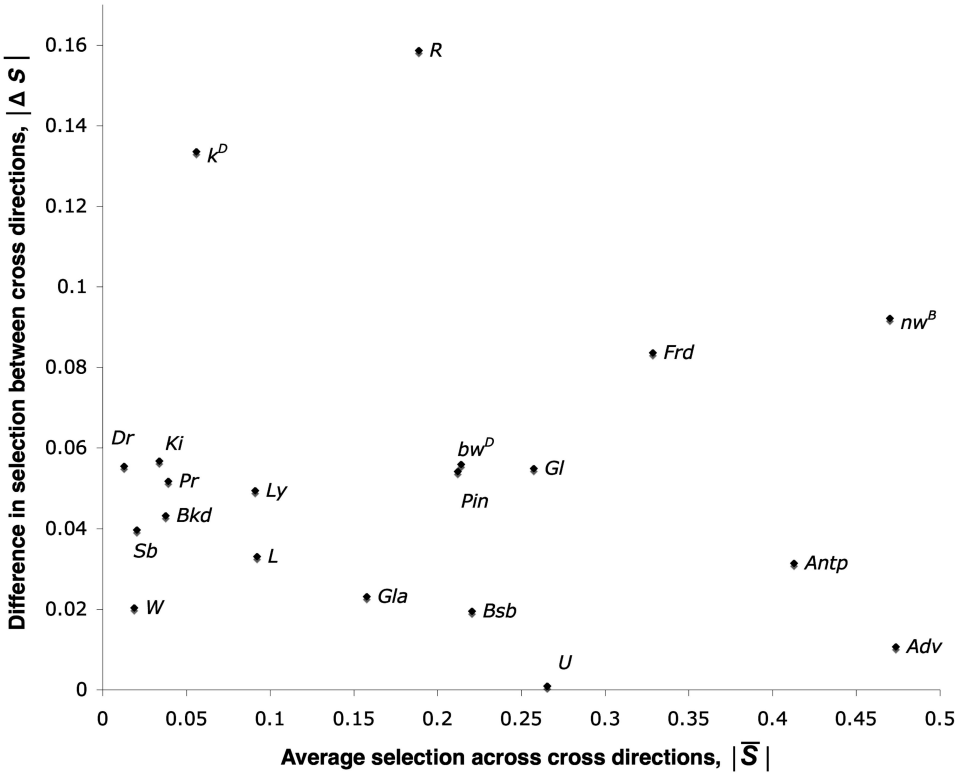

Figure 4: Difference in selection between reciprocal crosses, $|\Delta S|$, in relation to the average selection, $|\bar{S}|$. For each gene, we calculated $|\bar{S}| = |S_1 + S_2|/2$ and $|\Delta S| = |S_1 - S_2|$, where S_1 and S_2 are measures of the total selection experienced by a gene in the two cross directions (see "Material and Methods"). Table 1 reports the ratio of these measures as the standardized difference in selection between cross directions.

environments and found that epistasis became less positive under stress. This difference may be due to the fact that they used gene deletions (loss-of-function mutations) rather than the type of mutations used here. It is worth noting that selection in that study became weaker under stress, so while their results appear at odds with our own, both studies indicate that epistasis is more positive in the environment in which selection is stronger. Stress can also be induced by biotic factors, and the presence of parasites has been shown to increase the strength of selection on mutations in *E. coli* (Cooper et al. 2005). While average epistasis among mutations under parasitized versus nonparasitized conditions was not significantly different in that study, the direction of epistasis tended to become more positive under parasitized conditions.

Epistasis can be viewed as a change in selection on a focal allele in response to the state of some other locus. We find positive epistasis, which indicates that selection against a focal allele decreases in a bad genetic background. In contrast, we find that selection against a focal allele increases in a poor environment, which suggests that genetic and environmental perturbations may affect selection differently. However, a study in yeast found that both genetic and environmental stresses reduce selection (Jasnos et al. 2008). At this point, there are not enough data to know which pattern is more common or whether this discrepancy reflects some aspect of canalization and organismal complexity (Lenski et al. 1999; Sanjuán and Nebot 2008).

A surprising result is the prevalence and strength of parent-of-origin effects on deleterious mutations. The standardized change in selection due to cross direction is 15% or greater for 80% of the genes we examined. Although previous studies have reported evidence of parent-of-origin effects on selection, most of these studies involved only a few alleles (Clark and Feldman 1981; Clark and Bundgaard 1984; Beeman et al. 1992) or a single genotype (Ávila et al. 2006), which makes it difficult to assess the frequency of these effects. Parent-of-origin effects indicate that an individual's fitness depends on not only its own genotype but also how that genotype was assembled (i.e., which allele came from which parent). The mechanism for this type of parent-of-origin effect is unknown, but at least two explanations are possible. The first is ge-

nomic imprinting, whereby allelic expression depends on whether an allele was maternally or paternally inherited. However, confirmed cases of imprinting in *D. melanogaster* are extremely rare and are mostly limited to sex chromosomes (Maggert and Golic 2002; Ashburner et al. 2004).

We suspect that the most likely explanation involves maternal effects, which are common in most organisms (Roach and Wulff 1987; Mousseau and Fox 1998), including fruit flies and other insects (Mousseau and Dingle 1991). A recent study has also shown that maternal effects, rather than genomic imprinting, are the likely cause of parent-of-origin effects in gene expression in *D. melanogaster* (Wittkopp et al. 2006). However, simple additive maternal effects cannot explain our results. Our finding of parent-of-origin effects on allele frequencies, not just overall survival, implies that different offspring genotypes are differentially affected by maternal genotype. The prevalence of these effects among our genes indicates that this type of genotype-by-genotype epistasis may be common.

Although this high incidence of parent-of-origin effects has not previously been documented, it is easy to understand why it might occur. Just as selection can depend on the external environment, selection may also depend on the maternal environment. Although alternative maternal genotypes can be thought of as analogous to alternative environments, there are unique evolutionary implications of maternal-offspring epistasis because of the genetic associations that exist between mothers and offspring and that arise from Mendelian inheritance (Wade 1998; Wolf 2000).

We measured how aspects of selection against phenotypically dominant mutations depend on the environment and the presence of other mutations (epistasis), as well as cross direction (parent-of-origin effects). There is no a priori reason why the key patterns we observe (increased selection against unconditionally deleterious alleles under stress, more positive epistasis under stress, and high incidence of parent-of-origin effects) should apply only to the type of mutation we have studied. Nevertheless, it remains a challenge to test whether these patterns apply to other types of mutations and to understand why comparable patterns in unicellular organisms differ.

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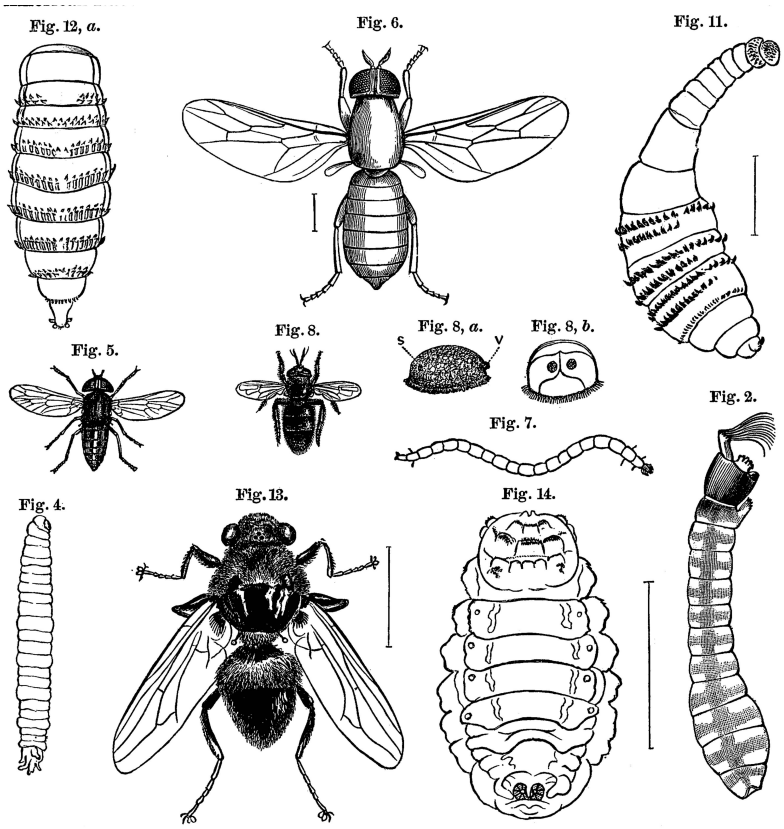

Figure 12a, larva of the bot-fly of the horse, *Gastrophilus equi*; figure 6, carpet-fly *Scenopinus pallipes* Say; figure 11, "in Cayenne, this revolting grub is called the 'Ver macaque'"; figure 5, horsefly or gadfly *Tabanus lineola* Fabr.; figure 8, *Microdon globosus* Fabr.; figure 8a, puparium; figure 8b, anterior view of pupa case; figure 7, carpet fly *Scenopinus pallipes* worm; figure 4, "a larva, which is, probably, a young Horse-fly, living in abundance on the under side of the stones in a running brook at Burkesville Junction, Va."; figure 13, the bot-fly of the ox, *Hypoderma bovis*; figure 14, larva of the bot-fly of the ox; figure 2, larva of a Labrador species of the black fly, about a quarter of an inch long, from "A Chapter on Flies" by A. S. Packard, Jr. (*American Naturalist*, 1869, 2:586–596, 597).