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The effects of competition on the strength and softness of selection

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Abstract

Deleterious alleles are constantly introduced into populations due to mutation. In subdivided populations, the impact of these mutations depends on the strength of selection as well as the softness of selection, that is, the extent to which fitness is governed by local rather than global competition. It is widely appreciated that the intensity and type of competition will affect selection on deleterious mutations but most empirical work has focused solely on the effects of competition on selection strength. However, competition has rarely been studied in the context of selection 'softness' even though competition is at the conceptual root of soft selection. All other things being equal, theory predicts that inter- and intraspecific competitions have opposing effects on the softness of selection. Using *Drosophila melanogaster*, we estimated the strength and softness of selection in a 'baseline' competitive environment as well as two additional competitive environments characterized by either additional intra- or interspecific competitors. We found that competitive environment had little effect on the average strength of selection. While the softness of selection was affected by the type of competition, the direction of change varied across tests of different genes, contrary to expectation. Although the 'hard/soft' selection paradigm implicitly assumes that all individuals are equally sensitive to the local competitive environment, we found this not to be the case. Wild-type individuals were more sensitive to changes in the genetic quality of their local competitors than mutant individuals.

Introduction

Mutations are constantly entering populations and the vast majority of those that affect fitness are deleterious (Keightley & Lynch, 2003). These deleterious alleles contribute to the standing genetic variance in fitness and are likely responsible for much of the depression in fitness observed with experimental inbreeding (Charlesworth & Charlesworth, 1999). The frequency of these deleterious alleles at equilibrium depends primarily on selection. Ideally, we would like to understand how and why selection works the way it does. Do certain types of environments alter selection predictably? Our thinking can be clarified by theoretical models but should be confirmed by empirical tests, starting with model systems.

In subdivided populations, the equilibrium frequency of deleterious alleles is determined by both the strength and softness of selection (Whitlock, 2002; Agrawal, 2010). The strength of selection is the proportional reduction in fitness of individuals carrying a mutant allele relative to the wild type. The softness of selection is the extent to which an individual's absolute fitness depends on the genetic quality of its local intraspecific competitors (Wallace, 1975; Whitlock, 2002). Selection often arises from the competition for resources, and our goal here is to examine how intra- and interspecific competitions affect the strength and softness of selection.

When density of either inter- or intraspecific competitors is high, there are fewer resources *per capita*, rendering the environment more stressful. Although it is commonly believed that selection typically becomes

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stronger under any type of stress, there is little basis for this position in theory or in data (Martin & Lenormand, 2006; Agrawal & Whitlock, 2010). However, intraspecific competition could be an exception to the statement above, predictably increasing the strength of selection. In a recent survey, Agrawal & Whitlock (2010) observed that studies testing the effect of various types of stress on selection often yielded mixed results but studies manipulating density more consistently found stronger selection. They postulated that intraspecific competition might be a different type of stress in that it is not experienced equally by all individuals; good-quality genotypes worsen the environment for poor quality genotypes by using their resources, accentuating the differences between good and bad genotypes. This logic would predict that intra- but not interspecific competition typically increases the strength of selection.

Competition should also affect the softness of selection. Since Wallace (1968, 1975) first coined it, the term 'soft selection' has been used to refer to situations where the absolute fitness of an individual depends on its genetic quality relative to the local average genetic quality as opposed to 'hard selection' where its fitness depends on the global average genetic quality. The 'soft vs. hard' distinction is important for a variety of phenomena including mutation load and inbreeding depression (Whitlock, 2002; Glemin *et al.*, 2003; Roze & Rousset, 2004; Theodorou & Couvet, 2006; Agrawal, 2010), social evolution (Grafen, 1984; Wade, 1985; Kelly, 1992; West *et al.*, 2002; Lehmann & Rousset, 2010; Van Dyken, 2010), disease evolution (Chao *et al.*, 2000; Débarre *et al.*, 2011) and the maintenance of polymorphism with spatial heterogeneity in selection (Levene, 1953; Dempster, 1955; Christiansen, 1975, 1985). Although hard and soft selections are often contrasted as mutually exclusive alternatives, it is more realistic to think of these as two points along a continuum of 'selection softness' (Whitlock, 2002; Agrawal, 2010). Intermediate levels of softness occur when an individual's fitness depends partly on the average genetic quality of its local neighbours and partly on the global average genetic quality.

Biologically, soft selection is thought to occur when individuals compete locally for resources rather than globally. If individuals consume resources locally and resources are limited, then an individual's fitness will depend strongly upon the competitive ability of its neighbours; the competitive abilities of distant conspecifics are unimportant. Consistent with this idea, a simple model of resource competition found that selection was softer, at least on some types of mutations, when there were fewer resources *per capita* (Agrawal, 2010). Thus, a simple prediction is that selection can be made softer by experimentally increasing intraspecific density while holding resource level constant.

Although intraspecific competition is predicted to make selection softer, interspecific competition should have the opposite effect. In the canonical case where there are no interspecific competitors in the metapopulation, selection is soft because mutants tend to compete against other mutants for local resources, whereas wild types tend to compete against other wild types due to genetic subdivision. When a mutant is slow to acquire resources, another mutant can then acquire those resources and thus mutants as a group do not suffer. The difference in acquisition ability between wild types and mutants does not translate into a difference in absolute fitness. In this scenario, an individual's fitness is strongly affected by the acquisition ability of its conspecific neighbours (i.e. selection is soft).

This logic does not hold if interspecific competitors are present across the metapopulation. If a mutant is slow to acquire resources, those resources may be consumed by a heterospecific competitor rather than by a conspecific mutant. Because mutants have lower competitive abilities, they will lose a greater fraction of their local resources to interspecific competitors than will wild-type individuals, assuming the intensity of interspecific competition is similar across all demes. As a result, the difference in acquisition ability between mutants and wild types will generate a difference in absolute fitness between these genotypes. In this scenario, an individual's fitness is less sensitive to the acquisition abilities of conspecific neighbours because heterospecifics play an important role in determining the competitive environment. Thus, interspecific competition effectively hardens selection on the focal species.

Although the softness of selection is expected to depend on the mechanisms of density regulation including competition, it is misleading to think extrinsic ecological factors alone determine the softness of selection for the entire genome. Different genes will experience different softnesses of selection depending on their phenotypic effects and how these interact with the ecological circumstances (Holsinger & Pacala, 1990; Kelly, 1994; Ravigné *et al.*, 2004; Agrawal, 2010). For example, genes affecting traits involved in the acquisition of limited resources will tend to experience softer selection than genes affecting the efficiency with which acquired resources are converted into fitness (Agrawal, 2010). Despite this among-gene variation, we still expect that increasing intraspecific competition should make selection softer, on average, whereas interspecific competition should have the opposite effect.

Some of these ideas were examined previously using *Drosophila melanogaster* as an empirical model (Laffafian *et al.*, 2010). In that study, the strength and softness of selection was estimated on eight genes at two very different densities. As expected, they found considerable variation among genes in both the strength and softness of selection. Contrary to prediction, they did not find selection to be softer at the higher density.

Here, we extend this approach to examine the effects of both inter- and intraspecific competition. Using a partially overlapping set of genes to those studied by Laffafian *et al.* (2010), we measured selection on eight mutations in *D. melanogaster* in three competitive scenarios: (i) 'baseline' environment (approximately 100 intraspecific competitors per vial), (ii) elevated intraspecific competition environment (approximately 200 intraspecific competitors per vial) and (iii) elevated interspecific competition environment (approximately 100 intraspecific competitors + 100 interspecific competitors per vial). Estimating the softness of selection requires that we measure the fitness of genotypes in demes that vary in local genetic quality. We accomplished this by creating demes containing 0%, 25% and 50% mutants within each competitive environment. Maximum likelihood estimates were then used to infer the strength and softness of selection. Several of the 'simple' predictions described above were not supported by the results, and we discuss reasons for why this may be so.

Materials and methods

Wild-type flies were obtained from a large outbred laboratory population of *D. melanogaster* originally collected in 1970 in Dahomey (West Africa) and maintained in the current laboratory since 2004. Eight deleterious and phenotypically dominant *D. melanogaster* mutants were obtained from the Bloomington Drosophila Stock Center. Mutations had visible effects on the eyes (*Gl* [33], *R* [34]), antennae (*Antp* [23]), wings (*Adv* [20], *Ly* [34], *U* [17]), bristles (*Bsb* [18]) or body colour (*Frd* [26]); the number within the square brackets are the number of generations the mutations have been backcrossed into the wild-type population. *Drosophila simulans* carrying a deleterious phenotypically recessive mutation that affected the colour of the eyes (*w*) were obtained from the San Diego Drosophila Stock Center and were maintained in current laboratory conditions for at least four generations. *Drosophila simulans* were homozygous for the white allele (*w/w*), so that they were easily distinguishable from *D. melanogaster*. All stocks were cultured at 25 °C on a 12L : 12D cycle with 70% RH.

For each 'competitive environment', we measured the egg-to-adult survivorship of flies in three demes that varied in the frequency of mutant genotypes. When selection is soft, an individual is expected to have a better chance of survival if its conspecific neighbours are poor competitors (i.e. mutants). For each gene, a 3 by 3 factorial design was performed where demes with three different genotype frequencies were tested within three different competitive environments (Table 1). Our baseline environment (*Base*) consisted of 100 *D. melanogaster* eggs. The '*Inter*' treatment introduced interspecific competitors and consisted of 100 *D. melanogaster* eggs and 100 *D. simulans* eggs, which were collected from crosses between female *w/w* to male *w/w*. The '*Intra*' treatment introduced additional intraspecific competitors and consisted of 200 *D. melanogaster* eggs. The frequencies of mutants in the different deme types (described below) of *Intra* are the same as those in *Base* (i.e. the addition was not purely wild-type individuals).

Table 1 Number of eggs in each treatment vial.

		Number of eggs		
Competitive environment	Deme type	*D.mel* (wt)*	*D.mel* (m)*	*D.sim*
Base	1	100	0	0
	2	75	25	0
	3	50	50	0
Inter	1	100	0	100
	2	75	25	100
	3	50	50	100
Intra	1	200	0	0
	2	150	50	0
	3	100	100	0

D.mel and *D.sim* represents *Drosophila melanogaster* and *Drosophila simulans*, respectively. wt and m represents wild-type and mutant individuals, respectively.

Within each environment, survival was assayed in three types of demes that differed with respect to genotype of the focal species (*D. melanogaster*). Type 1 demes contained 100% wild-type (+/+) eggs and were created by collecting the appropriate number of eggs from crosses between +/+ females to +/+ males. Type 3 demes consisted of approximately 50% +/+ and 50% mutant (M/+) eggs and were created by collecting the appropriate number of eggs from crosses between +/+ females to M/+ males. Type 2 demes consisted of approximately 75% +/+ and 25% M/+ eggs and were created by collecting half the appropriate number of eggs from each of the two crosses described earlier. The number of replicates for each type of deme at each competitive environment varied from 50 to 60 depending on the gene being examined. For each gene, the different assays (competitive environment × deme type) were performed over the same period in a balanced fashion, but each gene was assayed at different times; our comparisons are among competitive environments rather than among genes.

Eggs were collected by allowing flies to lay eggs on grape-agar medium seeded with live yeast pellets for *D. melanogaster* females and with yeast paste for *D. simulans* females. The eggs were then transferred, along with the thin layer of grape-agar medium on which they rested, into 13.5 dram (wide) vials containing 7 mL of standard yeast-sugar-agar food medium seeded with live yeast pellets. The larvae were then allowed to compete for resources within the vial and develop under normal culture conditions. The number, genotype and species of surviving adults were then recorded on days 11 and 15 after the initial set-up. In total, over

4000 replicate vials were examined, which involved counting over 690 000 eggs and scoring over 500 000 flies.

We chose to utilize the specific genes and densities in this study for logistical reasons. The mutations examined here have strong recessive effects on viability (homozygous lethal in most cases) but also have deleterious heterozygous effects on juvenile survival (Wang *et al.*, 2009). The alleles are also phenotypically dominant in adults, allowing for ease of differentiating between wild-type and mutant individuals. Although the large recessive fitness effects and dominant adult phenotypic fitness effects render these alleles different than most spontaneous mutations, there is no obvious reason why the way competition affects selection on juvenile viability should be unique to these types of mutations. Nonetheless, caution must be taken when extrapolating our results to spontaneous mutations in natural populations. Similarly, our results apply to competition effects measured at the specified densities and caution should be used in extrapolating these results to untested densities.

Fitness model

Following Laffafian *et al.* (2010), the absolute fitness of individual i in deme j was modelled as

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

ω_i represents the genetic quality of individual i, and $\bar{\omega}_j$ represents the average genetic quality of all individuals in deme j. The genetic quality of wild-type individuals and mutants are $\omega_+ = c$ and $\omega_m = c\ (1 - s)$, respectively; c is a constant representing the baseline fitness of wild-type individuals. The value of s measures the strength of selection, which is the proportional reduction in fitness of mutants relative to wild-type individuals. ψ_x represents the sensitivity of genotype x to the average quality within its deme. The soft/hard paradigm assumes $\psi_x = \psi$ for alI x, (i.e. mutant and wild types are both equally sensitive); we will return to this assumption later. Under this assumption, ψ is a measure of the softness of selection, where $\psi = 0$ indicates pure hard selection and $\psi = 1$ indicates pure soft selection.

In regard to this experiment, the fitness model in eqn (1) can be simplified to the following for both wild-type and mutant individuals:

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

The frequency of mutant x in deme j in represented by $f_{x,j}$ and has a value of 0, 0.25 and 0.5 for deme types 1, 2 and 3, respectively.

A maximum likelihood model was used to estimate the parameters in eqn (2) that would have been the most likely to have resulted in the observed number of surviving wild-type and mutant individuals for each vial [The survival of the *D. simulans* is not modelled. The interspecific competitor is regarded as an environmental effect that may alter the parameters of eqn (2) in *Inter*]. The likelihood model is described in detail in Laffafian *et al.* (2010). Briefly, the likelihood model is based on the binomial distribution to determine the probability of observing $n_{i,j,k}$ surviving adults in replicate k of genotype i in deme type j, given that there were initially $e_{i,j,k}$ eggs. This probability depends on the expected survivorship of genotype i in deme type j as given by eqn. (1). There are various sources of uncertainty (egg counts are not exact, genotype frequencies of sampled eggs will not exactly match expectations, replicate vials vary in quality) and these are built into the likelihood model as described in Laffafian *et al.* (2010). We fitted separate likelihood models for each gene. Because each of the genes examined were assayed at different time points, differences between parameter estimates for different genes could be due to true differences in selection among genes or due to of 'block' effects. Consequently, we do not attempt to examine differences in selection among genes in this study (see Laffafian *et al.*, 2010 for this type of analysis). Rather, our analyses focus on differences between competitive environments within a gene where there is no confounding difference of 'block'.

The full-likelihood model for a single treatment of a single gene had five parameters, k, s, ψ_{wt}, ψ_m and σ, where σ is a measure of the among-replicate variance in survivorship. However, for our initial analyses, we use a constrained model following the assumption of the soft/hard paradigm that $\psi_{wt} = \psi_m$; we refer to this as the 'equal-sensitivity' model. Other models, including the full model, were examined for specific tests and these are described in the Results where appropriate.

To find the maximum likelihood estimates, we used the *optim* function in R (R Development Core Team, 2011). To improve our ability in finding the global maximum likelihood, a two-step optimization procedure was used. The Nelder-Mead optimization method was first used until no further improvement was possible. The resulting sets of parameters were then used as the starting parameters for the Broyden–Fletcher–Goldfarb–Shanno optimization method to refine the search for the global maximum likelihood estimate. This was repeated 25 times for each model using different sets of random starting values; the best likelihood score was selected (and numerous starting values led to the same maximum likelihood estimates).

Results

The amount of stress that an environmental or a genetical condition applies onto a population is often measured by the reduction in the fitness of wild-type

individuals relative to their fitness in the absence of the stressor (Agrawal & Whitlock, 2010). The survival of wild-type individuals varied significantly among competitive environments (Fig. 1; $F_{2,1385} = 396.25$, $P < 10^{-15}$). A *post hoc* Tukey's HSD test revealed that the survival of wild-type individuals between the three environments were all significantly different from each other with survival in *Base* being highest and survival in *Intra* being lowest.

We are interested in whether the stressfulness of the environment affects the strength of selection. To test this idea, we examined the relationship between the estimated values for s and c using a general linear model including gene as a fixed effect. Recall that the value c in the likelihood model represents survival of wild-type individuals (in type 1 demes), and so it is inversely related to stress. We found little support for the intuitive prediction that stress would increase the strength of selection. Although the estimated values of c seem to be negatively correlated to the values of s, this effect was marginally nonsignificant (Fig. 2; $t_{15} = -1.873$, $P = 0.0807$). We investigated this further by comparing the values of the strength of selection, s, between the baseline environment (*Base*) and the environment with

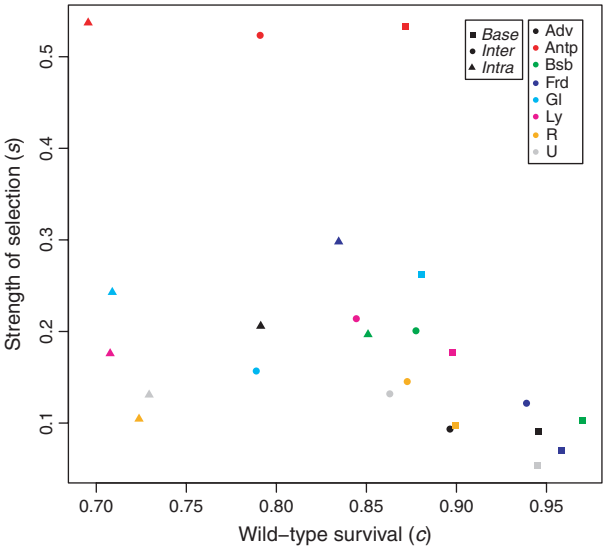

Fig. 2 The effects of environmental stress (inversely related to c) on the strength of selection (s). All values are parameter estimates from equal-sensitivity models. Square, circular and triangular points indicate values from the baseline, elevated interspecific and elevated intraspecific environments, respectively.

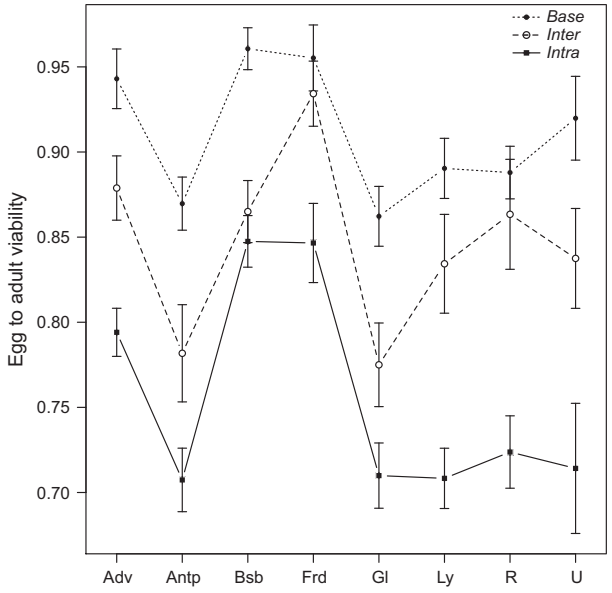

Fig. 1 Egg-to-adult viability of wild-type individuals in type 1 demes (100% wild types). Type 1 demes were tested simultaneously with other demes types for each gene examined, and in each competitive environment. The x-axis indicates the gene being examined at the corresponding time. Error bars indicate 95% confidence intervals. Data points are connected by lines to clearly show which set of points belong to the *Base*, *Intra* and *Inter* environmental treatments. Interpolation of the data points is not used to indicate any form of relationship among genes.

increased interspecific competition (*Inter*) or with increased intraspecific competition (*Intra*; Fig. 3). Paired t-tests indicated that neither increased inter- nor intraspecific competition significantly affected the strength of selection (*Base* vs. *Inter*: $t_7 = -1.12$, $P = 0.3015$; *Base* vs. *Intra*: $t_7 = -2.14$, $P = 0.0619$). Visual inspection of Figs 2 and 3 suggest that selection tends to be stronger under competition but neither analysis supports this claim statistically.

Concurrent with estimating s, we also estimated values for the softness of selection, ψ, for each gene in each environment using the equal-sensitivity models. In each case, we also examined a 'pure hard selection' model in which we constrained $\psi = 0$, then used a likelihood-ratio test to test whether the equal-sensitivity model ($\Psi \neq 0$) provided a significantly better fit. Of the 24 gene-environment combinations, only in six did an equal-sensitivity model not provide a significantly better fit than a pure hard-selection model, indicating that selection in our experiment was generally somewhat soft.

A major goal of this study was to examine how inter- and intraspecific competition affected the softness of selection. Figure 4 shows the ψ values for *Inter* and *Intra* compared to *Base*. To examine whether the interspecific competition affected the softness of selection, we analysed, for each gene, a model in which the softness parameter was constrained to be the same for both *Base* and *Inter* ($\psi_{Base} = \psi_{Inter}$; 'shared-sensitivity' model). For each gene, the log-likelihood of this model was compared to the sum of the log-likelihoods for the two

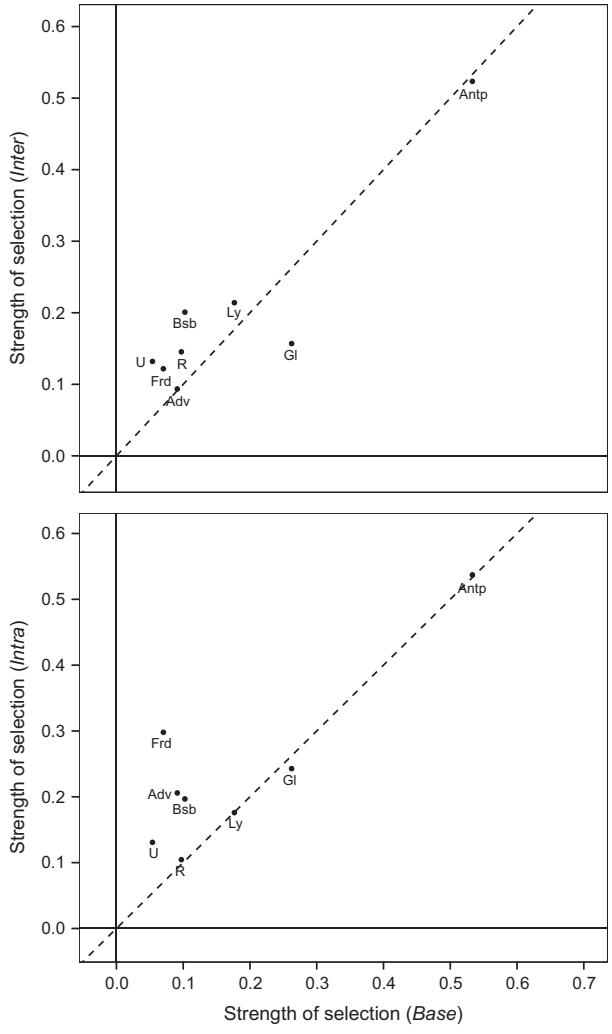

Fig. 3 The strength of selection at the baseline density compared with treatment with interspecific competitors (top) and with additional intraspecific competitors (bottom). The dashed line is the one-to-one line indicating equal strength at both density treatments.

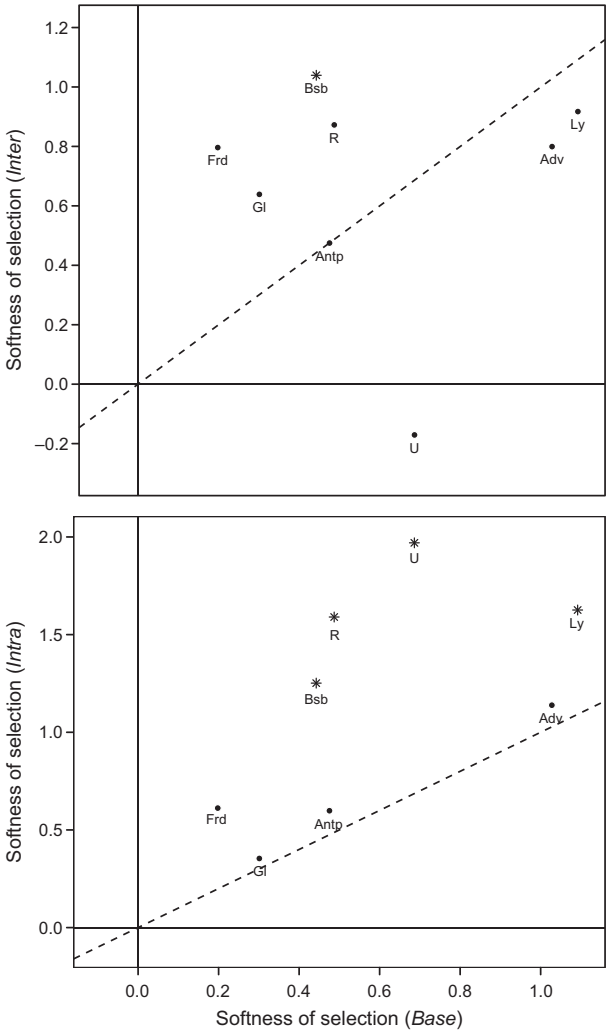

Fig. 4 The softness of selection at the baseline density compared with treatment with interspecific competitors (top) and with additional intraspecific competitors (bottom). The dashed line is the one-to-one line indicating equal softness at both density treatments. *Genes where the equal-sensitivity models provided a significantly better fit than their corresponding shared-softness model.

environment-specific equal-sensitivity models. For only one of the eight genes, *Bsb*, was there support for different softness values between *Base* and *Inter*. Moreover, the direction of this change was not as predicted by theory as selection was significantly softer, rather than harder, when the heterospecifics were present ($\chi^2 = 7.99$, d.f. = 1, $P = 0.0005$). A paired *t*-test of the ψ values indicated that overall, they were not significantly different between *Base* and *Inter* ($t_7 = 0.466$, $P = 0.66$).

Similar sets of contrasts were made between *Base* and *Intra* to examine the effect of increased intraspecific competition. For four of the eight mutations examined, the shared-sensitivity models yielded a significantly worse fit than models allowing for separate ψ-values between *Base* and *Intra* (*Bsb*: $\chi^2 = 14.67$, d.f. = 1, $P = 0.0001$; *Ly*: $\chi^2 = 6.08$, d.f. = 1, $P = 0.0137$; *R*: $\chi^2 = 6.13$, d.f. = 1, $P = 0.0133$; *U*: $\chi^2 = 3.91$, d.f. = 1, $P = 0.0480$). For all four of these genes, the increased intraspecific competition significantly softened selection, which is consistent with theoretical predictions. A paired *t*-test of the softness values between these two density treatments showed that, on average, across the eight genes, the increased intraspecific competition significantly increased the values of Ψ ($t_7 = 3.326$, $P = 0.0127$), indicating softer selection. However, it is clear from Fig. 4 that there was variation in how mutations respond to increased competition.

The values for Ψ reported above were obtained from equal-sensitivity models that assume wild types and mutants are equally sensitive to the genotypic composition of the deme, $\psi_{wt} = \psi_m = \psi$, in keeping with standard assumption of the soft/hard paradigm. We attempted to test this assumption by also examining an unconstrained model ($\Psi_{wt} \neq \Psi_m$) for each gene-environment combination and comparing its likelihood score to that of the corresponding equal-sensitivity model. We found that the full model fitted significantly better than the equal-sensitivity model for six of the 24 gene-treatments combinations, indicating that, for these cases, wild types and mutants differed significantly in their sensitivity to local genotypic composition. In all of these cases, the wild types benefited more from an increased frequency of mutants as neighbours than did the mutants themselves.

The likelihood approach used above may have limited power to test for differences in Ψ_{wt} and Ψ_m because there is much more information on wild-type survival than mutant survival (Recall, there are no mutants in type 1 demes and one-third as many mutants in type 2 demes). As an alternative approach, we decided to directly compare survival in type 2 and type 3 demes for wild types and mutants (D_{wt}, D_m, respectively). To estimate survival for this calculation, we used the number of observed adults of each genotype divided by the expected number of eggs placed into that deme for that genotype for the appropriate deme type. The values of D_i were then calculated by taking the logarithm of the ratio between the survival values in type 3 demes to the survival in type 2 demes. A positive D_i value indicated that survival of genotype i in type 3 demes was higher than that in type 2 demes and a negative value indicated the opposite. If changes in the frequency of mutant individuals had similar effects on both wild types and mutants then D_{wt} and D_m should have similar values. These values are plotted in Fig. 5.

The D_i values were analysed in a general linear model including gene, allele (mutant or wild type) and environment as fixed effects. Only the allele term was significant ($F_{1,37} = 56.49$, $P < 10^{-8}$), reflecting the fact that the D_{wt} values were typically greater than the corresponding D_m values. As shown in Fig. 5, the values of D_{wt} were generally positive, indicating that increasing the frequency of mutant individuals increased the survival of wild-type individuals. In contrast, the D_m values were typically closer to zero. A number of these values were negative, which could be due to measurement error of values close to zero or, alternatively, indicate that the survival of mutants decreased when their frequencies increased in the population.

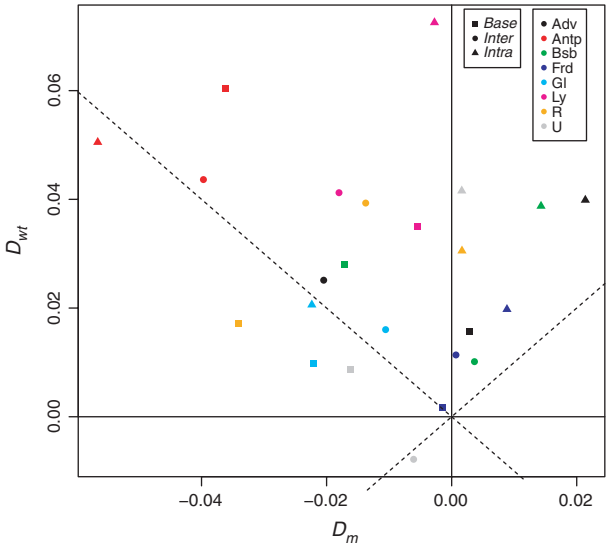

Fig. 5 Sensitivity to the genetic composition of local competitors for wild-type individuals (D_{wt}) and mutant individuals (D_m) for each gene from each density treatment. The dashed line indicates equal sensitivities for both genotypes. Square, circular and triangular points indicate values from the baseline, elevated interspecific and elevated intraspecific environments, respectively.

Discussion

Selection plays a large role in determining the fate of mutations. In subdivided populations, there are two aspects of selection that are important, the strength and the softness of selection (Wallace, 1975; Whitlock, 2002). The strength and the softness of selection both play major roles in determining inbreeding depression, mutation load, genetic variation and the evolution of mating systems (Levene, 1953; Christiansen, 1975; Agrawal & Chasnov, 2001; Whitlock, 2002; Glemin *et al.*, 2003; Roze & Rousset, 2004; Theodorou & Couvet, 2006; Agrawal, 2010). Ecological conditions have the potential to alter either of these aspects of selection, but there have been few empirical studies testing these effects, especially regarding the softness of selection (Whitlock, 2002; Agrawal, 2010; Agrawal & Whitlock, 2010). Using *Drosophila* species as the model system, our study aimed to test several basic predictions related to the effects of resource competition on the strength and softness of selection. Several of our results did not match theoretical predictions suggesting that changing densities may affect more than resource availability.

Competition for resources is quite common in nature (Connell, 1983; Gurevitch *et al.*, 1992). As densities in a population increase, resource limitation is likely to create a stressful environment. We found that increasing both the number of conspecifics and heterospecifics in the population created a more stressful environment. Increasing intraspecific competition had a larger effect than interspecific competition, likely due to the high juvenile mortality of *D. simulans* (data not shown) within our populations. Their deaths presumably result in more resources *per capita* for *D. melanogaster* in these

treatments compared to the treatments with additional *D. melanogaster*.

Unlike other forms of stress, intraspecific competition for resources may have predictable effects on the strength of selection (Agrawal & Whitlock, 2010). Under intense competition for local resources, high quality genotypes that develop slightly faster may render the environment less favourable to low quality genotypes by reducing the amount of resources available to the latter. The difference in fitness between these genotypes effectively increases through intraspecific competition. The effects of interspecific competition are less predictable however and will not necessarily have the same effect.

Although we found no statistically significant effect of competition on selection strength, there was a trend towards stronger selection under both types of competition, especially intraspecific. A similar study by Laffa fian *et al.* (2010) investigating the effects of intraspecific competition on the same system found that their high-density environment significantly increased the strength of selection. Their experiment compared environments that differed in densities by a factor of four, whereas our environments only differed by a factor of two. It is possible that our high-density environments were not competitive enough for a statistically significant effect to be detected. Although selection tends to be slightly stronger with intraspecific competition than interspecific competition in our study, it is not possible to attribute this to competition type because 'stress' also differs between these treatments, that is, our intraspecific competition treatment (*Intra*) was more stressful than our interspecific competition treatment (*Inter*).

As highlighted by Wallace (1975), competition for local resources is crucial for the concept of soft selection. As the intensity of competition increases from elevated densities, intuition suggests that the absolute fitness of an individual would depend more strongly on the genetic quality of its local competitors, that is, selection would become softer due to limiting resources. We found only some support for this prediction. While we tended to observe softer selection with intraspecific competition, the difference in the softness of selection between the baseline environment and one with increased intraspecific competition highly depended upon the gene being examined. However, variation among genes in our study needs to be interpreted with caution; different genes were assayed at different time points so block effects may contribute to the differences in estimates of selection among genes. This is different from the study by Laffafian *et al.* (2010), which assayed multiple genes simultaneously, and so they were able to conclude that genes responded differently to changes in intraspecific competition.

Population subdivision creates a situation where mutant individuals are more likely to compete against other mutants for local resources (Whitlock, 2002). This effectively shelters mutants from selection since there is little difference in competitive abilities between neighbours. Interspecific competition, however, should reduce this sheltering effect because mutant individuals are less competitive than wild-type individuals when facing heterospecifics. Interspecific competitors should tend to equalize the total competition experienced by individuals in demes where mutants are common vs. rare, effectively hardening selection (Agrawal, 2010). We found no evidence to support this prediction. The softness of selection did not vary significantly between the baseline environment and the environment containing interspecific competitors. This was true for all genes examined except for the *Bsb* gene. Selection on the *Bsb* gene was softer in the environment with hetero specifics, however, which was opposite to the predicted effect.

At best, we have tepid support for the prediction of intraspecific competition making selection softer and no support for the prediction of interspecific competition making selection harder. These predictions are based on rather straightforward arguments supported by simple ecological models (Agrawal, 2010), so why have they failed? The most likely reason is that it is not possible to directly manipulate 'competition', rather we manipulated density. The simple theoretical predictions are derived by imagining a change in a single variable in each case (i.e. 'resource abundance' for the intraspecific competition prediction or 'frequency of interspecific competitors' for the interspecific competition prediction), while leaving all other parameters the same. The simple model (Agrawal, 2010) shows that other variables are key to understanding the softness of selection, including the degree of 'early' juvenile mortality and the amount of resources that are wasted on juveniles that die. It is plausible that increasing total larval density (either by the addition of conspecifics or heterospecifics) changes the environment in numerous ways so that parameters other than just those we intended were altered unpredictably (i.e. the amount and timing of early deaths). In addition, *D. simulans* was a weak competitor (having low survival, results not shown), which may have limited our power to detect an effect of interspecific competition.

One major assumption of the hard/soft selection paradigm is that all genotypes are equally affected by the genetic quality of their local population (Wallace, 1968, 1975; Whitlock, 2002). Our results, however, suggest that this may not be true. In six of the 24 gene-environment combinations examined, the full likelihood model, which allowed for sensitivities to differ between mutant and wild-type individuals, provided a better fit to the observed data than one assuming the same sensitivities for the genotypes. Due to the limited power of the maximum likelihood approach for our data, we further analysed differences in sensitivity by calculating the relative survival across deme types for wild types

and mutants (D_{wt} and D_m, respectively). Relative to mutant individuals, wild-type individuals were generally more sensitive to the genetic composition of the deme ($D_{wt} > D_m$) and benefited more from a decreasingly competitive environment ($D_{wt} > 0$). In other words, wild-type individuals benefit from being surrounded by poor competitors but mutants tend to have low fitness regardless of their neighbours.

The benefit that mutants receive from being locally clustered into demes by population subdivision is reduced if they are less responsive than wild-type individuals to the quality of their neighbours. Population structure does not create a sheltering effect from selection for these mutants and their equilibrium frequencies will be reduced relative to the case where all genotypes were assumed to be equally sensitive (Laffafian *et al.*, 2010). Thus, the mutation load of the metapopulation is reduced under this scenario relative to one where all sensitivities are equal across genotypes.

These results have important implications for other evolutionary processes affected by the softness of selection. The sensitivity of a genotype to its local deme quality determines the extent to which it benefits from being surrounded by lower quality neighbours. The ability to utilize available resources under soft selection allows alleles involved in local adaptation to increase in frequency when rare within heterogeneous metapopulations (Levene, 1953; Christiansen, 1975; Débarre & Gandon, 2011). Most studies regarding protected polymorphisms in heterogeneous environments implicitly assume equal sensitivities among genotypes. However, the extent to which polymorphisms are protected will change when genotypes respond differently to their local deme quality. Differences in sensitivity among genotypes will determine how well the rare genotype can benefit from the selective deaths of common genotypes and *vice versa*.

Studies of social evolution have also invoked processes involving soft selection (Gardner and West, 2006; Lehmann & Rousset, 2010; Van Dyken, 2010). The benefit that altruists gain in local clusters is counteracted by an increase in the intensity of kin competition under soft selection (Queller, 1992; Taylor, 1992). This type of local density regulation prevents the evolution of altruism because demes containing altruists will not attain higher fitness than demes containing selfish individuals. Differential sensitivities among genotypes will likely change the outcome for the evolution of altruism as it will affect the extent to which selfish and altruistic individuals can benefit from the presence of altruists.

Our study tested several predictions relating the effects of competition on the strength and softness of selection. However, support for these predictions was limited, either due to a lack of power or because the differences among our density treatments caused environmental differences other than just competitive intensity. We also found evidence that genotypes experienced different sensitivities to the local quality of competitors. This suggests that the hard/soft selection paradigm may not accurately reflect how competition occurs even under simple laboratory conditions. Although evolutionary biologists frequently speculate (often with unwarranted certainty) about how selection is affected by ecological conditions, testing these claims often reveals surprising gaps in our knowledge.

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