

# The Effects of Selective History and Environmental Heterogeneity on Inbreeding Depression in Experimental Populations of *Drosophila melanogaster*

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**ABSTRACT:** Inbreeding depression varies considerably among populations, but only some aspects of this variation have been thoroughly studied. Because inbreeding depression requires genetic variation, factors that influence the amount of standing variation can affect the magnitude of inbreeding depression. Environmental heterogeneity has long been considered an important contributor to the maintenance of genetic variation, but its effects on inbreeding depression have been largely ignored by empiricists. Here we compare inbreeding depression, measured in two environments, for 20 experimental populations of *Drosophila melanogaster* that have been maintained under four different selection regimes, including two types of environmentally homogeneous selection and two types environmentally heterogeneous selection. In line with theory, we find considerably higher inbreeding depression in populations from heterogeneous selection regimes. We also use our data set to test whether inbreeding depression is correlated with either stress or the phenotypic coefficient of variation (CV), as suggested by some recent studies. Though both of these factors are significant predictors of inbreeding depression in our study, there is an effect of assay environment on inbreeding depression that cannot be explained by either stress or CV.

**Keywords:** inbreeding depression, stress, balancing selection, environmental heterogeneity, inbreeding load, variance in fitness.

## Introduction

Inbreeding depression, the reduction in mean fitness (and other related traits) in those progeny whose parents are related, is a phenomenon manifested nearly universally in outbreeding taxa. As inbreeding depression has the potential to dramatically influence fitness of individuals, population genetic structure, and population dynamics, it is

considered a major ecological and evolutionary force whose study is of central relevance to those working in the fields of plant and animal breeding, medical genetics, conservation biology, and evolutionary ecology. Since Darwin (1868, 1876), inbreeding depression has been measured in a wide variety of species and environments and we now know that it can vary considerably in magnitude (Lynch and Walsh 1997; Keller and Waller 2002). Understanding the source(s) of this variation has become a central focus in modern studies of inbreeding depression. Here we examine an important, but largely untested, prediction that differences in selective history among populations contribute to this variation. This prediction arises from the simple idea that different selective regimes result in different types of segregating variation.

The progeny of related parents are more genetically homozygous than progeny produced from random mating (i.e., outbreeding) and this results in inbreeding depression if homozygotes are less fit (on average) than heterozygotes. This difference in fitness between genotypes can be due to either heterozygote advantage or (partial) recessivity of deleterious alleles (i.e., “overdominance” and “dominance” explanations, respectively; Wright 1977). Of course, a locus can contribute to inbreeding depression only if there is segregating allelic variation of one of these two types. Thus, the nature of segregating variation is critical for inbreeding depression.

Much past work on the genetic basis of inbreeding depression has focused on distinguishing between the dominance and overdominance hypotheses, and it is generally believed that the former makes the biggest contribution to inbreeding depression (Charlesworth and Charlesworth 1999). Though deleterious recessives are thought to be responsible for most inbreeding depression, there are sev-

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eral possible reasons why such variants would be segregating in populations. The simplest explanation is that deleterious recessive alleles are present in populations because of the constant influx of new mutations (i.e., mutation-selection balance). Although there is little doubt that mutation-selection contributes to inbreeding depression, the magnitude of its contribution has not been firmly established. In fact, mutation-selection balance is inconsistent with the inference from several biometrical studies that indicate inbreeding depression is due to deleterious recessives segregating at relatively high frequencies (Charlesworth and Hughes 1999; Kelly and Willis 2001; Kelly 2003; Charlesworth et al. 2007).

Thus, there is an increasing recognition that processes other than mutation-selection balance must be responsible for preserving the segregating deleterious recessives thought to cause inbreeding depression. It is well known that a variety of forms of selection—including negative frequency-dependent selection, antagonistic pleiotropy, and environmental heterogeneity in selection—can help maintain allelic variation at levels that are higher than those expected under mutation-selection balance (Charlesworth and Charlesworth 2010). If the alleles maintained by selection have deleterious recessive effects on some fitness components in some environments, then such alleles can make a substantial contribution to inbreeding depression.

Here we focus on environmental heterogeneity in selection. If alternative alleles are favoured in different environments (“environmental antagonism”), genetic variance in fitness is increased because heterogeneous selection extends the persistence time of such alleles and possibly maintains a polymorphism indefinitely. Importantly, the alleles that are most easily maintained by environmental heterogeneity are those whose deleterious effects in the “wrong” environment are recessive (Levene 1953; Dempster 1955; Gillespie 1973, 1974; Felsenstein 1976). This means the alleles that are most likely to be maintained by heterogeneous selection have precisely the type of dominance that causes inbreeding depression. While loci with environmentally antagonistic alleles may be numerically rare across the genome, they can contribute substantially to inbreeding depression because polymorphisms at such loci can be maintained at much higher levels compared to loci with unconditionally deleterious alleles that are maintained only by mutation-selection balance.

Environmental heterogeneity can also contribute to inbreeding depression through conditionally neutral alleles. For example, consider a locus that is selectively constrained in environment A but neutral in environment B. For a population persisting only in environment A, a mutant allele would be maintained at a very low level because of the constant selection against it. For a population evolving

in both environments A and B, the mutant allele experiences less selection on average than it would under constant selection and is consequently able to reach higher frequencies. Thus, it can make a bigger contribution to inbreeding depression (assuming it is partially recessive), at least when measured in environment A.

Because of environmentally antagonistic variation and/or conditionally neutral variation, we can expect that populations that evolved in selective regimes with environmental heterogeneity express more inbreeding depression than those that evolve under constant selection. Despite its potential importance as a source of variation in inbreeding depression, environmental heterogeneity has received relatively little attention. One notable exception is the work of Ronce et al. (2009) who used a Fisherian landscape model to show how inbreeding depression is expected to differ in populations with a history of spatially heterogeneous selection. The assumptions underlying that model differ from some of the logic described above, but nevertheless, their work illustrates the potential importance of spatial heterogeneity to the study of inbreeding depression (see “Discussion”).

In addition to a population’s selective history, inbreeding depression can also depend on the specific environment in which it is assayed. A fairly large number of studies have measured inbreeding depression for a given population in two (or more) environments, often finding considerable differences between assay environments. Meta-analyses indicate that variation in inbreeding depression is positively correlated with environmental stress (where “stress” is operationally defined as a reduction in mean absolute fitness); that is, inbreeding depression tends to be greater in “stressful” environments than in “benign” environments (Armbruster and Reed 2005; Fox and Reed 2010). However, the reasons for this pattern remain unclear.

A positive correlation between the magnitude of inbreeding depression and the “stressfulness” of the environment would be expected if selection against deleterious mutations tends to be stronger in stressful environments. However, literature surveys examining selection on new mutations in different environments have yielded little support for this idea (Martin and Lenormand 2006; Agrawal and Whitlock 2010). An alternative hypothesis for the stress-inbreeding depression correlation is based on an interaction between selective history and test environment (Agrawal and Whitlock 2010). In many (though not all) cases, the “benign” test environment is one to which the population has adapted whereas the “stressful” test environment is one in which the population has little or no selective history. This difference between environments with respect to previous selective exposure leads to a bias in the type of segregating variation relevant to the ex-

pression of inbreeding depression. In effect, stress often results from a mismatch between selective history and the test environment. Alleles that are strongly selected in the standard environment will be very rare because of past selection, regardless of their effects in the novel environment. In contrast, alleles that are strongly selected in the novel environment but neutral or very weakly selected in the standard environment will not have been eliminated by past selection and so will be able to add substantially to inbreeding depression measured in the novel environment. This logic suggests that variation in inbreeding depression among environments is better understood with respect to the selective histories of populations in various environments rather than some intrinsic property of the environments themselves. Specifically, this logic predicts that for a given test environment, inbreeding depression will tend to be lower in populations with a selective history in that environment than those for which the environment is more novel.

In this study, we examined the effects of evolving under either heterogeneous or homogeneous environmental conditions on the subsequent expression of inbreeding depression and whether this depended on the specific environment in which fitness was assayed. Specifically, we measured inbreeding depression in 20 experimental populations of *Drosophila melanogaster* evolved under one of four different selective regimes and measured in each of two different test environments. The four selective regimes were: (i) a constant cadmium-supplemented environment, (ii) a constant salt-supplemented environment, (iii) a temporally varying environment, and (iv) a spatially varying environment. We use these populations to address two specific questions. First, is inbreeding depression greater in populations from environmentally variable regimes than in constant selective regimes? Second, in a given environment, is inbreeding depression lower for the populations that experienced constant selection in that environment rather than constant selection in the alternative environment? In addition, we use this data set to test for other patterns recently discussed in literature. Specifically, we ask whether inbreeding depression in our data set is correlated with either stress or the phenotypic coefficient of variation (Fox and Reed 2011). Finally, we test whether either of these putative explanatory variables is sufficient to account for the differences in inbreeding depression between test environments.

## Material and Methods

### *Establishment of Initial Genetically Variable Fly Stocks*

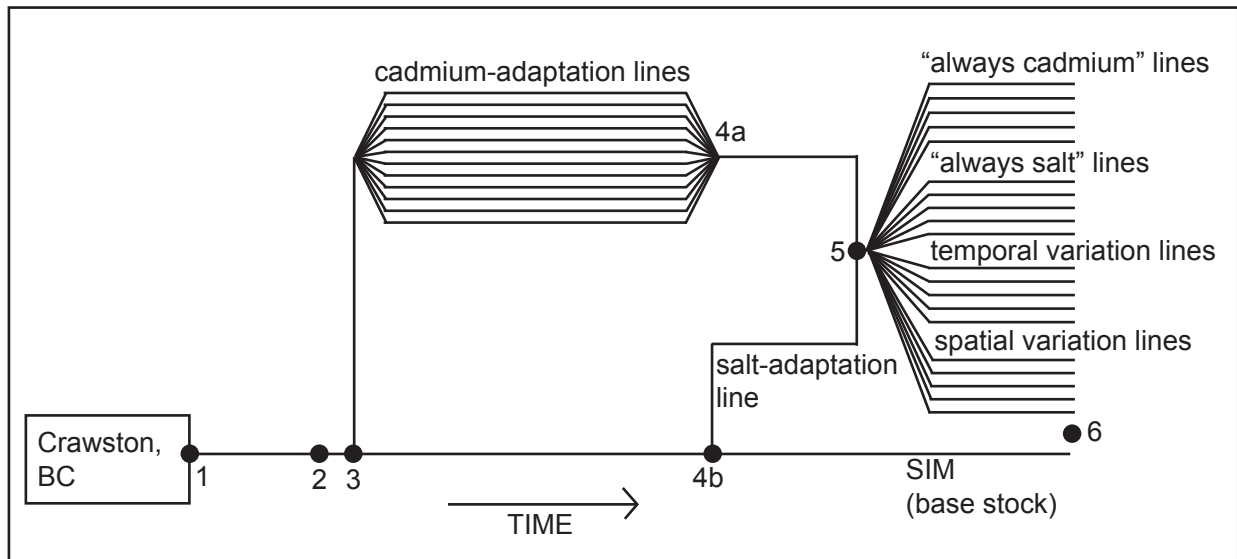
Figure 1 illustrates the origin and selective histories of all stocks described herein. A stock population of *Drosophila*

*melanogaster* was generated from a sample of mated female flies collected from an orchard near Cawston in the Similkameen Valley, British Columbia, in September 2005. This stock, “SIM,” was subsequently expanded to a large population size (~2,000–4,000 adults) by S. Yeaman at the University of British Columbia (see Yeaman et al. 2010). In June 2007, a large sample was transferred to the University of Toronto, where it has been maintained in population cages under constant conditions (25°C, 70% relative humidity, 12L : 12D photoperiod) with overlapping generations on a standard cornmeal/sugar/killed-yeast/agar medium (hereafter referred to as “medium”).

From the SIM stock, 12 replicate populations (each with a population size of at least 1,000 adults generation<sup>-1</sup>) were generated (by C. C. Spencer), and reared in experimental environments where the standard medium had been supplemented with cadmium. This experimental evolution began in July of 2007, and in April 2008 flies from the replicate populations were pooled to create a single population. At the same time, a replicate stock of SIM was created (by A. Wang) that was subsequently reared on medium that had been supplemented with salt. During the course of the experimental evolution, the amount of salt or cadmium added to the medium was progressively increased, such that by the start of the experimental evolution project (described below) the concentrations had reached 33 mg L<sup>-1</sup> and 75 μg L<sup>-1</sup>, respectively, and the populations had become adapted to their specific environmental conditions (C. C. Spencer and A. Wang, unpublished data).

In October of 2009, 448 adult flies of each sex were collected as they eclosed as virgins from both the cadmium and salt-adapted populations. Males and females from different populations were mated en masse, and the eggs produced from these crosses were cultured at a density of 150–200 eggs vial<sup>-1</sup> on standard medium and allowed to develop in the incubator for 14 days. At that time, the eclosed adult F1 offspring were collected. A total of 350 sets of flies, consisting of 4 males and 4 females produced from matings of cadmium-adapted sires to salt-adapted dams, and 4 males and 4 females produced from matings of salt-adapted sires to cadmium-adapted dams were assembled. These sets of flies were haphazardly grouped into 20 “populations” of 14 sets of flies each, which were then assigned to one of four experimental treatments (see below), resulting in five replicate populations (each with adult  $N = 448$ ) per treatment.

In each generation of subsequent culture, populations were maintained on a discrete 14-day culture schedule in vials. In each generation, cultures were initiated (on day 1) by placing replicate sets of 150–200 eggs into 14 vials containing 10 mL of treatment-specific medium (see below). These vials were then incubated for 11.25 days. At



**Figure 1:** Schematic diagram the origin and subsequent history of the base (*SIM*) and offshoot populations of *Drosophila melanogaster* laboratory “system” used in this experiment. Each branch represents a population (branch lengths not to scale), and numbers indicate key events. 1, *SIM* population generated from a sample of mated females collected from an orchard near Cawston, British Columbia (September 2005); 2, approximately 45 generations later (June 2007), a sample of *SIM* was transferred to the University of Toronto; 3, shortly thereafter (July 2007), 12 replicate populations were generated and reared on cadmium-supplemented medium; 4a, in April 2008, flies from the replicate populations (that had experienced ~20 generations of selection) were pooled to create a single population that was cultured on cadmium-supplemented medium; 4b, at the same time, a population was derived from *SIM* that was reared on medium that had been supplemented with salt; 5, after 36 generations (October 2009), flies from cadmium-adapted and salt-adapted lines were mixed to create 20 replicate populations that were assigned to one of four different selective treatments; 6, inbreeding depression for survivorship and female fecundity in these experimentally evolved populations was measured on salt-supplemented using flies collected during the eighteenth generation of selection and on cadmium-supplemented medium using flies collected during the nineteenth generation of selection.

that time, all eclosed adults were lightly anesthetized with  $\text{CO}_2$  and mixed among replicate vials. Any individuals failing to complete development by this time are automatically excluded from contributing to the next generation. This mimics natural situations where environmental deterioration or seasonal changes impose hard limits on development time. From the pool of eclosed adults, sets of 16 male and 16 females were collected according to treatment-specific protocols (see below) and placed into 14 new vials containing fresh medium, whose surface had been seeded with a limited amount of live yeast ( $10 \text{ mg vial}^{-1}$ ). These second set of vials were then returned to the incubator for an additional 2 days during which time adults compete for food and/or mates. Next, the flies were transferred to a final set of 14 “oviposition” vials containing fresh medium but no live yeast, for a period of 18 h before being discarded. Finally the eggs laid in these last vials were culled by hand to a density of  $150\text{--}200 \text{ eggs vial}^{-1}$  and were used to found the next generation of culture.

#### Experimental Treatments

The populations used here are part of a comprehensive ongoing study, designed to examine the role of environmental heterogeneity on the maintenance of genetic variation. Populations were either cultured in a manner in which the environment was homogenous or heterogeneous. In total, there were four different selective regimes used for this study. In the homogenous treatments, (“always cadmium” and “always salt”), the flies were cultured such that they experienced only one of the two types of supplemented medium (cadmium or salt, respectively). In the two heterogeneous treatments used here, populations experienced both cadmium- and salt-supplemented food, but in different ways. In the “temporal variation” treatment, flies were cultured in alternating generations on either cadmium- or salt-supplemented medium. In each generation of the “spatial variation” treatment, 7 (of 14) vials contained cadmium-supplemented medium and the other 7 vials contained salt-supplemented medium. We imposed a “soft” selection regime (*sensu* Wallace 1968,

1975) by ensuring that an equal number of eclosed males and females were obtained from both the salt and cadmium natal vials on day 11.25, before being mixed and distributed among vials.

#### *Inbreeding Depression Assays*

Inbreeding depression for survivorship and female fecundity was measured twice in each of the populations: once on salt-supplemented medium, and once on cadmium-supplemented medium, with the former assay using flies collected during the eighteenth generation of selection (June 2010) and the latter assay using flies obtained from the nineteenth generation. Both sets of assays began by first rearing flies for two generations on nonsupplemented medium (to avoid potentially confounding maternal effects). In the first generation of this culture, flies were cultured according to the standard schedule described above. In the second generation of flies, virgin females and males were collected as they eclosed as adults on days 9–10 of the culture cycles and randomly placed into single female-male pairs to found 32 independent lineages per population. Eggs from each lineage were collected as usual on the fourteenth day of the culture cycle, and the offspring of both sexes were collected as virgins as they eclosed 9–10 days later and stored separately.

From each of the 32 lineages derived in each population, we created two sets of “families” by housing a female either with her brother (“inbred family”) or with an unrelated male from one of the other lineages from same population (“outbred family”) following a round-robin design. These single female-male pairs were housed for 3 days in test tubes containing 5 mL of supplemented medium and 625  $\mu\text{g}$  of live yeast (applied to the surface of the medium) before being transferred to a new test tube containing supplemented medium whose surface had been scored to facilitate egg-laying by females. Eighteen hours later, adults were discarded, and the eggs in each test tube were counted before being returned to the incubator. Thirteen days later, all eclosed adults were removed from the test tubes, counted (to measure survivorship), and a single adult female was placed into a new test tube containing supplemented medium with a scored surface. These test tubes were incubated for 18 h before females were discarded, and the eggs that had been laid were counted (to measure fecundity).

The mean egg-to-adult survivorship and female fecundity of inbred and outbred lineages were calculated for each of the 20 populations on both medium types. We calculated inbreeding load as  $B = [\ln(\text{mean outbred performance}) - \ln(\text{mean inbred performance})]/F$ , where  $F$  is the inbreeding coefficient, which is equal to 0.25 in our study. Inbreeding load, a commonly used measure of the

effects of inbreeding (Charlesworth and Charlesworth 1987; Fox and Reed 2011) is related to inbreeding depression ( $\delta$ ) by  $\delta = 1 - W_{\text{Inbred}}/W_{\text{Outbred}} = 1 - e^{-FB}$ .

#### *Data Analysis*

All of our analyses were performed in R, version 2.12.2 (R Core Development Team 2011). In our first set of analyses, we examined inbreeding load from each assay environment separately. In each case, we first tested for significant variation among different selective regimes. Specifically, in R we applied “anova” to the model  $\text{lm}(B_x \sim -1 + \text{selective regime})$ , where  $B_x$  is the inbreeding load in assay environment  $X$ ; the intercept term ( $-1$ ) was included to obtain the proper contrasts as described below. We performed two contrasts by using the “glht” function. First, we compared the two heterogeneous selection treatments with the two homogeneous selection treatments. Second, we compared the two homogeneous selection treatments with one another. We did not perform the third orthogonal contrast comparing the two heterogeneous treatment because we had no strong a priori prediction.

In our second set of analyses, we used mixed model regression analyses to analyze all 40 measures of inbreeding depression in survival (20 populations  $\times$  2 test environments). Our measures of survival, which are based on many individuals per family, are much less noisy than our measures of fecundity, which are based on the number of eggs laid by a single daughter per family. For this reason, we have considerably less power to examine inbreeding load in fecundity so we perform these analyses only on the survivorship data. Our basic model (model 1) included selective treatment and test environment as fixed effects and population, nested within selective treatment as a random effect. Following recent themes in the literature regarding variation in inbreeding depression, we then examined models that included stress (Fox and Reed 2011) and the coefficient of variation, CV (Waller et al. 2008).

The “stress” of population  $i$  in environment  $j$  was measured as  $1 - (\text{mean outbred survivorship of population } i \text{ in environment } j) / (\text{best survivorship of any population in either environment})$ . As discussed by (Fox and Reed 2011) there is a potential autocorrelation problem because both  $B$  and stress depend on outbred survivorship (i.e., measurement error in outbred survivorship affects estimates of  $B$  and stress in opposite ways). However, they noted this autocorrelation effect is quite small unless sample sizes are very small and, more importantly, is in the opposite direction to the expected (and observed) effect.

Waller et al. (2008) argued that the phenotypic variance of outbred types might serve as a better predictor of inbreeding depression across environments than stress. Be-

cause  $CV = (\text{standard deviation})/\text{mean}$ , estimates of the CV should be negatively autocorrelated with estimates of  $B$  simply due to measurement error of the mean outbred fitness. Thus, the autocorrelation is in the opposite direction to the hypothesized (and observed) effect. In the models reported here,  $CV^2$  was included rather than CV because the former had a better fit.

Additional models were created by adding to the basic model. In model 2 stress was added, in model 3 we added  $CV^2$  to the basic model, and in model 4 both stress and  $CV^2$  were added to the basic model. Each model was analyzed using the “lme” function with the “ML” method to allow for comparison of likelihood scores among models with different fixed effects. Formally, model 1 was generated by the statement “lme(fixed = B ~ test environment + selective regime, random = ~1 | population ID / selective regime, method = “ML”).” Other models were generated by adding the appropriate terms to the “fixed” statement. Akaike Information Criterion (AIC) scores were used in comparing between nonnested models (e.g., model 2 vs. 3).

## Results

### *Comparison of Inbreeding Loads within Each Environment*

Mean outbred and inbred survivorships and inbreeding loads  $B$  are shown in figure 2. Fecundity measures and corresponding inbreeding loads are shown in figure 3. Both figures illustrate a clear pattern of adaptation; for a given test environment, populations with a selective history in that same environment performed better than those populations without a selective history in that test environment. A one-way ANOVA was used to test for differences in inbreeding loads among the four treatments using survivorship and female fecundity data collected on both salt-supplemented or cadmium-supplemented medium. Inbreeding load differed significantly across the treatments in all analyses that were performed (survivorship on salt:  $F_{3,16} = 10.32$ ,  $P = .0005$ ; survivorship on cadmium:  $F_{3,16} = 12.34$ ,  $P = .0002$ ; fecundity on salt:  $F_{3,16} = 4.19$ ,  $P = .023$ ; fecundity on cadmium:  $F_{3,16} = 3.36$ ,  $P = .045$ ).

For each fitness component in each environment, we used planned contrasts to test two major questions: (i) Do populations from heterogeneous selection regimes show greater inbreeding load than populations from homogeneous selection regimes (i.e.,  $\Delta_{\text{Het-Hom}} > 0$ )? (ii) Between the two homogeneous selection regimes does the population adapted to the test environment show less inbreeding load than the nonadapted one (i.e.,  $\Delta_{\text{Adapted-Not}} < 0$ )? The results of these contrasts are given in table 1.

We found strong support for the prediction of higher

inbreeding load in populations from the heterogeneous selection regimes. The contrast was significant for survival in both test environments and for fecundity in the cadmium test environment. For fecundity in the salt test environment, the contrast was in the predicted direction but was marginally nonsignificant ( $P = .075$ ).

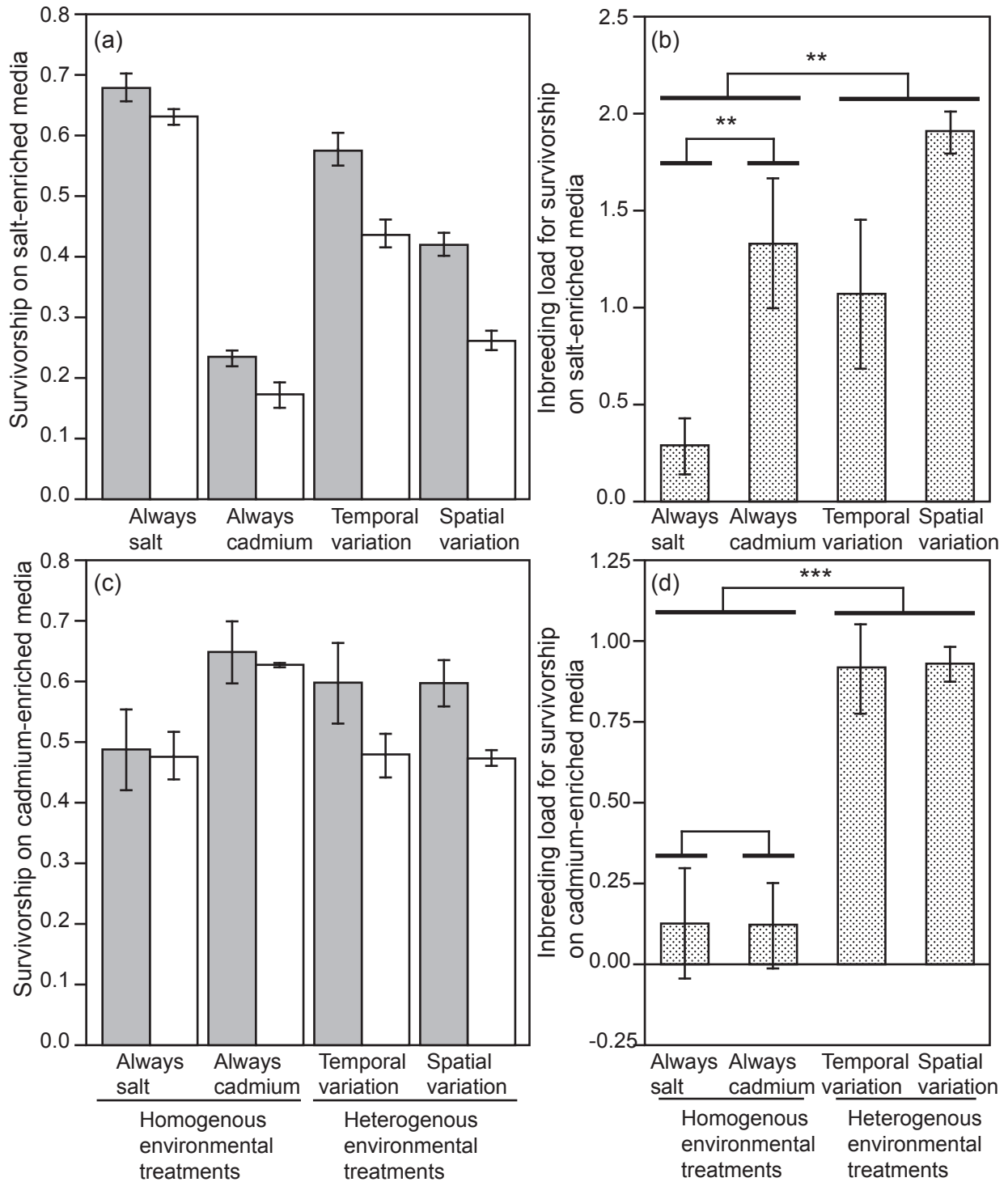
The evidence for lesser inbreeding loads in populations adapted to a particular environment was less conclusive. In the salt test environment, the populations adapted to salt had significantly less inbreeding load than the populations adapted to cadmium for both survivorship and fecundity. However, in the cadmium test environment, there was no difference between the cadmium- and salt-adapted populations for either fitness component.

### *Statistical Models of Inbreeding Load*

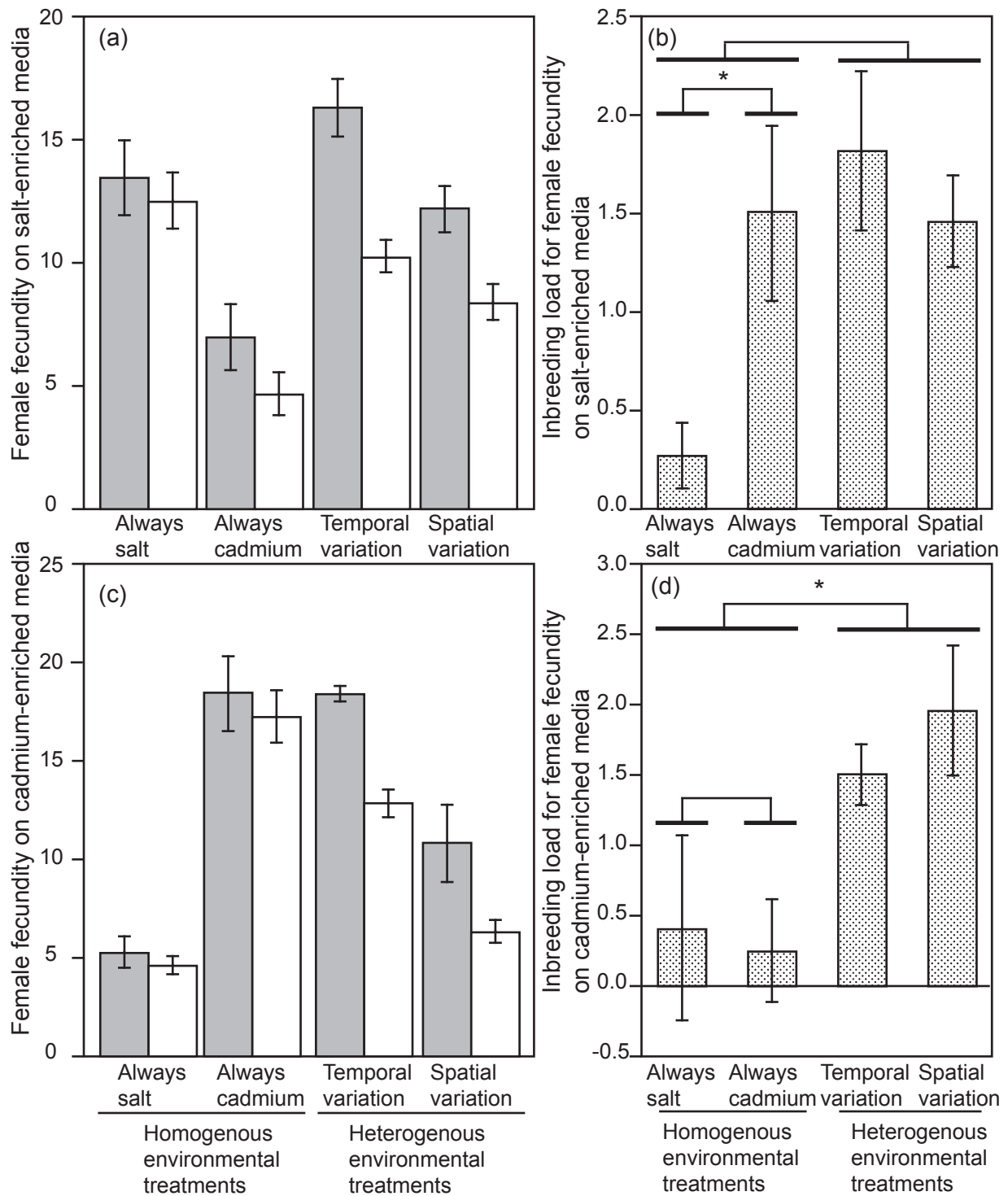
We examined a series of statistical models of inbreeding load for survival (table 2). Our basic model (model 1) includes selective treatment and test environment as fixed effects and population nested with selective treatment as a random effect. The addition of either stress (model 2 vs. 1:  $\chi^2 = 8.80$ ,  $df = 1$ ,  $P = .003$ ) or  $CV^2$  (model 3 vs. 1:  $\chi^2 = 9.99$ ,  $df = 1$ ,  $P = .002$ ) significantly improves the likelihood (fig. 4). Of the four models examined, the best model according to AIC scores is the one including  $CV^2$  (model 3) rather than the full model including both stress and  $CV^2$  (model 4). In this data set,  $CV^2$  and stress are strongly correlated ( $r = 0.90$ ) so there is little power to differentiate between them as explanatory variables (note the similar AIC scores of models 2 and 3). Finally, we found that test environment remains significant even when including stress or  $CV^2$  in the model (e.g., model 3 vs. a reduced version of this model in which the test environment term is removed:  $\chi^2 = 6.31$ ,  $df = 1$ ,  $P = .012$ ).

## Discussion

Inbreeding depression reflects both the dominance relationships ( $h$ ) and selective effects ( $s$ ) of alleles contributing to the standing genetic variation in fitness. Classically, the amount of environmental heterogeneity over a population's selective history is thought to be important in determining its level of segregating variation for fitness (see Yeaman et al. 2010), but this aspect of selective history has received very little attention in the context of inbreeding depression. Here we investigated the role of a selective history involving environmental heterogeneity on the magnitude of inbreeding depression as assessed in two environments. Both selective history and assay environment had strong effects on the expression of inbreeding depression.



**Figure 2:** Effects of inbreeding and environment on survivorship of *Drosophila melanogaster* cultured under four different selective regimes. *Left column*, survivorship in “outbred” families (filled bars) and “inbred” families (open bars); *right column*: inbreeding load [ $\ln(\text{mean outbred survivorship}) - \ln(\text{mean inbred survivorship})$ ]/0.25]; *top row*: performance on salt-supplemented medium; *bottom row*: performance on cadmium-supplemented medium. Data expressed as mean  $\pm$  SEM,  $n = 5$  per column. Horizontal bars indicate contrasts described in table 1; two asterisks,  $P \leq .01$ , and one asterisk,  $P \leq .001$ .



**Figure 3:** Effects of inbreeding and environment on female fecundity of *Drosophila melanogaster* cultured under four different selective regimes. *Left column:* fecundity in “outbred” families (filled bars) and “inbred” families (open bars); *right column:* inbreeding load [ $\ln(\text{mean outbred fecundity}) - \ln(\text{mean inbred fecundity})$ ]/0.25]; *top row:* performance on salt-supplemented medium; *bottom row:* performance on cadmium-supplemented medium. Data expressed as mean  $\pm$  SEM,  $n = 5$  per column. Horizontal bars indicate contrasts described in table 1; one asterisk,  $P \leq .05$ .



**Table 1:** Planned contrasts of inbreeding load in survivorship and female fecundity based on data collected on cadmium-supplemented and salt-supplemented medium

Trait	Test environment	More inbreeding load in populations from heterogeneous selection regimes, $\Delta_{\text{Het-Hom}} > 0?$			Less inbreeding load in populations adapted to test environment, $\Delta_{\text{Adapted-Not}} < 0?$		
		Estimate (SE)	<i>t</i> value	<i>P</i>	Estimate (SE)	<i>t</i> value	<i>P</i>
Survival	Cd	1.59 (.26)	6.08	$3.2 \times 10^{-8}$	-.00 (.19)	-.02	1
	NaCl	1.36 (.42)	3.25	.010	-1.04 (.30)	-3.51	.006
Fecundity	Cd	2.81 (.91)	3.09	.014	-.16 (.64)	-.25	.96
	NaCl	1.50 (.67)	2.25	.075	-1.24 (.47)	-2.63	.035

### *Selective History and the Genetic Basis of Inbreeding Depression*

Selective history affects the types of alleles expected to underlie standing genetic variation in fitness. Traditionally, most studies of inbreeding depression have focused on two possible sources of genetic variation in fitness, heterozygote advantage and mutation-selection balance, with most studies supporting the latter over the former (Charlesworth and Charlesworth 1999). While it is often acknowledged that other forms of balancing selection may contribute to inbreeding depression, these have received comparatively little attention. Recently, several authors have drawn attention to balancing selection in the form of environmental heterogeneity as a potentially important contributor to inbreeding depression (Epinat and Lenormand 2009; Ronce et al. 2009; Cheptou and Donohue 2011).

Few, if any, natural populations exist under constant conditions; environments inevitably vary over space and/or time. Environmental heterogeneity may be important for two reasons. First, environmental heterogeneity will result in weaker selection against deleterious alleles that are only selected in one environment, so they are expected to be at a higher frequency than when they experience constant negative selection. Second, balancing selection can maintain environmentally antagonistic polymorphisms. Moreover, the alleles most likely to be maintained by antagonistic environmental selection are those whose deleterious effects are recessive (Levene 1953; Dempster 1955; Gillespie 1973, 1974; Felsenstein 1976), which means they will be able to contribute to inbreeding depression.

Because real populations differ in the extent to which they experience environmental heterogeneity, we should expect variation in inbreeding depression to correlate with selective history. We found strong support for this idea in our experimental populations coming from different selective regimes. Populations from heterogeneous selection regimes had higher inbreeding loads than populations from homogeneous selection regimes in all four comparisons (for survivorship and fecundity, measured in each

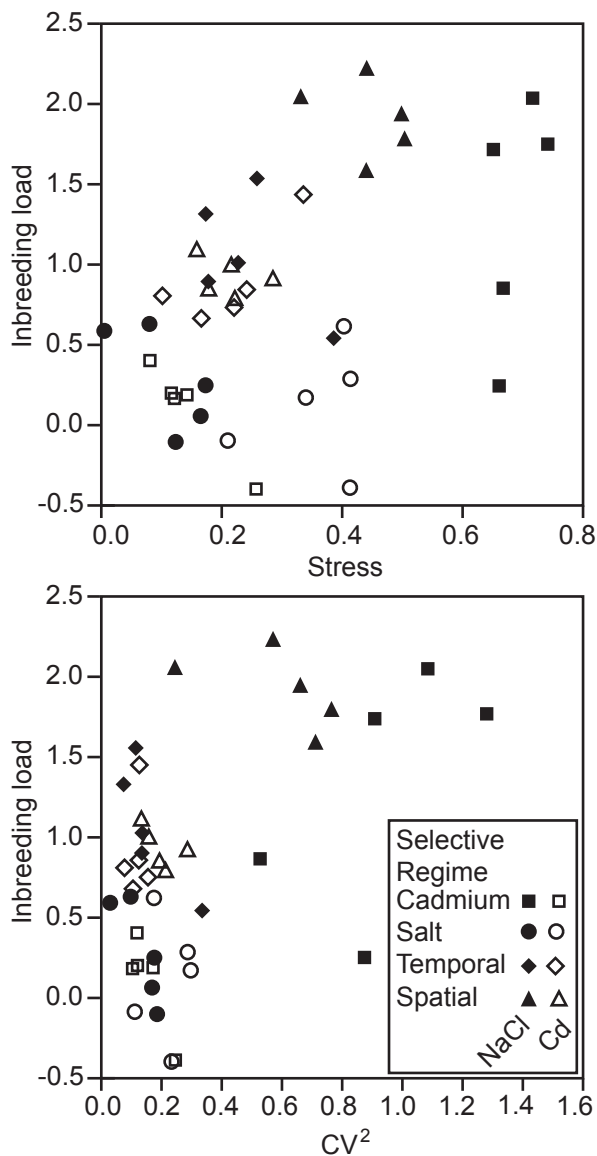
of two environments), statistically significantly so in three of the four.

While our results show inbreeding depression is greater in populations with heterogeneous populations, we cannot definitively determine whether this is due to alleles maintained by environmentally antagonistic balancing selection or conditionally deleterious alleles. Environmentally antagonistic alleles provide a more parsimonious explanation for the higher average levels of inbreeding depression observed in the heterogeneously selected populations compared to the homogeneously selected populations. Because balancing selection can maintain very high levels of polymorphism, even a few such loci can substantially increase the total inbreeding depression. Moreover, it is not clear that conditionally neutral alleles should result in more inbreeding depression for the heterogeneously selected treatments than the homogeneously selected treatments when the contrast involves consideration of both adapted and nonadapted types of homogeneous treatments for a given assay environment (as was done here). For example, alleles that are conditionally deleterious in cadmium (and neutral in salt) are expected to be relatively common in the “always salt” populations but very rare in the “always cadmium” population. When assayed in cadmium, inbreeding depression due to cadmium-specific deleterious

**Table 2:** Statistical models of inbreeding load in survivorship

Model	CV <sup>2</sup>	Stress	log L	df	AIC
1	...	...	-22.1	8	60.3
2	...	Y	-17.7	9	53.5
3	Y	...	-17.1	9	52.3
4	Y	Y	-17.0	10	54.0

Note: Model 1 includes selective treatment and test environment as fixed effects and population, nested within selective treatment as a random effect. All other models include these terms, plus one or more additional terms as indicated by a “Y” in the appropriate column. For each model, we report the log likelihood (log L), the degrees of freedom (df) and the Akaike Information Criterion (AIC) score. CV = coefficient of variation.



**Figure 4:** Inbreeding load, stress, and  $CV^2$  of survivorship in *Drosophila melanogaster* cultured under four different selective regimes, and measured on both salt-supplemented and cadmium-supplemented media. Inbreeding load in survival is plotted against stress (top) and  $CV^2$  (bottom).

alleles is expected to be high in the former but low in the latter. Heterogeneously selected populations are expected to harbor such alleles at frequencies intermediate between the two homogeneous treatments and thus express an intermediate level of inbreeding depression. Instead, we found the highest levels of inbreeding depression in the heterogeneous treatments, which is instead suggestive of environmentally antagonistic alleles maintained by balancing selection in these treatments.

Nevertheless, we suspect that conditionally neutral alleles are also important. In fact, such alleles were the impetus behind our prediction regarding the effect of adaptation on inbreeding depression. In each test environment (and for each life-history trait), we asked whether the populations adapted to that environment had less inbreeding depression than the populations adapted to the alternative environment. We expected this would be so because past selection will have reduced the frequency of alleles with environment-specific deleterious effects only from those populations having evolved in that environment. The evidence here was less clear. When evaluated in the salt environment, levels of inbreeding load for the always-salt populations were significantly lower (for both survival and fecundity) than for the always-cadmium populations. However, when evaluated in the cadmium environment, levels of inbreeding load for populations from the always-salt and always-cadmium treatments were quite similar, despite the fact that the outbred fitness values differed considerably between these two types of populations. In total, none of our tests yielded evidence opposite to our prediction about the role of adaptation, but only two of the four tests resulted in significant support for the prediction, and both of these were from the same environment. The reason for this asymmetry between test environments is unclear. Either there are few or no cadmium-specific deleterious alleles, or alternatively, such mutations exist but their deleterious effects are additive so they do not contribute to inbreeding depression. Our ancestral cadmium population evolved for  $\sim 2$  years in cadmium before the experiment, whereas our ancestral salt population evolved for  $\sim 1$  year prior to the experiment. For this reason, cadmium-specific deleterious alleles may have been at lower initial frequency than salt-specific deleterious alleles.

In sum, the differences between the homogeneous and heterogeneous treatments seem most consistent with the presence of environmentally antagonistic alleles whereas the differences between the two homogeneous treatments suggests the presence of conditionally neutral alleles. It seems likely that a variety of allelic types contribute to inbreeding depression in our populations, including unconditionally deleterious, conditionally deleterious, and environmentally antagonistic alleles.

#### Comparison to Theoretical Predictions

Recently, Ronce et al. (2009) used a Fisherian landscape model to investigate how environmental heterogeneity might contribute to inbreeding depression. They made two predictions that are relevant to our data. First, they predicted that there should be greater inbreeding depression in populations adapted to a given environment than in

populations adapted to an alternative environment. Additionally, they predicted that populations experiencing gene flow from alternative environments should have less inbreeding depression than ones in which no gene flow occurs. Our data do not match either of these predictions and it is worth considering why this is so.

In their model, fitness is determined by a trait under stabilizing selection. Mutations affect the phenotype additively but dominance for fitness arises because of the curvature of the Gaussian fitness landscape that maps phenotype to fitness. The curvature is more negative close to the optimum than away from it, leading to stronger dominance and thus greater inbreeding depression for adapted populations than for nonadapted populations. In their model, the same trait that is under stabilizing selection in one environment is also under stabilizing selection in the alternative environment but with a different optimum. This assumption of their model precludes the possibility that there are alleles that are selectively neutral in the one environment but deleterious in the other; as discussed above such alleles are potentially responsible for some of the patterns that we observed. In landscape parlance, conditionally neutral alleles would only exist if there were dimensions in which the fitness surface is flat in one environment but under stabilizing selection in another environment. We suspect that their assumption about the absence of such alleles is the primary reason why their prediction for inbreeding depression in adapted versus nonadapted populations is opposite to our predictions and corresponding results.

Ronce et al. (2009) also examined inbreeding depression in a population experiencing migration from a population under selection for a different optimal phenotype (an “island-mainland” scenario). Their prediction of reduced inbreeding depression with environmental heterogeneity stems from the fact that populations experiencing gene flow from another habitat will be further from the optimum than a well-adapted population and this will reduce inbreeding depression because the fitness landscape’s curvature is less negative (and so alleles are less recessive) further from the optimum. It is important to note that the scenario they model is not the same as either of our heterogeneous environmental selection regimes; in particular, the pattern of gene flow between habitats included in their model is only one-way. With bidirectional gene flow (as in our heterogeneous populations), the antagonistic alleles maintained by balancing selection will be more strongly biased towards those alleles with recessive deleterious effects. Moreover, the one-dimensional model used by Ronce et al. (2009) constrains the relationship between dominance and selection ( $h$  and  $s$ ), thus restricting the type of mutations that could be segregating within a population.

While our results do not match the predictions of Ronce et al. (2009), this may be due to some of the underlying assumptions of their model as well as subtle, but important, differences between the scenarios envisioned by them and those being examined here. The model of Ronce et al. (2009) is an important first step in our theoretical understanding of inbreeding depression in different environments but further development is needed.

#### *Stress, CV, and Inbreeding Depression*

There is a strong and growing body of evidence that inbreeding depression increases under stress (Armbruster and Reed 2005; Fox and Reed 2010). Indeed, we observe this effect in our own data set. Recently, Waller et al. (2008) proposed that an additional and possibly better predictor of inbreeding depression than mean absolute fitness (i.e., stress) would be the variance (or CV) in fitness. Likewise, we found strong support for an effect of  $CV^2$  on inbreeding depression in our data set. In our data set, stress and  $CV^2$  are strongly correlated, making it difficult to distinguish between them, though the model with  $CV^2$  performs slightly better than the one with stress.

While both CV and stress have been hypothesized to be positively related to inbreeding depression—a claim supported empirically here and elsewhere (Waller et al. 2008; Fox and Reed 2011)—it is unclear why this should be so. Neither of these ideas are directly formulated in terms of the population genetic parameters that directly underlie inbreeding depression (allele frequency,  $q$ , selection  $s$ , and dominance  $h$  for each gene). However, it is easy to see why CV would be related to these parameters.

In essence, the CV hypothesis amounts to arguing that the amount of variance within a group (among outbred types) should be a good predictor of the amount of variance between groups (between inbred and outbred groups). Like inbreeding depression, genetic variance increases with genic variance ( $pq$ ) and the magnitude of effect ( $s$ ). For example, if  $\bar{s}$  is larger in a new environment, then we expect an increase in genetic variance as well as inbreeding depression in the new environment. If selection remains the same on average but some genes become more strongly selected and others more weakly selected, the genetic variance for fitness can still increase; if those genes that experience higher  $s$  in a new environment tend to be the more polymorphic ones (as expected for alleles that are neutral in their selected environment but are deleterious in the environment), then genetic variance and inbreeding depression will be higher in the new environment. In other words, many of the types of changes that increase the genetic variance among outbred genotypes in a novel environment are also expected to increase inbreeding depression.

Although stress and CV are significant predictors of inbreeding depression, neither can account for all of the “explainable” variation. There is a significant effect of the assay environment on inbreeding depression that is left unaccounted for by the other factors we considered. Specifically, inbreeding depression tends to be greater in the salt environment, even after removing the effect of stress or CV as well as selective history.

A recent survey of studies of selection against new mutations found a number of examples where average selection differed significantly between test environments (Agrawal and Whitlock 2010). In some cases, average selection was significantly stronger in the more stressful environment (Remold and Lenski 2001; Vassilieva et al. 2000), and in other cases the reverse was true (e.g., Kishony and Leibler 2003; Jasnos et al. 2008). In other words, average selection differed significantly between particular environments but not in a way that is reliably predicted by stress. Our result here that selection against inbred types is stronger in one particular environment fits with this theme. However, the theme itself—some particular environments are more selective than others for unknown reasons—is an unsatisfying one that requires explanation. This remains an important challenge for those pursuing a general understanding of inbreeding depression as well as selection more broadly.

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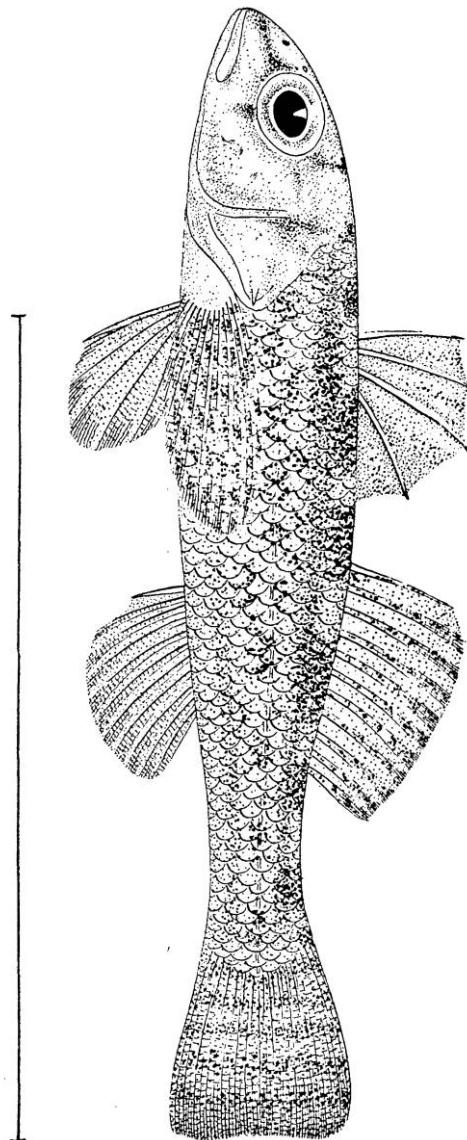
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