doi:10.1111/evo.12527

Variation in the strength of inbreeding depression across environments: Effects of stress and density dependence

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Received June 30, 2014
Accepted September 1, 2014

In what types of environments should we expect to find strong inbreeding depression? Previous studies indicate that inbreeding depression, δ, is positively correlated with the stressfulness of the environment in which it is measured. However, it remains unclear why stress, per se, should increase δ. To our knowledge, only "competitive stress" has a logical connection to δ. Through competition for resources, better quality (outbred) individuals make the environment worse for lower quality (inbred) individuals, accentuating the differences between them. For this reason, we expect inbreeding depression to be stronger in environments where the fitness of individuals is more sensitive to the presence of conspecifics (i.e., where fitness is more density dependent). Indeed, some studies suggest a role for competition within environments, but this idea has not been tested in the context of understanding variation in δ across environments. Using *Drosophila melanogaster*, we estimated δ for viability in 22 different environments. These environments were simultaneously characterized for (1) stressfulness and (2) density dependence. Although stress and density dependence are moderately correlated with each other, inbreeding depression is much more strongly correlated with density dependence. These results suggest that mean selection across the genome is stronger in environments where competition is intense, rather than in environments that are stressful for other reasons.

KEY WORDS: **Competition, deleterious mutations, density dependence, inbreeding depression, selection, stress.**

Inbreeding depression has been estimated hundreds of times in a wide variety of taxa. From this body is work, it is clear that inbreeding depression is common but also that it is highly variable in magnitude. Understanding this variation is one of the major challenges facing modern research on inbreeding depression.

Closely related species, or even different populations of the same species, can differ considerably in their degree of inbreeding depression (e.g., Brewer et al. 1990; Dole and Ritland 1993). Such differences are typically attributed to differences in the levels or types of segregating polymorphism that exist because of different demographic or selective histories (Barrett and Charlesworth 1991; Husband and Schemske 1996; Byers and Waller 1999; Fowler and Whitlock 1999; Crnokrak and Barrett 2002; Busch 2005). A second dimension of variation in the inbreeding depression literature emerges from studies in which inbreeding depression has been measured on the same population in multiple environments, yielding substantially different values. What factors are responsible for this "among-assay environment" variation? Can we predict in which types of environments the expression of inbreeding depression will be greatest?

To clarify our thinking, it is useful to consider a standard single-locus model with fitnesses of AA, Aa, and aa as 1, $1 - hs$, and $1 - s$, in which the frequencies of the A and a alleles are p and q. From this model, inbreeding depression is approximately

$$\delta = 2F(1 - {}^1\!/_2 h)s\pi, \quad (1)$$

where F is the degree of inbreeding (i.e., the inbreeding coefficient), $\pi = pq$ is the level of polymorphism. The approximation is accurate provided that either s or q is small. From equation (1), we would infer that a difference in δ measured in two environments is due to changes in h or s because F and π are held constant when measuring δ on the same population in multiple environments.

EVOLUTION
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The single-locus result in equation (1) can be extended to n loci across the genome under the assumptions of multiplicative fitness effects and no linkage disequilibria:

$$\delta = 1 - \exp\left[-2nF\left(\frac{1}{2} - \bar{h}\right)(\bar{s}\bar{\pi} + \mathrm{Cov}[s, \pi])\right] \times \exp\left[2nF(\bar{\pi}\mathrm{Cov}[h, s] + \bar{s}\mathrm{Cov}[h, \pi] + E[\tilde{h}\tilde{s}\tilde{\pi}])\right], \quad (2)$$

where the symbols accented by a tilde denote deviations of the variable from its mean (e.g., $\tilde{s} = s - \bar{s}$). A major component of equation (2) is similar to equation (1), simply by using mean values for h, s, and π. However, equation (2) contains additional covariance terms that are not present in equation (1), the importance of which will be discussed later.

These equations formalize the intuitive idea that δ will be greater in those environments where deleterious alleles tend to be more recessive (lower $\bar{h}$) and where selection tends to be stronger (larger $\bar{s}$), holding all else equal. Unfortunately, little is known about h (Phadnis and Fry 2005; Agrawal and Whitlock 2011; Manna et al. 2011, 2012) and even less about how it changes across environments. But what about selection, s? Are there environments where mean selection $\bar{s}$, and thereby δ, are expected to be especially strong?

An intuitively appealing idea is that selection tends to be stronger in stressful environments and, indeed, inbreeding depression has been associated with stress (Fox and Reed 2010). However, reviews of studies measuring mutational fitness effects show that stress per se does not reliably increase selection (Martin and Lenormand 2006; Agrawal and Whitlock 2010); rather some types of stresses seem to increase $\bar{s}$, whereas others weaken it. A hypothesis emerging from the review of Agrawal and Whitlock (2010) is that environments in which fitness is more strongly density dependent (rather than simply more stressful) should have stronger average selection. This is because the effects of density often do not fall equally on all individuals. Those individuals that start off with a minor advantage can "get out ahead of the crowd," exploiting resources and worsening conditions for those around them, thereby accentuating their initial advantage (i.e., increasing s). This is the same logic underlying the idea of "competitive dominance and suppression" with respect to the intensity of intraspecific competition in plants (Weiner 1985; see Discussion).

The key idea is that stronger density dependence typically reflects more intense intraspecific competition, and that the effects of this heightened competition fall disproportionately on weaker individuals. However, if the density dependence of fitness is increased (due to abiotic stress, predators, interspecific competitors, or for any other reason) in a manner that does not create conditions where better types (e.g., outbreds) can make the environment worse for lesser types (inbreds), then we do not expect selection to be stronger. Certainly, one could conceive of such scenarios (e.g., random deaths by a virulent pathogen), but we suspect that environments with stronger density dependence will often, directly or indirectly, increase intraspecific competition in a manner that increases selection. For example, the addition of predators to an environment may increase the strength of density dependence and the strength of selection even though the addition of predators does not intuitively appear to increase intraspecific competition. This could occur if better genotypes are able to find safe foraging sites sooner, forcing lesser genotypes to forage in areas with higher predation risk. Alternatively, better genotypes may find food more quickly, reducing the abundance of food so lesser genotypes are forced to spend more total time searching for food and being exposed to predation. In either case, better genotypes worsen the environment experienced by lesser genotypes. To be clear, it is not a mathematical truism that increased density dependence will always result in stronger selection but it seems likely that this will often, though not always, be the case.

If density dependency increases $\bar{s}$, it should also increase δ. Here we test that idea by measuring inbreeding depression for survival in *Drosophila melanogaster* in 22 laboratory environments, which we also characterize with respect to stressfulness (*S*) and density dependency (*DD*). We find that inbreeding depression is stronger in environments where fitness is more density dependent whereas the effects of stress on δ are much less consistent.

Methods

For this experiment, we used *D. melanogaster* from a laboratory population originally collected in 1970 from Dahomey, Africa and maintained in the current laboratory at a moderate population size (1000–3000) for ~9 years prior to the experiment. Using standard cytogenetic techniques, we isolated 90 lines, each homozygous for chromosome 3, which represents ~40% of the coding genome. The remainder of the genome in each line should have contained normal levels of heterozygosity for this population. In the assays described below, inbred genotypes were created by crossing to females from males of the same line, yielding an inbreeding coefficient of $F \approx 0.4$. After a mating period, females (but not males) from within-line crosses were pooled into a cage to lay eggs on grape-juice agar plates. Outbred flies were created by crossing females to males of a different line using a round-robin design (e.g., ♀1 × ♂2, ♀2 × ♂3, …, ♀90 × ♂1). Females (but not males) from between-line crosses were pooled in a cage for egg laying. The assays also involved a competitor stock that had been constructed by introgressing to fixation *DsRed* (which codes for a red fluorescent protein) into a smaller "copy" of the main laboratory population described above. Eggs were collected in a similar manner from this *DsRed* population. In all cases, only eggs laid within 36 h of females being separated from males were used to

minimize the risk of unfertilized eggs due to sperm limitation. Eggs were collected less than 24 h after being laid and washed into phosphate buffered saline (PBS) solution in a microcentrifuge tube. After additional rinsing with PBS, the excess solution was removed. Using a wide-bore tip, eggs were pipetted into wide fly vials (28.5 mm × 95 mm) to create, approximately, the desired densities. Based on preliminary trials, we pipetted volumes of 4.5, 5.5, and 6.5 μL to obtain 100 ± 10, 130 ± 15, and 160 ± 15 eggs (note measurement errors reported here are approximate ranges, not formal statistics).

The assays were performed in 22 environments that varied with respect to thermal regime, chemical composition, food abundance, water availability, and substrate texture. These are listed in Table 1. Four types of assays were formed in each environment.

1. Focal density with outbreds (FO): ∼130 outbred eggs + ∼130 *DsRed* competitor eggs.
2. Focal density with inbreds (FI): ∼130 inbred eggs + ∼130 *DsRed* competitor eggs.
3. Low density with outbreds (LO): ∼100 outbred eggs + ∼100 *DsRed* competitor eggs.
4. High density with outbreds (HO): ∼160 outbred eggs + ∼160 *DsRed* competitor eggs.

The competitor flies were used to help standardize the competitive environment experienced by inbred and outbred flies (i.e., inbred flies did not compete only against other inbred flies within vials).

The number of wild-type and *DsRed* adult flies reaching adulthood was scored by counting and clearing adult flies soon after peak emergence (e.g., 11 days post setup) and a few days later when emergence should be near complete (e.g., 15 days post setup). The actual days used for scoring varied between environments, but was constant across assay types within an environment. In the analyses reported, we consider total number of wild-type or *DsRed* flies (i.e., summing across both scoring days).

Thirty replicates were performed of each assay type in each environment. All replicate assays for a given environment were initiated within a 5-h period. (Different environments were assayed over a two-month period following isolation of the 90 chromosome 3 lines.) In total, 463,089 flies were scored.

For each environment, the data from the four types of assays were used to calculate (1) the stressfulness *S*, (2) the density dependency *DD*, and (3) the inbreeding depression δ. As described below, the assays performed at different densities (LO and HO) or with inbred flies (FI) are used to make inferences about the environment for a largely outbred population reared at the focal density under the conditions given in Table 1.

As in other literature relating selection and stress (see Agrawal and Whitlock 2010 and references therein), we define stress as the reduction in absolute fitness (or, in our case, survival) below its maximal value. We are interested in stress at the focal density so we estimated stress as $\hat{S} = 1 - (\bar{n}_{FO,wt} + \bar{n}_{FO,DsRed})/(2 \times 130)$, where $\bar{n}_{FO,g}$ is the mean number of adult flies of genotype *g* (wild-type or *DsRed*) emerging from *FO* vials. (For various reasons, it might make sense to calculate *S* based on only wild-type or only *DsRed* flies. Stress values calculated as such are strongly correlated [$\rho > 0.9$] with each other and with the measure described above. We used the measure described because it is based on the most data and should thus have least measurement error.)

In the present context, density dependency refers to the sensitivity of the fitness metric (survival) to density. Moreover, we are interested in the proportional change in survival to meaningfully compare *DD* between environments with very different survival. Ideally, the degree of density dependency would be measured as the tangent to the curve relating survival to density (after rescaling so that survival at nonfocal densities is measured proportional to survival at the focal density). As this is not practical, we instead approximated true *DD* via a crude linear regression centered on the focal density. We first calculated the average combined survival of wild-type and *DsRed* flies, separately for LO, FO, and HO assays. These values were divided by the mean for FO to make measures proportional to the focal density. The slope of the regression of these values on density was used as the measure of *DD*; we multiplied the slope by −100 so that larger (and positive) values of *DD* represent stronger density dependence, that is, the percent decrease in survival, relative to that at the focal density, per capita increase in density. (We also calculated alternative versions of *DD* using only data from wild-type flies and from only *DsRed* flies. In addition we calculated *DD* using only data from LO and HO, standardizing to the average survival across the two extreme densities to avoid using any data from FO. All six of these alternate measures were highly correlated [$\rho > 0.9$] but we use the measure of *DD* described because it is based on the most data.)

Inbreeding depression was calculated as $\hat{\delta} = 1 - \bar{n}_{FI,wt}/\bar{n}_{FO,wt}$. We bootstrapped over replicates to obtain standard errors and 95% confidence intervals for δ, *DD*, and *S*.

We calculated relationships among the variables using two approaches. First, we used standard parametric tests (Pearson moment correlations and simple linear regression). However, these tests are not entirely appropriate as the data are not normally distributed and there is some (weak) correlational structure to the measurement error among the variables. For this reason, we also constructed customized likelihood analyses that use more appropriate distributions and explicitly incorporate the correlated measurement error. The details of these likelihood analyses are

Table 1. Description of environments.

Label	Environment
A	"Regular" high-nutrition food based on a standard sugar–yeast–agar recipe, with 10 mL of food per vial; vials housed at 25°C. These conditions most closely resemble typical laboratory cultures. All other environments described with respect to this one unless otherwise stated
B	$CdCl_2$ (50 $\mu g\ L^{-1}$) added to cornmeal recipe (see L)
C	Caffeine (0.25 $g\ L^{-1}$) added to cornmeal recipe (see L)
D	Vials housed at 17°C (final count was done on day 19)
E	Vials placed in 4°C incubator for one hour three days after vial setup
F	Vials placed in 4°C incubator for 1.5 h three days after vial setup
G	Vials placed in 4°C incubator for two hours three days after vial setup
H	Vials placed in 4°C incubator for one hour five days after vial setup
I	Vials placed in 4°C incubator for 1.5 h five days after vial setup
J	Vials placed in 4°C incubator for two hours five days after vial setup
K	A reduced volume of cornmeal-based food (see L), only 3 mL/vial
L	An alternative high-nutrition food recipe based on cornmeal, sugar, and yeast
M	The cornmeal recipe (see L) was made with 25% less water, resulting in harder and drier media
N	Double the normal amount of agar added to the cornmeal recipe (see L), resulting in harder food
O	Cornmeal recipe (see L) made with the addition of ethanol (100 mL 95% ethanol per L of food)
P	Vials placed in 38°C water bath (38°C) for two hours five days after setup
Q	Vials housed at 28°C
R	The cornmeal recipe (see L) was made with 25% more water, resulting in softer and wetter media
S	Cornmeal recipe (see L) supplemented with NaCl (15 $g\ L^{-1}$)
T	"Used food"; prior to experiment, ~200 eggs were placed in each vial and larvae were allowed to develop for three days before killing all larvae via 30+ h at 4°C
U	Cornmeal recipe (see L) supplemented acetic acid (200 mL acetic acid per L of food)
V	Cornmeal recipe (see L) supplemented with urea (4 $g\ L^{-1}$)

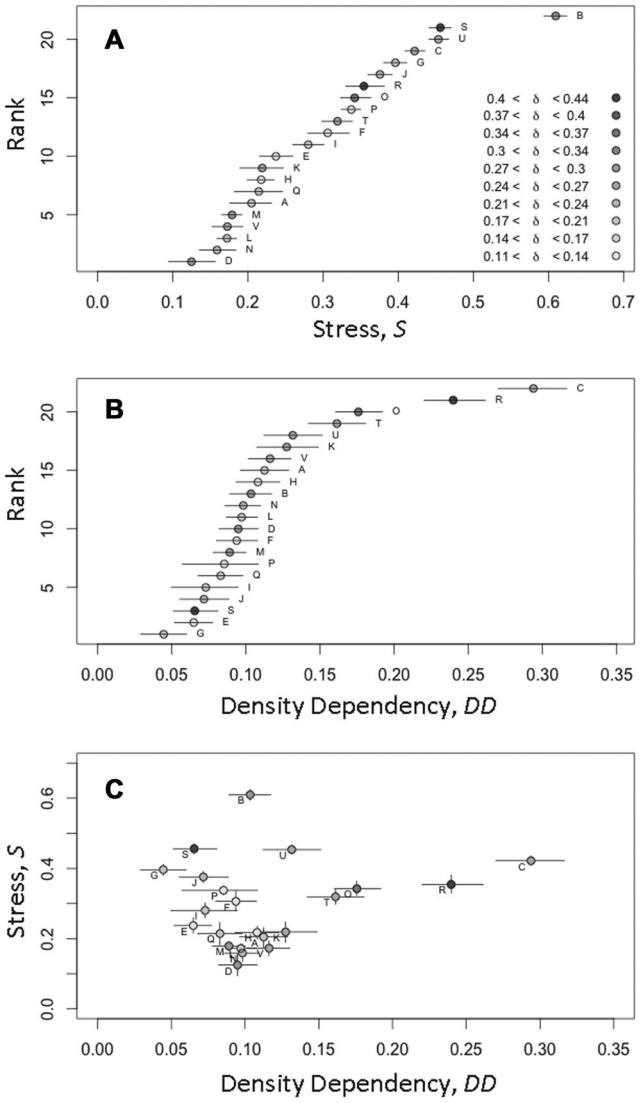

Figure 1. Variation among environments with respect to stress (*S*) and density dependence (*DD*). (A) Stressfulness of the environments plotted in rank order. (B) Density dependence of the environments plotted in rank order. (C) Joint distribution of stress and density dependence (Pearson: r = 0.20, *P* = 0.38; likelihood: *r* = 0.15, *P* = 0.47). Error bars are bootstrap 95% confidence intervals. Points are shaded with respect to levels of inbreeding depression.

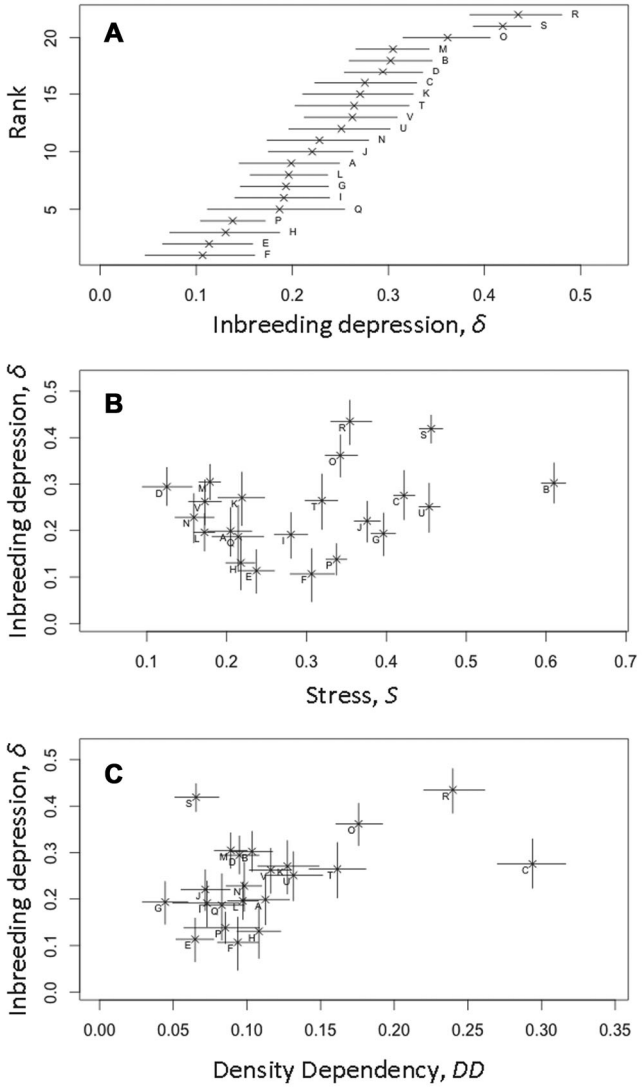

Figure 2. Variation in inbreeding depression. (A) Inbreeding depression for each environment plotted in rank order. (B) Bivariate distribution of inbreeding depression and stress (Pearson: r = 0.31, *P* = 0.17; likelihood: *r* = 0.24, *P* = 0.18). (C) Bivariate distribution of inbreeding depression and density dependence (Pearson: r = 0.45, *P* = 0.03; likelihood: *r* = 0.46, *P* = 0.03). Error bars are bootstrap 95% confidence intervals.

given in the Supporting Information. In all cases, the results from the two approaches were qualitatively the same and with quantitatively similar *P*-values.

Results

The set of environments examined here varied considerably in stress and density dependency. Estimates of stress varied over a sixfold range ($0.13 \leq \hat{S} \leq 0.61$, Fig. 1A), as did estimates of density dependence ($0.05 \leq \widehat{DD} \leq 0.29$, Fig. 1B). These two environmental attributes were only weakly and nonsignificantly correlated (Fig. 1C; Pearson: r = 0.20; $P = 0.38$; likelihood: $r = 0.15$; $P = 0.47$).

Estimates of inbreeding depression varied over a fourfold range ($0.11 \leq \hat{\delta} \leq 0.44$, Fig. 2A). Because past studies (e.g., Fox and Reed 2010) have examined how inbreeding depression is correlated to stress only, we first examine the relationships of δ with each environmental attribute separately. The correlation between inbreeding depression and stress was weak and nonsignificant (Fig. 2B; Pearson: r = 0.31; $P = 0.17$; likelihood: $r = 0.24$;

Table 2. Model results examining effects of stress *S*, density dependence *DD*, and their interaction on inbreeding depression δ.

	Simple linear regression		Likelihood	
Effect	Estimate	*P*-value	Estimate	*P*-value
S	0.01	0.95	0.01	0.97
DD	1.60	0.007	1.61	0.002
$S \times DD$	−11.6	0.03	−11.9	0.03

Model is structured to examine effects of *S* and *DD* with respect deviations from their mean values so that the main effects alone show the expected change in δ from increasing *S* or *DD*, assuming no correlated change in the other variable (see Supporting Information for details).

$P = 0.18$). In contrast, inbreeding depression was significantly correlated with density dependence (Fig. 2C; Pearson: r = 0.45; $P = 0.03$; likelihood: $r = 0.46$; $P = 0.03$).

Next, we examined a model in which inbreeding depression was a function of both stress and density dependence (Table 2). The main effect of stress on inbreeding depression was almost zero and nonsignificant. The main effect of density dependence was much stronger and significant. Much of the (weak) correlation between inbreeding depression and stress shown in Fig. 2B was likely due to the (weak) correlation of stress with density dependence, as the main effect of *S* is near zero when *DD* is included in the model. The stress × density dependence interaction term was also significant. This is likely due to two environments, "G" and "S," with low-density dependence and high stress that had moderate to strong inbreeding depression. However, it is clear from Figure 2C that the strongest pattern in the data is an increase in the strength of inbreeding depression with density dependence (see also Fig. 1C).

Discussion

Our main finding is that inbreeding depression is stronger, on average, in those environments where density dependence is stronger. Presumably, this is because density dependence increases $\bar{s}$ (Agrawal and Whitlock 2010), Alternatively *DD* might reduce $\bar{h}$ or affect δ via one of the covariance terms in equation (2), however, we are aware of no reason to expect such effects.

Our work is motivated by the idea that *DD* reflects the intensity of competition and that the latter determines how small differences in genetic quality are translated into differences in fitness (but see caveats in Introduction). Of course, competition can only magnify differences in genetic quality if individuals that differ in genetic quality actually compete. Consistent with this notion, several important studies have found inbreeding depression to be much larger when inbred and outbred plants are grown in mixed cultures rather than when each type is grown separately ("mixed versus pure" [hereafter, MvP] experiments; Schmitt and Ehrhardt 1990, Carr and Dudash 1995; Cheptou et al. 2001; Cheptou and Schoen 2003; Koelewijn 2004). These results have been interpreted as evidence of "dominance and suppression" in plant competition (Weiner 1985), which is based on the same idea that underlies the prediction that $\bar{s}$ should increase with density dependence. MvP experiments provide an elegant means of revealing the consequences of competition within a single environment, but such experiments do not provide a measure of competition per se. As pointed out by Cheptou et al. (2001), an MvP experiment will fail to find the expected effect if the intensity of competition is weak within the chosen assay environment, but this explanation would be unknown because competition is not quantified in a typical MvP experiment. Similarly, if an MvP experiment were to be performed in multiple environments, it would be difficult to interpret differences between environments because the intensity of competition is not quantified in any way that can be compared across environments. Our complementary approach using *DD* as an index of competitive intensity in each environment allows us to examine whether competition intensity can explain some of the variation in δ among environments.

In discussing "dominance and suppression," Weiner (1985) argued that when plants are grown at high density, intense competition will increase the variation among individuals as the winners of competition leave the losers with little resources. This can be viewed as tying into the "phenotypic variability" hypothesis (Waller et al. 2008), which proposes that inbreeding depression should be greatest in environments where there is more phenotypic variability. As discussed elsewhere (Long et al. 2013), the phenotypic variability hypothesis is akin to arguing that the variation within groups (e.g., among outbred individuals) should be a predictor of the variation between groups (i.e., between inbreds and outbreds), a reasonable proposition that is supported by data (Waller et al. 2008; Fox and Reed 2010; Long et al. 2013). In proposing the phenotypic variability hypothesis, Waller et al. (2008) were not explicit about why some environments would express more phenotypic variability than others or through which population genetic parameters the effect might be mediated. The ideas discussed here on density dependence, competition, and their effects on mean selection offer one set of answers to these questions.

Among studies measuring δ in multiple environments, our study is unique with respect to the number set of environments (22) examined. For example, among the 30+ studies included in the literature survey by Fox and Reed (2010), most examined inbreeding depression in only two environments and the maximum was six. Although we hope to gain a broader perspective about the nature of selection by examining a larger set of environments, it is worth considering the assumptions underlying the inferences made from our study. Our set of environments is not

a random sample from the universe of all possible environments. Rather we are limited to choosing among environments that are feasible to manage in large numbers in the laboratory. This set of environments may be unrepresentative of all possible laboratory environments as well as those in which flies are likely to encounter in nature. Moreover, our environments are not equally unique. For example, we have several environments that employ cold shock treatments, but that differ in the age and duration at which it is applied. Although seemingly similar, such environments can differ significantly in stress and/or density dependence, often more so than environments with qualitatively different stressors. Unlike sampling individuals from a population, it is unclear how to best select a random set of environments because the "population" of environments is ill defined. Although we caution readers to be aware of these issues, we see no reason that our choice of environments should bias our results. The idea we are testing—that inbreeding depression should increase with density dependence—should apply across all types of environments and there is no a priori reason why it should be more pronounced in the environments used here.

In our experiment, δ is not significantly correlated to stress (S) whereas the literature survey by Fox and Reed (2010) showed these two factors to be strongly correlated. The reason for this discrepancy is unclear but several possible explanations exist. Although our dataset covers quite a wide range of stress levels (Fig. 1), it is only about half the range covered by the meta-analysis of Fox and Reed. The latter also has more datapoints, so for both reasons their power to detect a relationship is greater. In our data there is a positive, albeit nonsignificant, correlation between S and δ. However, the relationship between S and δ is largely accounted for by the correlation between DD and S, as revealed by the near zero main effect of S on δ in the full model. Some, perhaps much, of the S–δ relationship reported by Fox and Reed (2010) may have been better accounted for by density dependence, had it been measured.

Second, in many studies, the "stressful" environment is also a novel environment. Past selection is expected to have reduced polymorphism levels at sites that are strongly selected in the normal environment, whereas sites deleterious in the novel environment, but neutral (or weakly selected) in the normal environment, will not have been purged by past selection; consequently, inbreeding depression will tend to be higher in novel habitats (Agrawal and Whitlock 2010). This can be seen in equation (2) where past selection is expected to make Cov[s, π] negative, reducing δ, in the native environment, whereas Cov[s, π] will be closer to zero in a novel habitat so that inbreeding depression will be greater. Consistent with this, Long et al. (2013) found that a set of replicate fly populations adapted to a $CdCl_2$-enriched medium had much lower inbreeding depression when assayed on the $CdCl_2$-enriched medium than on an NaCl-enriched medium. Further, fly populations adapted to the NaCl-enriched medium had much lower inbreeding depression than $CdCl_2$-adapted populations when assayed on the NaCl-enriched medium. Thus, it is not surprising that some novel (and stressful) environments will result in strong δ, even if density dependence is weak.

However, not all stressful environments are novel. This is most clearly exemplified by plant studies finding stronger inbreeding depression in the field than in the greenhouse (e.g., Johnston 1992; Eckert and Barrett 1994; Koelewijn 1998), despite the greenhouse being more novel but much less stressful. Perhaps density dependency is higher in the field than the greenhouse. On the other hand, stress may affect inbreeding depression beyond what can be accounted for by either density dependence or novelty.

One possibility is that some "simple" benign environments such as the greenhouse may require the function of only a minimal set of genes (e.g., n_{min} genes under selection). Stressful environments (e.g., the field) may be more "complex," requiring proper function of more genetic pathways to achieve high fitness (e.g., $n_{min} + n_{stress\_response}$ genes under selection) leading to higher $\bar{s}$ because of more genes with $s \neq 0$. If the number of genes that respond to a stress ($n_{stress\_response}$) depends primarily on the type of stress (e.g., heat shock vs. nutritional stress) rather than the severity of the stress, then there would be no general relationship between S and either $\bar{s}$ or δ. However, stressful environments would nonetheless tend to have higher $\bar{s}$ or δ than the "simple" benign environment, assuming such an environment exists (e.g., perhaps the greenhouse for plants).

The relationship between stress and δ reported by Fox and Reed (2010) is one of the most compelling patterns to arise from the vast inbreeding depression literature. The challenge now is to understand it. Going forward, we advocate for studies that examine environmental factors whose variation can be logically predicted to create variation in the population genetic factors that affect δ (see eq. 2). Our experiment represents one step forward but we remain far from a thorough understanding of all of the among-environment variation in δ.

ACKNOWLEDGMENTS

We thank S. Kang, Y. Jeon, and A. Wang for preliminary work testing the suitability of various potential assay environments and D. Choi for assistance in scoring flies during the experiment. This manuscript was improved through useful discussions with S. Barrett and C. Balough as well as helpful comments from D. Schoen and an anonymous reviewer. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

DATA ARCHIVING

The doi for our data is 10.5061/dryad.95pp7.

LITERATURE CITED

Agrawal, A. F., and M. C. Whitlock. 2010. Environmental duress and epistasis: How does stress affect the strength of selection on new mutations? Trends Ecol. Evol. 25:450–458.

———. 2011. Inferences about the distribution of dominance drawn from yeast gene knockout data. Genetics 178:553–566.

Barrett, S. C. H., and D. Charlesworth. 1991. Effects of a change in the level of inbreeding on the genetic load. Nature 352:522–524.

Brewer, B. A., R. C. Lacy, M. L. Foster, and G. Alaks. 1990. Inbreeding depression in insular and central populations of *Peromyscus* mice. J. Hered. 81:257–266.

Busch, J. W. 2005. Inbreeding depression in self-incompatible and self-compatible populations of *Leavenworthia alabamica*. Heredity 94:159–165.

Byers, D. L., and D. M. Waller. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Annu. Rev. Ecol. Syst. 30:479–513.

Carr, D. E., and M. R. Dudash. 1995. Inbreeding depression under a competitive regime in *Mimulus guttatus*: consequences for potential male and female function. Heredity 75:437–445.

Cheptou, P.-O., and D. J. Schoen. 2003. Frequency-dependent inbreeding depression in *Amsinckia*. Am. Nat. 162:744–753.

Cheptou, P.-O., J. Lepart, and J. Escarré. 2001. Inbreeding depression under intraspecific competition in a highly outcrossing population of *Crepsis sancta* (Asteraceae): evidence for frequency-dependent variation. Am. J. Bot. 88:1424–1429.

Crnokrak, P., and S. C. H. Barrett. 2002. Purging the genetic load: a review of the experimental evidence. Evolution 56:2347–2358.

Dole, J., and K. Ritland. 1993. Inbreeding depression in two *Mimulus* taxa measured by multi-generational changes of the inbreeding coefficient. Evolution 47:361–373.

Eckert, C. G., and S. C. H. Barrett. 1994. Inbreeding depression in partially self-fertilizing *Decodon verticillatus* (Lythraceae): population-genetic and experimental analyses. Evolution 48:952–964.

Fowler, K., and M. C. Whitlock. 1999. The variance in inbreeding depression and the recovery of fitness in bottlenecked populations. Proc. R. Soc. Lond. B Biol. Sci. 266:2061–2066.

Fox, C. W., and D. H. Reed. 2010. Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. Evolution 65:246–258.

Husband, B. C., and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54–70.

Johnston, M. O. 1992. Effects of cross and self-fertilization on progeny fitness in *Lobelia cardinalis* and *L. siphilitica*. Evolution 46:688–702.

Koelewijn, H. P. 1998. Effects of different levels of inbreeding on progeny fitness in *Plantago coronopus*. Evolution 52:692–702.

———. 2004. Sibling competition, size variation and frequency-dependent outcrossing advantage in *Plantago coronopus*. Evol. Ecol. 18:51–74.

Long, T. A. F., L. Rowe, and A. F. Agrawal. 2013. The effects of selective history and environmental heterogeneity on inbreeding depression in experimental populations of *Drosophila melanogaster*. Am. Nat. 181:532–544.

Manna, F., G. Martin, and T. Lenormand. 2011. Fitness landscapes: an alternative theory for the dominance of mutation. Genetics 189:923–937.

Manna, F., R. Gallet, G. Martin, and T. Lenormand. 2012. The high-throughput yeast deletion fitness data and the theories of dominance. J. Evol. Biol. 25:892–903.

Martin, G., and T. Lenormand. 2006. The fitness effect of mutations across environments: a survey in the light of fitness land- scape models. Evolution 60:2413–2427.

Phadnis, N., and J. D. Fry. 2005. Widespread correlations between dominance and homozygous effects of mutations: implications for theories of dominance. Genetics 171:385–392.

Schmitt, J., and D. W. Ehrhardt. 1990. Enhancement of inbreeding depression by dominance and suppression in *Impatiens capensis*. Evolution 44:269–278.

Waller, D. M., J. Dole, and A. J. Bersch. 2008. Effects of stress andphenotypic variation on inbreeding depression in *Brassica rapa*. Evolution 62:917–931.

Weiner, J. 1985. Size hierarchies in experimental populations of annual plants. Ecology 66:743–752.

Associate Editor: M. Cristescu