Environmental stress, inbreeding, and the nature of phenotypic and genetic variance in *Drosophila melanogaster*

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Fifty-two lines of *Drosophila melanogaster* founded by single-pair population bottlenecks were used to study the effects of inbreeding and environmental stress on phenotypic variance, genetic variance and survivorship. Cold temperature and high density cause reduced survivorship, but these stresses do not cause repeatable changes in the phenotypic variance of most wing morphological traits. Wing area, however, does show increased phenotypic variance under both types of environmental stress. This increase is no greater in inbred than in outbred lines, showing that inbreeding does not increase the developmental effects of stress. Conversely, environmental stress does not increase the extent of inbreeding depression. Genetic variance is not correlated with environmental stress, although the amount of genetic variation varies significantly among environments and lines vary significantly in their response to environmental change. Drastic changes in the environment can cause changes in phenotypic and genetic variance, but not in a way reliably predicted by the notion of 'stress'.

Keywords: environmental stress; genetic homeostasis; genetic variance; inbreeding; phenotypic variance; population bottlenecks

1. INTRODUCTION

Important quantitative genetic properties of a population may be changed in multiple ways by changes in the environment it inhabits. Environmental change can affect the average expression of traits, the amount of genetic variance and environmental variance for traits, the heritability of those traits and the level of inbreeding depression (reviewed in Hoffmann & Parsons 1991). Moreover, the effect of environmental change has sometimes been assumed to be greatest in stressful environments, i.e. those to which the organism is not well adapted (Hoffmann & Merilä 1999).

Inbreeding, and the loss of genetic variance or heterozygosity that can result, are well known to affect the variance among individuals of the expression of traits. Lerner (1954) proposed that fewer heterozygous individuals might express more phenotypic variance (V_P) even if genetic variance were held constant, and this notion has received some support (reviewed in Fowler & Whitlock 1999a). Inbred individuals may be less able to develop consistently in stressful environments, and if so, an interaction between inbreeding and environmental stress could exaggerate patterns of change in phenotypic variance. While there is some support for this idea (Van Esbroeck & Bowman 1998; Callaghan *et al.* 1998; Imasheva *et al.* 1999; Stanton *et al.* 2000; Bubli *et al.* 2000), not enough is known to predict a general pattern.

Changes in the environment can also change the additive genetic variance (V_A), but the extent to which these

changes are correlated with the amount of 'stress' caused by the environmental change is subject to much debate (reviewed in Hoffmann & Merilä 1999). In some experiments, V_A increases with greater stress or environmental novelty (Holloway *et al.* 1990; Pigliucci *et al.* 1995; Imasheva *et al.* 1998), in some there is no change (Bubli *et al.* 1998; Hoffmann & Schiffer 1998; Merilä & Fry 1998) and in others there is a decrease (Kasule 1991; Ebert *et al.* 1993; Blows & Sokolowski 1995; Kawecki 1995; Sgrò & Hoffmann 1998a,b). Note that many of these studies obtain different results for different traits.

Inbreeding usually reduces mean fitness relative to outbred individuals (inbreeding depression), but there is conflicting evidence on whether inbreeding depression is exaggerated in stressful environments (relationship supported: Hauser & Loeschke (1996), Bijlsma *et al.* (1999); relationship unsupported: Dahlgaard & Loeschke (1997), Dahlgaard & Hoffmann (2000)).

Previously, we have examined variation in phenotypic and quantitative genetic properties among replicate inbred lines derived from a common source population and tests were carried out under standard and relatively benign environmental conditions (Fowler & Whitlock 1999a,b; Whitlock & Fowler 1999; Phillips *et al.* 2001; Whitlock *et al.* 2002). We investigated how manipulation of a pair of environmental stresses (reduced temperature and increased larval density) affects variation within and between lines. Specifically, in this paper we question whether increased environmental variation has a large effect on the expression of phenotypic variance of inbred lines and whether environmental stress changes expression of components of variance in repeatable ways.

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Figure 1. *Drosophila melanogaster* wing characteristics measured in this study. Ten landmarks were measured for each wing, labelled from 1 to 10. The measurements were consistently made from the same point of the junction of the wing veins. The characters used were (A) wing area (the area of the polygon defined by vertices at points 1, 2, 3, 4, 5, and 10), and the angles formed by the points (B) 5–7–4, (C) 8–7–6, (D) 2–9–3, (E) 2–1–5 and (F) 2–3–5 (with the vertex listed as the middle point). Area is measured in mm² and the angles are measured in radians.

2. MATERIAL AND METHODS

(a) Pedigrees

Full details of the derivation of the experimental populations are given in Fowler & Whitlock (1999a). Briefly, the Dahomey population of *Drosophila melanogaster* was used to found 52 bottlenecked lines, each started from a single pair of randomly chosen flies and subsequently maintained at a relatively large population size. These lines were created in three groups, 2–3 months apart, each of which contained two batches of lines separated by a day in their schedules. We will refer to these six batches as ‘sets’ consisting of eight or ten bottlenecked lines per set. Each set was accompanied by a control, outbred line, formed by the offspring of 400 random pairs and maintained at a large size under the same husbandry as the inbred lines.

(b) Environmental challenges

At the fifth generation after the bottlenecks, a random sample from each line was raised in each of four differing environments. Female flies from each line laid eggs on grape-juice medium (Fowler & Partridge 1986), and first-instar larvae were transferred after 25 ± 1 h to culture vials. Two temperature environments were used: 18 and 25 °C, in otherwise identical walk-in growth chambers. Within each temperature environment, two density environments were used: low density (a standard 35 ml fly food vial with 3.75 ml of food carrying 25 first-instar larvae) and high density (a 5 ml vial with 1.5 ml of food carrying 100 larvae). Thus, the density treatments differed by a factor of 10. In each environment, four vials were set up per bottlenecked line and 12–14 vials per control line.

The offspring from each vial were counted as a measure of larval to adult survivorship. Subsequently, both wings from eight females from each vial were mounted on slides and measured with a computerized digitizer for a series of landmarks (see figure 1). These landmarks were transformed into a measure of the wing area and five measures of the angles formed by wing veins (details in Fowler & Whitlock 1999a). The values for left and right wings were averaged for each character to create the value given to that individual.

3. RESULTS

(a) Changes in fitness and mean phenotype across environments

(i) Are high density and low temperature stressful?

There were significant differences in survivorship among the environments (table 1). Flies grown at 18 °C were *ca.* 3% less likely to survive to adulthood than those grown at 25 °C ($p < 0.01$), whereas larvae grown at high density were *ca.* 14% less likely to survive to adulthood than those at lower density ($p < 0.0001$). p -values were calculated by analysis of variance of the log-transformed survivorship of replicate vials. No interaction terms were significant.

(ii) How do temperature and density stress affect trait means?

The average values of the six different phenotypes change across all of the environments, but the magnitude of this change is only large for wing area. A three-way analysis of variance was performed for the mean phenotypes from each vial from the control flies, with factors ‘set’, ‘temperature’ and ‘density’. There were significant differences among the sets, reflecting subtle differences in the environments over the course of the calendar year. Each character varied significantly across temperature and density, with all p -values 0.001 or less. The changes were substantial for wing area (flies at 18 °C were, on average, 21% larger than those at 25 °C; flies at high density were 16% smaller, on average, than those at low density). Angle 2–9–3 was reduced by 4%, on average, either due to the shift to a cold temperature or the shift to high density, but all other significant changes were 3% or less. The interaction between density and temperature was significant at the $p < 0.001$ level for wing area and angles 5–7–4, 2–9–3 and 2–1–5.

(iii) Does environmental stress change inbreeding depression?

There was substantial inbreeding depression (defined as the difference in fitness between outbred and inbred individuals divided by the fitness of outbred individuals) in these four environments (table 1). On average, the probability of surviving from first instar to adult was *ca.* 11% lower for an individual from an inbred line than one from a control line. This difference was highly significant (two-factor ANOVA, $p < 0.0001$). However, the degree of inbreeding depression did not depend upon the environment (inbreeding by environment interaction: $p = 0.88$). Environmental stress did not exacerbate the deleterious effects of inbreeding.

(b) Inbreeding and changes in phenotypic variation with stress

Here, we examine the changes in V_P due to changes in the macroenvironment. We distinguish between V_{env} , variance in phenotype caused by gross changes in the environment (such as the temperature and density changes imposed here) and V_E , the variance in phenotype caused by a subtle difference in the developmental environment (not controlled for in this experiment).

(i) Does V_P change with stress and is this change greater in inbred lines than the controls?

Each line provides an independent replicate for the amount of V_P in each environment. Using the natural log

Table 1. Larval-to-adult survival probability for the different environments (mean across lines (s.e.)).

		18 °C	25 °C
low density	control	0.949 (0.007)	0.974 (0.007)
	inbred	0.838 (0.011)	0.863 (0.01)
	inbreeding depression	11.7%	11.4%
high density	control	0.818 (0.017)	0.827 (0.021)
	inbred	0.717 (0.012)	0.748 (0.01)
	inbreeding depression	12.3%	9.5%

Table 2. The distribution of the variance among environments (CV, coefficient of variation.)

trait	control mean V_{env}	relative V_{env} ^a	inbred mean V_{env}	estimated $V(V_{\text{env}})$ among inbred lines ^b	CV (V_{env})
wing area	0.036 892	8.31	0.032 956	2.5×10^{-5}	15
5–7–4	0.000 091	0.06	0.000 132	2.0×10^{-8}	108
8–7–6	0.000 938	0.22	0.001 104	5.3×10^{-7}	66
2–9–3	0.000 099	0.70	0.000 101	5.3×10^{-10}	23
2–1–5	0.000 082	0.31	0.000 099	1.1×10^{-9}	33
2–3–5	0.000 034	0.04	0.000 08	2.6×10^{-9}	64

^a The relative V_{env} given here is the mean control V_{env} divided by the phenotypic variance within environments of the same traits in the controls.

^b The estimated variance across inbred lines in V_{env} was calculated by the pooled mean over sets of the observed $V(V_{\text{env}})$ minus the error variance in V_{env} as estimated from 1000 bootstraps.

of variance within lines and within environments, a two-way ANOVA was performed with the factors of inbreeding status and environment. All characters were significantly affected by inbreeding status (except angle 8–7–6, where $p = 0.06$). Wing area increased in V_P with temperature stress (by 80% on average, $p < 0.001$) and density stress (by 155%, $p < 0.001$). Angle 2–9–3 increased significantly in V_P only in high density (by 24%, $p = 0.0009$) and no other trait changed significantly in V_P over environments. In no case, however, was the interaction term between inbreeding and environment significant. The change in phenotypic variance with stress for inbred lines was no greater, on average, than for control lines.

(ii) *Is there more variance among environments within lines in inbreds than the controls?*

For each trait, a mixed-model nested ANOVA was performed for each line to estimate the amount of variance among the four environments (V_{env}), the amount of variance among vials within environments and the residual variance among individuals within vials. We then compared the estimates of V_{env} for the six control lines with those from the 52 bottlenecked lines (results in table 2). None of the traits was significantly different between inbred and control lines (Wilcoxon signed-rank tests), although this may reflect the low number of control lines. All of the shape characters had more V_{env} on average in the inbred lines than in the control lines, but none was significant.

(iii) *Is there more variance among lines in V_P in some environments?*

The mean change in V_P shows what may happen, on average, with a change in environment, but the variance in V_P across inbred lines reveals what *can* happen in isolated lines. Therefore, we questioned whether the variance

among inbred lines in phenotypic variance was a function of environmental stress. First, we assessed whether the macroenvironment affected the variance among lines in V_P , testing statistical significance by bootstrapping over individuals and using the residuals from vial means to remove line and vial effects. In those traits where the environment changed the distribution of V_P , we tested whether this change was correlated (Spearman's rank test) with stress levels (percentage mortality) in each environment. This test used the six independent estimates of the variance in V_P from the control sets.

Wing area was the only factor where the environment affected the variance in V_P ($p < 0.0001$), but this difference was very large. The variance among lines in V_P was almost 20 times greater in the most stressful (cold, high density) environment than in the least (warm, low density). Furthermore, this difference was strongly correlated with stress (Spearman's $\rho = 0.80$, $p < 0.0001$). For wing area, there is a much greater probability of a large increase in V_P after a population bottleneck if the population is grown in a stressful environment. However, this increase in the variance in V_P disappears if the average change in V_P is taken into account. The variance ratio (the V_P of a trait expressed as a ratio of the V_P of the same trait in the controls from the same environment; Fowler & Whitlock (1999a)) did not vary significantly across environments, nor did the variance of this variance ratio. The variance among lines in V_P changed with stress, but only because it scaled with the mean changes in V_P across environments.

(iv) *Do the inbred lines vary in their sensitivity to the different environments?*

A bootstrap analysis was performed to test whether the substantial variation among lines in the extent of V_{env} was

Figure 2. The distribution of variance among environments (V_{env}). The histograms plot, for each character, the distribution of V_{env} for the 52 inbred lines. This distribution includes sampling error. The error variance was estimated by bootstrapping over residuals from line means for the inbred data within sets. Approximately 0.65, 0.73, 0.70, 0.42, 0.48 and 0.47 of the variance in these distributions is due to true variance among lines in V_{env} for (a) wing area, angles, (b) 5–7–4, (c) 8–7–6, (d) 2–9–3, (e) 2–1–5, and (f) 2–3–5, respectively. The variances plotted in this figure have been scaled by multiplying by 10^2 , 10^4 , 10^3 , 10^4 , 10^4 and 10^4 , respectively.

significant (table 2). Pseudo-datasets were created by resampling 10 000 times from a dataset consisting of the residuals from line means, to avoid confounding among-line average differences with within-line effects. The pseudo-data for each pseudo-line were created by resampling vials randomly with respect to line within sets but from the original environment. Variance among lines in V_{env} was calculated as the pooled variance of the sets. Of the 10 000 pseudo-datasets, none of three characters (area and angles 5–7–4 and 8–7–6) had more variance among lines than the actual data. The other traits also had statistically significant variance in V_{env} (angle 2–9–3, $p < 0.0043$; 2–1–5, $p < 0.0007$; 2–3–5, $p < 0.017$). The distribution among inbred lines in V_{env} is shown in figure 2. The error variance for the estimates of V_{env} was calculated from the bootstrap, which demonstrated that more than half of the variance observed among lines was not due to sampling error.

In particular, the amount of variance among lines in V_{env} for wing area was much greater (*ca.* 200 times) than the variance among lines in V_{P} described in Fowler & Whitlock (1999a). For the other traits, the variance among lines for V_{env} was of the same order of magnitude as the variance among lines in within-line variance.

(v) *Is the variance among macro-environments correlated with V_{E} ?*

Estimates of V_{E} (the variance within lines within environments, which is not due to V_{A}) for these lines have been made previously (Whitlock & Fowler 1999) and revealed significant variation in V_{E} . We tested whether the genotypes that are more sensitive to subtle environmental differences are also those that are most sensitive to large environmental perturbation? However, for each trait, the correlation between V_{env} and V_{E} was small and non-significant ($p > 0.2$ in all cases.).

(c) *Additive genetic variance and heritability under environmental stress*

We can estimate V_{A} , V_{E} and V_{P} of these traits in each environment. Since the individuals within a line have a known genetic relationship (the inbreeding coefficient, $F \sim 0.3$; see Whitlock & Fowler (1999)), the among-line variance is an estimate of the product of $2F$ and V_{A} , assuming that dominance variance is negligible. The variance within lines should be $V_{\text{E}} + (1 - F)V_{\text{A}}$, thus allowing an estimate of V_{E} . V_{P} is estimated from the sum of V_{A} and V_{E} .

For each environment, the six sets provide independent estimates of these parameters, which are subjected to

Table 3. The average slopes of the regression of variance component estimates on the percentage mortality across the four environments. (In parentheses are the p -values from two-tailed tests, uncorrected for 18 multiple comparisons.)

variance component	wing area	angle 5–7–4	angle 8–7–6	angle 2–9–3	angle 2–1–5	angle 2–3–5
V_A	0.0026 (0.31)	–0.0018 (0.18)	–0.0056 (0.08)	0.000 21 (0.043)	0.000 030 (0.89)	–0.0005 (0.12)
V_E	0.0217 (0.018)	–0.0044 (0.46)	0.002 (0.27)	0.000 08 (0.23)	–0.000 095 (0.49)	0.000 17 (0.74)
h^2	–1.69 (0.10)	–0.62 (0.45)	–0.89 (0.10)	0.50 (0.27)	0.37 (0.50)	–0.44 (0.34)

Table 4. Genetic and environmental variances and heritabilities in each environment.

trait	temperature	density	$V_A \times 10^4$ (s.e.)	$V_E \times 10^4$ (s.e.)	h^2 (s.e.)
area	18	100	29.4 (4.4)	52.4 (13.3)	0.41 (0.08)
	18	25	27.5 (6.5)	11.0 (4.6)	0.68 (0.14)
	25	100	13.8 (3.8)	29.1 (4.9)	0.29 (0.06)
	25	25	12.1 (2.7)	10.7 (2.9)	0.53 (0.12)
5–7–4	18	100	7.8 (1.2)	6.7 (0.6)	0.52 (0.06)
	18	25	10.0 (2.3)	23.9 (18.7)	0.53 (0.15)
	25	100	5.4 (0.6)	8.1 (0.4)	0.40 (0.03)
	25	25	8.6 (2.2)	6.3 (2.0)	0.56 (0.14)
8–7–6	18	100	10.6 (3.1)	30.3 (3.8)	0.26 (0.07)
	18	25	15.1 (2.5)	28.6 (3.9)	0.35 (0.06)
	25	100	16.6 (4.0)	27.6 (4.5)	0.38 (0.09)
	25	25	25.7 (7.4)	23.0 (5.7)	0.51 (0.12)
2–9–3	18	100	0.98 (0.36)	0.63 (0.31)	0.58 (0.19)
	18	25	0.57 (0.27)	0.66 (0.20)	0.41 (0.18)
	25	100	0.97 (0.42)	0.64 (0.26)	0.52 (0.17)
	25	25	0.74 (0.33)	0.52 (0.25)	0.52 (0.19)
2–1–5	18	100	1.7 (0.2)	0.8 (0.2)	0.70 (0.09)
	18	25	1.9 (0.5)	0.8 (0.4)	0.67 (0.14)
	25	100	1.5 (0.4)	1.1 (0.2)	0.56 (0.11)
	25	25	1.4 (0.4)	1.2 (0.4)	0.52 (0.14)
2–3–5	18	100	4.3 (0.9)	4.3 (0.7)	0.49 (0.09)
	18	25	5.1 (1.4)	4.5 (1.2)	0.51 (0.12)
	25	100	4.7 (1.0)	3.5 (0.5)	0.55 (0.07)
	25	25	5.2 (0.8)	3.1 (0.9)	0.63 (0.09)

analysis of variance to discover sources of change in these values over environments.

(i) *Does stress predict genetic or environmental variance?*

We can test whether variation in stress across the environments (see § 3b) causes predictable changes in either genetic or environmental variance for these characters. For each set in each environment, the variance components for each trait were regressed on the percentage survival of the control populations. The error of the slopes of these regressions should be normally distributed, so we can investigate, with t -tests, whether the six different sets have slopes which average significantly different from zero for a variance component of a given trait.

There was no significant evidence that the extent of stress predicted the magnitude of either genetic or environmental variance for any trait (see table 3). Similarly, heritability (h^2) was not consistently affected by stress for any trait (p -values ranged from 0.10 to 0.50).

(ii) *What is the variance across environments in V_A and V_E ?*

Using the independent measures of V_A , V_E and h^2 from each set, ANOVA revealed significant variation across environments for some traits (table 4). For wing area, V_A

varies as a function of temperature (increasing at 18 °C by a factor of 2.2, $p < 0.001$) but not density, while V_E varies as a function of density but not temperature (increasing at high density by a factor of 3.75; $p < 0.001$). Heritability for wing area shows marginally significant variation ($p < 0.02$). For other traits, the evidence is more equivocal. V_A varies across density treatments for angle 2–9–3 (increasing by 50% at high density; $p = 0.01$), and V_E may vary for angles 2–1–5 (decreasing by 35% at low temperature, $p < 0.02$) and 2–3–5 (increasing by 34% at low temperature, $p < 0.05$). (All p -values given in this paragraph are uncorrected for 24 multiple comparisons.)

Note that wing area has a greater V_A in the most stressful environment than in the least stressful (by a factor of 2.4; $p < 0.01$). There is an even greater change in V_E for this pair of environments (increasing by 4.9 fold; $p < 0.01$), so that h^2 is actually reduced in the stressful environment, although not significantly ($p = 0.43$).

4. DISCUSSION

We have found that changes in temperature or density environments affect the expression of wing size and morphology in *Drosophila melanogaster*. Inbred lines and control lines were affected equally by stress, both in their

fitness and expression of the V_P of these characters. However, changes in the environment affected different inbred lines in different ways. Moreover, the expression of genetic variance was not correlated with the level of environmental stress, as measured by the survivorship in the different environments.

Different inbred lines varied significantly in the amount of V_P within and among environments. These changes are interesting in the context of models of the evolution on complex adaptive landscapes. It has been shown that an increase in the V_P of a population can lead to a qualitative change in the adaptive landscape, such that valleys between alternative adaptive peaks can disappear, thereby allowing evolution to proceed to a new, higher peak (Kirkpatrick 1982). We have suggested that the V_P is quite likely to increase substantially in the populations that result from population bottlenecks (Fowler & Whitlock 1999a), either by an increase in the sensitivity to the environment (as suggested by Lerner 1954) or by genetic drift causing random changes in genetic variance (Whitlock 1995). This bottleneck-induced change in V_P can be an effective mechanism for evolution on rugged landscapes and has been called the variance-induced peak shift model, or VIPS (Whitlock 1995).

There are three reasons why our results point to a strong role for heterogeneous environments in the evolution of bottlenecked populations. First, we have seen here that stressful conditions increase the heterogeneity of phenotypic variance. As natural populations are quite likely to experience more stressful environmental conditions than found in a standard fly vial at 25 °C and under constant density, the amount of variability in V_P is likely to be much greater in nature than suggested by our previous work (Whitlock & Fowler 1996; Fowler & Whitlock 1999a). The current results show that the distribution among lines in V_P is likely to be much greater under more realistic, more diverse conditions.

Second, founder populations in natural settings are unlikely to always find the same environments. Variation in the exact environment of replicate founder populations in nature could have large effects on the V_P expressed by those populations. Such environmental heterogeneity would substantially increase the probability of peak shifts by the VIPS model.

Third, a single population in nature probably experiences more environmental heterogeneity than the constant conditions used in our previous experiments. This means that a single population will have a V_P determined in part by the component defined here as V_{env} , the variance among environments. As the lines varied substantially in their sensitivity to the environmental differences, this environmental heterogeneity results in another source of heterogeneity of V_P among lines. Results obtained in constant conditions (Fowler & Whitlock 1999a) are thus a substantial understatement of the amount of variance among lines in V_P expected under more natural conditions.

There has been substantial confusion in the literature over claims that 'stressful' environments increase 'genetic variation' (Hoffmann & Merilä 1999). Stress is rarely quantified, as has been done here, to show that an environment called 'stressful' actually reduces fitness. Often these environments are merely different from the

standard rearing environment of the test species. It is not clear whether stress or merely novelty may engender changes in expression of genetic variance.

Furthermore, many measures of genetic variability that are not equivalent have been employed. The most basic measure of genetic variability is V_A , being the statistic that predicts the rate of response to selection. V_A is often expressed in a standardized way, the 'evolvability' (the additive genetic coefficient of variation) or the narrow sense heritability (V_A divided by V_P). However, it is evident that these measures can change, even if V_A remains constant. The evolvability can change simply because the trait mean changes. The narrow sense heritability can change if just the environmental variance (V_E) changes (which would change V_P in the denominator of h^2). It has often been found that h^2 has changed significantly in stressful environments, but that this change was a function of a different V_E , with V_A relatively constant. Here, we present a dramatic example: V_A for wing area increased by a factor of 2.4 between the least- and most-stressful environments, but h^2 actually *decreased* in the same comparison because of the large increase in V_E .

V_A is a more appropriate measure of the evolutionary capacity of a population than heritability. If, for example, the slope of the relationship between fitness and phenotype stays constant independent of the distribution of phenotypes, then the contribution of changes in the genetics of a population to the response to selection are given by the changes in V_A . Changes in heritability would not predict the changes in response to selection with such constant selection. In mathematical terms, if the response to selection is given by $\Delta\bar{z}$ and the slope of the regression of fitness on trait value is β , then $\Delta\bar{z} = V_A\beta$ (Lande 1976). If the heritability changed because of a change in V_E , the response to selection would not change provided that β was constant. We would need to know how V_A changed to understand the future response to selection.

Finally, other studies have suffered from the possibility of alternative interpretations. Parent-offspring studies, in which parents and offspring are raised in different environments, can produce results affected by genotype by environment interactions; most studies of vertebrate populations encounter this difficulty (see Merilä & Fry (1998) for a well documented example). In our study, genetic variation is determined by the variation among lines with known genetic relationship and all individuals raised simultaneously in the same environment. Thus genotype by environment interaction cannot account for changes in genetic variance observed.

We have used a series of experiments to investigate the effects of population bottlenecks and inbreeding on genetic and phenotypic variation. Previously, we found that the V_A , on average, decreases in keeping with the simple additive expectation after a population bottleneck, but that different inbred lines individually have extremely different V_A (Whitlock & Fowler 1999), V_E (Whitlock & Fowler 1999), $\mathbf{G}$ matrices (Phillips *et al.* 2001) and V_P (Fowler & Whitlock 1999a). This study shows that the variance components of inbred lines are even more unpredictable if the environment changes as well. Inbred lines, on average, did not differ significantly in their sensitivity to macroenvironmental change from control lines, but they showed substantial variation among lines in this

sensitivity. The phenotypic or genetic variance of an inbred line will, therefore, be even more difficult to predict as the environment changes; conversely, environmental change allows another avenue for variation in crucial evolutionary parameters.

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