The Changes in Genetic and Environmental Variance With Inbreeding in *Drosophila melanogaster*

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ABSTRACT

We performed a large-scale experiment on the effects of inbreeding and population bottlenecks on the additive genetic and environmental variance for morphological traits in *Drosophila melanogaster*. Fifty-two inbred lines were created from the progeny of single pairs, and 90 parent-offspring families on average were measured in each of these lines for six wing size and shape traits, as well as 1945 families from the outbred population from which the lines were derived. The amount of additive genetic variance has been observed to increase after such population bottlenecks in other studies; in contrast here the mean change in additive genetic variance was in very good agreement with classical additive theory, decreasing proportionally to the inbreeding coefficient of the lines. The residual, probably environmental, variance increased on average after inbreeding. Both components of variance were highly variable among inbred lines, with increases and decreases recorded for both. The variance among lines in the residual variance provides some evidence for a genetic basis of developmental stability. Changes in the phenotypic variance of these traits are largely due to changes in the genetic variance.

MORPHOLOGICAL evolution by natural selection proceeds at a rate dependent on the amount of additive genetic variance for a trait (Fisher 1958). The pace of evolution is also affected by the variation in phenotypes due to environmental effects, because any increase in the phenotypic variance can change the shape of the mean fitness function and potentially change the topology of the adaptive landscape (Kirkpatrick 1982; Whitlock 1995a). Furthermore, qualitative changes in the genetic basis of phenotypic variance can result in genetically divergent populations even with uniform phenotypic selection (Cohan 1984; Goodnight 1995). Understanding the nature of phenotypic variation and its components is one of the central goals of evolutionary biology.

One factor long known to affect the amount and nature of phenotypic variation is genetic drift. Periods of small population size, and the genetic drift and inbreeding that occur as a result, can affect the amount and nature of variation within populations in many ways. Phenotypic variation does change dramatically as a result of bottlenecks and inbreeding for a wide range of traits and species (Fowler and Whitlock 1999). However, the magnitude and even direction of these changes is extremely variable, ranging from 10-fold declines to 6-fold increases. The causes of these changes in phenotypic variance (V_P) and of its components remain relatively unexplored.

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There are essentially two opposing factors to how and why V_P changes with inbreeding. Traditionally, the quantitative genetic view is that the amount of genetic variance would be reduced by inbreeding. The assertion made from this theory is that inbred individuals should be less genetically variable as a result of genetic drift [an assumption that formed the basis of using inbred lines as research organisms (see McLaren and Michie 1954)]. On the other hand, others have championed the view that inbred individuals would be *more* diverse, even within inbred lines, because more homozygous individuals would be less stable in their development. The effects of variation in the environment would therefore be increased in inbred individuals (Lerner 1954). Consequently, inbred lines would have a higher environmental variance (V_E) and a higher V_P. A considerable number of studies of V_P were produced, which showed highly variable results (Lerner 1954; Wright 1977; Fowler and Whitlock 1999). The reasons for the observed changes remain obscure, however, because few studies have partitioned the variance of inbred lines.

The amount of genetic variation is, for simple models, expected to decrease (Wright 1951; Falconer 1981). Recently, the traditional view of changes in the additive genetic variance (V_A) from inbreeding has been challenged by several researchers who have theoretically and empirically discovered that, contrary to the simple expectation, the amount of V_A can increase after inbreeding. Additive genetic variance can increase after a bottleneck if there are certain kinds of dominance variance (Robertson 1952; Tachida and Cockerham 1989; Willis and Orr 1993), because on average the effects of rare recessive or overdominant alleles can be

more additive after the genetic drift that accompanies small population size. Furthermore, epistatic variance will, on average, partially be transformed into additive genetic variance after bottlenecks (Bryant *et al.* 1986; Cockerham and Tachida 1988; Goodnight 1988; Whitlock *et al.* 1993; Whitlock 1995b).

Furthermore, recent theory demonstrates that the change in genetic variance following population bottlenecks will in some circumstances be itself extremely variable, so that some lines might increase in V_A after bottlenecks even if on average V_A decreases (Avery and Hill 1977; Lynch 1988; Whitlock 1995a). The magnitude of this variance among populations in the genetic variance is determined by many factors, some of which are quite difficult to measure or predict, including the effective population size, the number of loci affecting the trait, the distribution of allelic effects, and the nature of genetic interaction within and between loci (Whitlock 1995a). There is little data on the true magnitude of this variance in variance, but the fact that genetic variance potentially can substantially vary from one population to another has profound consequences on evolution on complex landscapes, evolution of structured populations, and experimental design.

Many empirical studies have shown that the amount of additive genetic variance can increase, on average, after population bottlenecks (Bryant *et al.* 1986; López-Fanjul and Villaverde 1989; Bryant and Meffert 1993, 1995, 1996; Fernández *et al.* 1995; Wade *et al.* 1996). Many of these studies deal exclusively with fitness components, and only four studies with relatively few replicate inbred lines deal with morphological variation. Bryant *et al.* (1986) have shown that several morphological characters in the housefly can increase in additive genetic variance after population bottlenecks, but the other studies that examined morphological characters [bristle number in *Drosophila melanogaster* (Frankham 1980), pupal size in *Tribolium castaneum* (Wade *et al.* 1996), and wing characters in *Bicyclus anynana* (Brakefield and Saccheri 1994)] have not found increases in V_A. Courtship behavior in houseflies similarly does not show any increase in V_A after bottlenecks (Meffert 1995). None of these studies were conducted on a sufficient scale to measure the variability in the changes in genetic variance.

Studies of the changes in genetic variance from inbreeding should be conducted on a much larger scale than they have been traditionally, to reduce the possibility of Type I errors in our measures of the effects of bottlenecks (Lynch 1988). We set out to perform such a large experiment, to measure the changes in V_A from inbreeding, the changes in the environmental variance, and the variance of these changes.

We have previously demonstrated that the phenotypic variance for several characters changes as a result of population bottlenecks, increasing in some lines and decreasing in others (Fowler and Whitlock 1999), which allowed us to investigate further the causes of these changes in variance. Here we report a large-scale experiment on bottlenecked populations of the fruit fly, *D. melanogaster*, which consists of 52 inbred populations and 6 outbred populations, with an average of 90 parent-offspring families measured in each inbred line and over 320 families for each outbred line. Approximately 80,000 flies have been measured for six morphological traits to examine the effects of inbreeding and small population size on phenotypic variance.

MATERIALS AND METHODS

Stocks, derivation of inbred lines, and family structure: The flies used in this study were taken as a random sample from a large outbred population of *D. melanogaster* collected in Dahomey (now Benin) in 1970. This stock has since been maintained at a large population size in cage culture and exhibits significant levels of genetic variation (Wilkinson *et al.* 1990; Whitlock and Fowler 1996). This strain is well adapted to laboratory conditions, which prevents strong selection for a novel environment from confounding or obscuring the results. All flies were kept at $25 \pm 1°$ with a constant illumination cycle of 12 hr light followed by 12 hr dark. All handling of the flies was performed at room temperature using either CO_2 anesthesia or ice anesthesia. Unless stated otherwise, flies were maintained in standard food vials (75×25 mm diameter) with 7 ml of medium.

The derivation of the inbred lines is the same as that reported in Fowler and Whitlock (1999). Fifty-two inbred lines were derived from a single outbred source population divided into three batches separated in time by ~3 mo. These lines were each created from the offspring of a single virgin pair of randomly chosen and randomly mated flies, so that the inbreeding coefficient of these lines was originally $\sim F = 0.25$. At the same time, two outbred populations were derived for each batch, each from the offspring of 200 randomly chosen pairs, including all of the parents of the inbred lines. For each of the inbred and outbred lines, flies from the F_2 generation were allowed to lay eggs on grape juice medium for collection of same-age, constant density sets of larvae to grow into the F_3 generation, which were measured for a set of six morphological traits (see below and Figure 1) and used as the parents of the present study.

For each inbred line, 120 pairs of virgin adults were paired at random (except that they were never taken from the same rearing vial, to eliminate the possibility of sib mating). For each of the six outbred lines, ~400 such pairs were created. On 2 consecutive days, pairs of adult flies (4–7 days posteclosion) were allowed to lay eggs in a fresh food vial for 24 hr, which produced two separate vials of offspring for each family. After this the pairs of parents were collected and frozen at $-20°$ for later measurement. The offspring of these families were then raised in these medium density vials until they emerged as adults, when they were collected and frozen.

Traits and measurement techniques: For each family, both wings from both parents were mounted on microscope slides for measurement of various size and shape characteristics of the wings, using propan-2-ol and Aquamount fixation. A dissecting microscope with *camera lucida* attachment and a Quora A3 digitizing tablet attached to a Macintosh computer were used to electronically measure the wings (see Fowler and Whitlock 1999 for more details). The right wings from each of four female offspring from each of the two vials per family were mounted and measured. Thus there were eight daughters

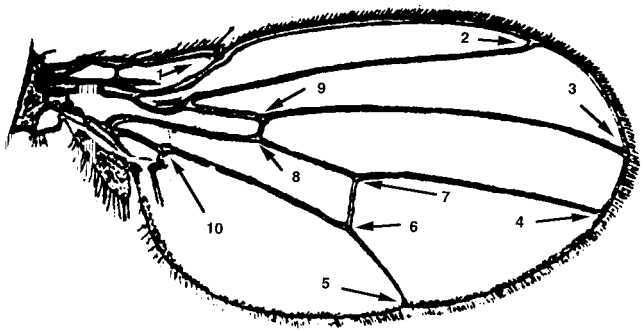

Figure 1.—The landmarks used to generate characters. Ten landmarks were measured for each wing, labeled from 1 to 10. The measurements were consistently made from the same point of the junction of the wing veins. The characters used were wing area (the area of the polygon defined by vertices at points 1, 2, 3, 4, 5, and 10), and the angles formed by the points 5-7-4, 8-7-6, 2-9-3, 2-1-5, and 2-3-5 (with the vertex listed as the middle point).

measured from each family. Only complete families were used (some pairs produced no offspring; breakage or loss of any component of a family also resulted in the whole family being discarded), which meant that an average of 90 families per inbred line and a total of 1945 families from outbred lines were analyzed.

The wings were measured for 10 landmarks each (see Figure 1), which were converted into six size and shape characters for analysis. The size of the wing was estimated by the area of the polygon defined by six points around the perimeter of the wing. The other five characters were angles whose vertices are defined by the intersections of the veins of the wings. The legend to Figure 1 lists these five characters. Angles were used because of their geometrical independence of size; therefore they are a useful measure of shape.

As reported in Fowler and Whitlock (1999), a subset of the parental flies in each line was also measured for an estimate of fitness, the productivity of a pair of flies over a 72-hr period. The means of these fitness measures were used to examine the possible relationship between changes in variance components and fitness.

STATISTICAL METHODS

Midparent-offspring analyses: The additive genetic variance for a trait can be estimated by twice the covariance between the average of the parents and the average of the offspring (Falconer 1981). Parents were chosen at random within lines, so there was no correlation between the phenotypic values of the parents within lines. Hence the variance among the midparent values should be half of the phenotypic variance. The residual variance (which we refer to as V_E) was estimated as the difference between this V_P estimate and the V_A estimate. This residual variance includes the environmental variance, as well as some of the nonadditive genetic variance terms. Parent-offspring analysis was performed separately for each of the 52 inbred lines and for the 1945 control outbred families.

Statistical testing: All statistical tests, unless otherwise noted, were conducted by resampling families from the control populations (with the mean of each batch subtracted from each individual value to eliminate batch effects). The pseudosamples were taken in the same sampling structure as the actual data (including the outbred population comparisons), and the parameters were estimated in the same way for each pseudosample. The distribution of these pseudostatistics was used as the null distribution for testing the null hypotheses and generating standard errors.

For example, the variance among lines in V_A was tested against the null hypothesis that all lines had the same V_A by sampling with replacement from the control families the equivalent number of families for each line, to make a pseudodata set the same size as the actual data set. The variance in V_A was calculated for this pseudodata set, and the process was repeated 10,000 times. The P value was then calculated as the proportion of pseudostatistics lying at or beyond the observed value. A similar procedure was followed for each of the statistical tests, unless otherwise noted. Standard errors of the estimates were calculated from the standard deviation of the distribution of pseudostatistics.

TABLE 1

Variance components of the outbred population

Trait	V_A	SE (V_A)	V_E	SE (V_E)	h^2
Wing area	12.12	1.26	5.28	0.77	0.64
Angle 5-7-4	9.09	0.39	5.42	0.23	0.64
Angle 8-7-6	21.83	0.79	22.66	0.88	0.52
Angle 2-9-3	0.90	0.04	0.37	0.02	0.71
Angle 2-1-5	1.97	0.13	0.56	0.35	0.81
Angle 2-3-5	5.76	0.35	1.59	0.12	0.81

All variances and standard errors have been multiplied by 10^4 for ease of viewing.

RESULTS

The variance components of outbred flies: The variance components of the control outbred populations are given in Table 1. There is substantial additive genetic variance, with heritabilities for the different traits ranging from 0.5–0.8. Only the variance components for wing area showed significant variance among batches.

The distribution of variance components among inbred lines: The additive genetic variance and the residual variance were calculated for each of the 52 inbred lines, from an average of 90 full families in each line. The distributions of the V_A estimates (standardized by the same estimate in the controls) are given in Figure 2, and many of the statistics describing these distributions are given in Table 2. Similarly, Figure 3 and Table 3 give the results for the V_E estimates.

The V_A distributions have several interesting properties. First, there is no evidence for any average increase in V_A in the bottlenecked lines. Instead, for each of the six characters, V_A decreased significantly ($P \ll 0.001$ for each character, t-test on variance ratios) from the outbred populations. The average decline in V_A over all traits was ~32%. This inbred V_A was less than the additive expectation derived from accounting for only the drift effects of the bottleneck generation itself for all of the characters and was significantly less for two characters (wing area, $P = 0.008$, and angle 2-3-5, $P = 0.0094$). The effective population size of the inbred lines would

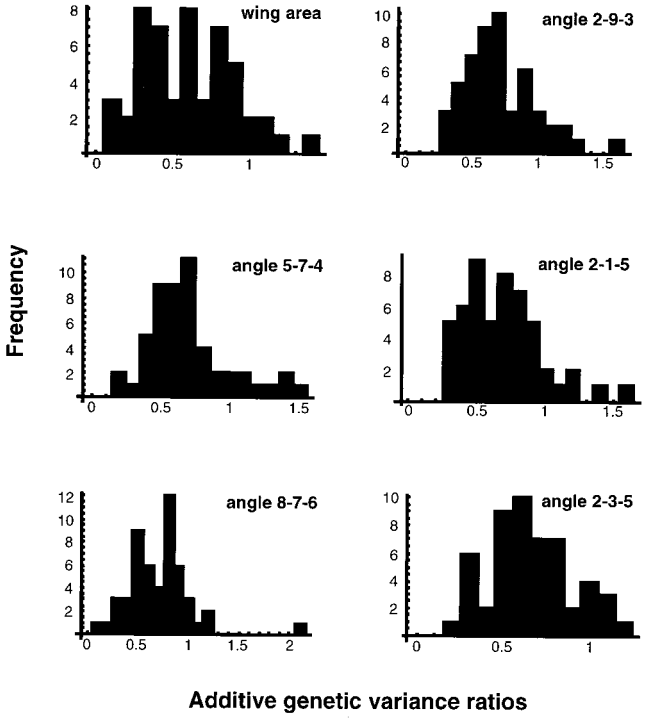

Figure 2.—The distribution of additive genetic variance among lines for each character. The variance ratio is the ratio of the additive genetic variance for each inbred line standardized by the same estimate in the outbred populations.

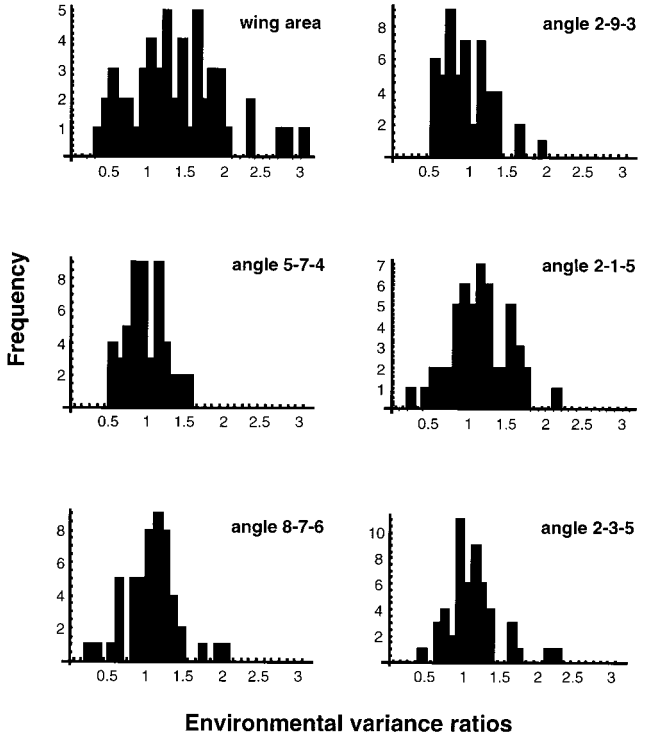

Figure 3.—The distribution of environmental variance among lines for each character. The variance ratio is the ratio of the environmental variance for each inbred line standardized by the same estimate in the outbred population.

have been lower than the census size during the intervening generations after the bottleneck, allowing for more drift. Effective population sizes in laboratory populations are often a small fraction of their census population size (Briscoe *et al.* 1992). To account for the change in V_A by a strictly additive model, the effective population size during the F_1 and F_2 generations would have had to have been ~7% of the census size, a figure consistent with estimates of N_e/N made from changes in heterozygosity (Frankham 1995).

There is significant variation among the lines in the extent of genetic variation (see Table 2). The standard deviation across lines of V_A, corrected for the variance in V_A expected due to sampling error, is 25–37% of the mean amount of V_A.

With an average decline in the amount of additive genetic variance and a large variance among lines in the amount of change in V_A, it becomes interesting to ask whether there are any lines that display evidence of an increase in V_A for any character. In fact, of the 312 character/line combinations, 34 increased in variance

TABLE 2

The distribution of additive genetic variance (V_A) among the inbred lines

Trait	Mean V_A ($\times 10^4$)	$V(V_A)$[a] ($\times 10^8$)	P value $V(V_A)$	Expected $V(V_A)$ due to error[b] ($\times 10^8$)	Estimated $V(V_A)$[b] ($\times 10^8$)	CV (V_A)[c]	Mean h^2	Mean variance ratio for V_A
Wing area	7.2	14.0	<0.001	7.04	7.00	36.7	0.44	0.60
Angle 5-7-4	6.3	6.89	<0.001	3.25	3.64	30.2	0.53	0.70
Angle 8-7-6	15.6	45.0	<0.001	24.2	20.9	29.3	0.42	0.71
Angle 2-9-3	0.64	0.06	<0.001	0.033	0.026	25.5	0.42	0.71
Angle 2-1-5	1.3	0.31	<0.001	0.13	0.18	31.5	0.68	0.68
Angle 2-3-5	3.8	1.88	<0.001	0.97	0.91	25.1	0.65	0.66

[a] $V(V_A)$, variance among lines in V_A.
[b] The expected $V(V_A)$ due to sampling error alone, estimated from 1000 replicates of resampling from the control families, was subtracted from the observed $V(V_A)$ to estimate the true variance in additive genetic variance across lines.
[c] CV, coefficient of variation.

TABLE 3

The distribution of environmental variance (V_E) among the inbred lines

Trait	Mean V_E ($\times 10^4$)	$V(V_E)$[a] ($\times 10^8$)	P value $V(V_E)$	Expected $V(V_E)$ due to error[b] ($\times 10^8$)	Estimated $V(V_E)$[b] ($\times 10^8$)	CV (V_E)[c]	Mean variance ratio for V_E
Wing area	7.3	10.6	<0.0001	5.1	5.6	32.2	1.38
Angle 5-7-4	5.2	2.79	0.016	1.9	0.92	18.3	0.96
Angle 8-7-6	22.2	36.2	0.016	24.4	11.82	15.5	0.98
Angle 2-9-3	0.43	0.0193	0.015	0.013	0.0064	18.6	1.14
Angle 2-1-5	0.60	0.0348	0.86	0.044	−0.0095		1.08
Angle 2-3-5	1.8	0.295	0.85	0.37	−0.077		1.11

[a] $V(V_E)$, variance among lines in V_E.
[b] The expected $V(V_E)$ due to sampling error alone, estimated from 1000 replicates of resampling from the control families, was subtracted from the observed $V(V_E)$ to estimate the true variance in the residual variance across lines.
[c] CV, coefficient of variation.

relative to the controls. For one of these, the V_A for angle 8-7-6 in one line, the increase was significant even with a conservative correction for multiple comparisons ($P < 0.01$, Dunn-Šidák correction for 312 multiple comparisons). Eight of the 312 character/line combinations increased significantly in variance relative to the additive expectation with $F = 0.3$ (the Dunn-Šidák correction for multiple comparisons is again included). These data provide evidence that V_A can either increase or decrease as a result of inbreeding, but for these characters, a decrease is much more likely.

In contrast to genetic variance, the amount of residual variance increased significantly with inbreeding for four of the six characters (see Table 3). On average, V_E increased by 11% relative to the outbred flies. This average change in the residual variance, however, is accompanied by substantial variation among lines in V_E for four characters (Table 3). The coefficient of variation of the change in V_E ranges from 16 to 32% for the characters with significant change in variance.

Large numbers of lines increased in V_E relative to the controls (209 of 312 comparisons). Three of the 312 comparisons decreased significantly at $P < 0.01$, which is almost exactly the expected Type I error rate. The two characters that do not have significant average increases in variance, angles 5-7-4 and 8-7-6, had some lines that did increase in variance. Hence V_E can either increase or stay the same, with an increase being more likely.

These changes in V_A and V_E are only slightly correlated; for each of the angle characters there is a positive covariance among lines in the two variance components (see Table 4), but this covariance is only marginally significant, as tested by a bootstrap, when corrected for multiple comparisons. The covariance between the raw estimates of the variance components was corrected by subtracting the expected error covariance, which was strong and negative because of the way V_E is defined as the difference between V_P and V_A. The changes in the mean values of these traits were not correlated with changes in V_A or V_E for any of the traits, as tested by simple correlations.

Variance components and fitness: The fitness measures demonstrated significant inbreeding depression (on average, a 28% reduction in fitness) and substantial variation among lines (K. Fowler and M. C. Whitlock, unpublished results). None of the characters show a significant relationship between V_A and fitness across

TABLE 4

The covariance of genetic and environmental variance components

Trait	$\mathrm{Cov}(V_A, V_E)$[a]	Expected error $\mathrm{Cov}(V_A, V_E)$[a]	Adjusted $\mathrm{Cov}(V_A, V_E)$[a]	P value $\mathrm{Cov}(V_A, V_E)$	Estimated correlation (V_A, V_E)
Wing area	−3.99	−2.57	−1.42	0.166	−0.23
Angle 5-7-4	0.50	−0.91	0.59	0.0968	0.32
Angle 8-7-6	7.4	−1.3	8.7	0.0142	0.55
Angle 2-9-3	0.0019	−0.0018	0.0037	0.2084	0.29
Angle 2-1-5	0.016	−0.0084	0.025	0.0218	
Angle 2-3-5	0.0056	−0.10	0.11	0.2272	

[a] All covariance and variance terms are shown multiplied by 10^8 for convenience.

TABLE 5

Correlations (among lines) of the variance components of different traits

	Correlations among V_A's				
	V_A angle 5-7-4	V_A angle 8-7-6	V_A angle 2-9-3	V_A angle 2-1-5	V_A angle 2-3-5
V_A area	0.1396	0.0339	0.0041	0.0687	0.1378
V_A angle 5-7-4		0.0907	0.0806	0.4141**	−0.0139
V_A angle 8-7-6			0.1209	0.0848	−0.0083
V_A angle 2-9-3				0.3164**	0.0375
V_A angle 2-1-5					−0.0056
	Correlations among V_E's				
	V_E angle 5-7-4	V_E angle 8-7-6	V_E angle 2-9-3	V_E angle 2-1-5	V_E angle 2-3-5
V_A area	0.0612	0.2718	0.1344	0.2916*	−0.0832
V_A angle 5-7-4		0.3686**	−0.0569	0.1504	0.0329
V_A angle 8-7-6			−0.0077	0.0622	−0.1900
V_A angle 2-9-3				0.3617**	−0.1752
V_A angle 2-1-5					−0.0688

* $P < 0.05$; ** $P < 0.01$.

lines. A pooled measure of variance, the sum of the V_A of each character standardized by the V_A of the controls, is also not significantly related to fitness ($P = 0.14$).

Similarly, there is no strong relationship between V_E and fitness for the angle characters. Fitness is significantly correlated with V_E for wing area ($r = 0.33$, $\beta = 25{,}209$, $P < 0.01$), with lines with higher V_E having lower fitness.

Correlations among variance components: These changes in the variance components are also correlated across traits. Twelve of the 15 correlations among V_A measures are positive, which is significantly many ($P = 0.035$, two-tailed sign test). Table 5 shows the estimated correlations.

The changes in V_E are also weakly correlated across some traits. There is thus some support for the idea that the developmental stability of an individual is correlated across characters.

DISCUSSION

This project had three goals: to understand the changes in genetic variance with bottlenecks, to measure the changes in environmental variance that might come with inbreeding, and to discover the reasons for the distribution of change in morphological phenotypic variance (as reported in Fowler and Whitlock 1999). We wanted to understand these changes not only as an average, but also to measure the variability among lines in these changes.

Additive genetic variance has been observed to increase as a result of population bottlenecks (Bryant *et al.* 1986; López-Fanjul and Villaverde 1989; Fernández *et al.* 1995; Meffert 1995; Wade *et al.* 1996). The present study repeats this type of analysis, with a much higher level of replication, on six morphological characters in Drosophila. *None* of these six characters increased in V_A on average relative to the outbred population. In fact, the additive genetic variance declined on average according to the expectation from classical additive theory. Clearly, the amount of genetic variance does not always increase after population bottlenecks.

There are several possible reasons for the discrepancy between these and previous results. First, the traits used here are all morphological characters, whereas in most cases (all except for the housefly studies) the characters that have been shown to increase in V_A after bottlenecks are fitness components. In fact, the only study to date that has compared the changes in variance for a fitness component to that in a morphological trait (Wade *et al.* 1996) has shown a much stronger decrease in V_A for the morphological trait after bottlenecks. The genetic architecture of fitness may be much more complicated than that of morphology, and therefore fitness components may behave differently. In particular, fitness is affected by many more genes than other traits. Thus linkage disequilibrium may play a more important role in the changes in variance, as disequilibria between closely linked loci take longer to dissipate and drift-induced linkage disequilibrium has been suggested as a possible reason for the increases in genetic variance (Lynch 1988). In addition, genetic variation for fitness components is more likely than morphology to be due to the effects of rare, recessive alleles, which are known to be able to contribute to an increase in genetic variance after bottlenecks (Robertson 1952). Furthermore, the genetic variance for fitness is known to increase with stress, such as that imposed by less favorable environments (see, *e.g.*, Hoffmann and Parsons 1991; Kondrashov and Houle 1994). This "stress" seems to

exaggerate the fitness differences between genotypes and therefore to increase the genetic variance, *even without gene frequency change*. Because inbreeding depression due to some loci may act as a source of stress at other loci, it is possible that the increases in genetic variance observed after inbreeding for fitness components may not reflect gene frequency change directly, but rather may merely be a by-product of inbreeding depression. We therefore expect that fitness components would behave differently after inbreeding than most morphological characters.

The only studies in the past that have shown increases in V_A after bottlenecks for morphological characters are those by Bryant and Meffert that use houseflies, but other researchers have performed similar experiments without finding the same results. Frankham (1980) found a decrease in the response to selection for abdominal bristle number in inbred lines of Drosophila in keeping with the simple additive expectation. Wade *et al.* (1996) measured V_A after bottlenecks for pupal weight in *T. castaneum*; the change in variance for pupal weight was in keeping with the $(1 - F)$ expectation of simple additive theory. Brakefield and Saccheri (1994; I. Saccheri, personal communication) looked for changes in V_A after bottlenecks for several wing characteristics of butterfly wings and also found decreases rather than increases.

There is one important difference between this study and these previous studies, and that is the scale of the replication. Lynch (1988) has suggested that the level of replication among lines from Bryant *et al.*'s (1986) study was too low and therefore there may have been a Type I error. Similarly, the negative results reviewed above may have underestimated the increases in variance because of their relatively low levels of replication. The present study corrects this potential problem. It is interesting to note that there were substantial numbers of lines in the present study in which the amount of V_A did increase, sometimes significantly.

One of the most important results of this study is that the change in genetic variance after a population bottleneck is not uniform, but rather the replicate lines are extremely variable in how much genetic variation they expressed. The range in additive genetic variance was substantial: for one character, angle 8-7-6, V_A ranged from 14 to 211% of that of the outbred control. The other characters showed lower, but similar, ranges. It is clear that knowing the average change in genetic variance attributable to a population bottleneck is insufficient to predict what will happen in any given population. Any evolutionary process that depends on rare events [such as the variance-induced peak shift model (Whitlock 1995a)] will be greatly affected by this variability among populations in the variance components. This variability in the changes in variance has been predicted by theory (Avery and Hill 1977; Lynch 1988; Whitlock 1995a), yet these are the first data that unambiguously demonstrate this effect. Whatever factors cause the changes in additive genetic variance are also influenced by drift in gene and genotype frequency.

Furthermore, it is also clear that knowing the change in variance in a small number of lines is insufficient to predict the expected change in variance. Measures of genetic parameters in inbred populations will therefore require large replication both within and among lines to get reasonable standard errors. It is interesting to note, however, that the standard errors of estimates of the mean variance change are not so high as predicted by Lynch (1988). Our standard errors are ~6% of the mean, even with 52 lines derived from two individuals each. Lynch (1988) predicts that the standard errors would be about three times that large.

The changes in environmental variance that we have observed are in the same direction as found previously by Lerner (1954) and others (King 1918; Mather 1950; Rasmusen 1952; Grüneberg 1954; McLaren and Michie 1954; Sheldon *et al.* 1964): the sensitivity of development to environmental differences tends to increase with inbreeding. This change averaged an ~11% increase. Measurements of changes in V_E have been made in the past from comparisons of extremely inbred lines and hybrids among them. These increases in V_E from extreme inbreeding averaged ~58% when the inbreeding coefficient was $F = 1$ (Fowler and Whitlock 1999), which is not significantly different from three times the change we observe in V_E here (where $F \approx 0.32$). Thus the changes in morphological variance due to changes in V_E for these characters are consistent with those due to a wide variety of characters in a wide variety of organisms.

The changes in V_E are not constant across lines, however. The amount of change in V_E varies significantly for most characters across lines, which is evidence for genetic variability for developmental homeostasis, when we assume that most of the residual variance is due to environmental factors and not nonadditive genetic variance. Such heritability of developmental stability has been posited before and measured indirectly (see, *e.g.*, Mather 1950; Reeve 1960), but we believe this represents the first direct demonstration of such genetic variance.

The changes in environmental variance are also weakly correlated across some traits. The lines that are more developmentally stable for one trait are likely to be more stable for other traits. This type of correlation across characters in developmental stability is unusual; in the past many studies have looked for a correlation of the fluctuating asymmetry of different traits and have not found it or found it to be very small indeed (see Whitlock 1996). V_E is quite likely to be a more powerful measure of the developmental stability of an organism, and therefore this correlational pattern can be seen here, albeit weakly.

Finally, what can we say about the causes of changes

in the phenotypic variance? V_P changes because of changes in both V_A and V_E, with changes in V_A tending to reduce V_P and the opposite for changes in V_E. Most of the variation in V_P after mild inbreeding is explained by differences in V_A, however. The V_P of these lines changed in an extremely variable way, but on average was reduced by inbreeding (Fowler and Whitlock 1999). This average decline in variance reflects the fact that the heritability of these characters is quite high and therefore that V_P is composed in large fraction by V_A, coupled with the fact that the proportional change in V_A is greater than that of V_E (an average change of -32% for V_A *vs.* 11% for V_E). Furthermore, there was more variance among lines in the amount of V_A than there was of V_E (the coefficients of variation were on average 30% *vs.* 14%, respectively). Thus V_A changed more on average and was more variable than V_E, and so was a much more important cause of the differences in V_P.

In summary, inbreeding causes changes in the phenotypic variance, as a result of changes in both the additive genetic and other components of variation. On average, the changes in these variance components are in accordance with simple theory: a decline in genetic variance nearly in proportion to the inbreeding coefficient and an increase in environmental variance. These averages belie significant variability among populations in the changes in genetic and environmental variance components. Theoretical investigations of the consequences of small population size must account for this heterogeneity among populations.

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