Inbreeding Changes the Shape of the Genetic Covariance Matrix in *Drosophila melanogaster*

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ABSTRACT

The pattern of genetic covariation among traits (the **G** matrix) plays a central role in determining the pattern of evolutionary change from both natural selection and random genetic drift. Here we measure the effect of genetic drift on the shape of the **G** matrix using a large data set on the inheritance of wing characteristics in *Drosophila melanogaster*. Fifty-two inbred lines with a total of 4680 parent-offspring families were generated by one generation of brother-sister mating and compared to an outbred control population of 1945 families. In keeping with the theoretical expectation for a correlated set of additively determined traits, the average **G** matrix of the inbred lines remained proportional to the outbred control **G** matrix with a proportionality constant approximately equal to $(1 - F)$, where F is the inbreeding coefficient. Further, the pattern of covariance among the means of the inbred lines induced by inbreeding was also proportional to the within-line **G** matrix of the control population with a constant very close to the expectation of $2F$. Although the average **G** of the inbred lines did not show change in overall structure relative to the outbred controls, separate analysis revealed a great deal of variation among inbred lines around this expectation, including changes in the sign of genetic correlations. Since any given line can be quite different from the outbred control, it is likely that in nature unreplicated drift will lead to changes in the **G** matrix. Thus, the shape of **G** is malleable under genetic drift, and the evolutionary response of any particular population is likely to depend on the specifics of its evolutionary history.

THE short-term response to both artificial and natural selection is influenced primarily by two factors: the amount of genetic variation for a trait and the strength of selection on that trait (Falconer and Mackay 1996). When the evolution of suites of many traits is considered, the pattern of genetic association or covariance among traits can also have a significant impact on the multivariate response to selection (Lande 1979). The genetic coupling between traits generated by pleiotropy and/or linkage means that selection acting on one trait can cause correlated responses in any trait that shares some genetic correlation with the trait under selection. When coupled with an analysis of the pattern of multivariate selection (Lande and Arnold 1983), the course of quantitative evolution can be theoretically predicted from the joint knowledge of the genetic variance/covariance matrix (**G**) and the selection gradients ($\boldsymbol{\beta}$; Lande 1979). Using this theory to predict the actual response to selection, however, has run into several difficulties. In particular, the genetic and environmental parameters described by **G** and $\boldsymbol{\beta}$ are subject to change because of changing environmental conditions, change induced by previous responses to selection, or other changes in the genetic system of the population. Genetic drift has long been known to change the amount of genetic variance. The effects of drift on the **G** matrix, though, are not expected to change the orientation of the matrix but merely to change its overall magnitude. Drift, on average, will scale the entire matrix by a scalar factor of $1 - F$, where F is the inbreeding coefficient (Wright 1951; Lande 1980a). This result, if true, is important, because it implies that drift would not affect the trajectory of evolution but merely the pace at which evolution would proceed.

This theoretical result, however, refers only to the *average* changes in **G** that result from drift. Currently there are no theoretical predictions about the distribution of changes in the **G** matrix caused by drift. It is well known that the additive genetic variances (the diagonal elements of **G**) change on average in predictable ways, but the distribution of such changes can be quite broad (Avery and Hill 1977; Lynch 1988; Zeng and Cockerham 1991; Whitlock 1995), which is a result that was confirmed empirically (Whitlock and Fowler 1999). We would therefore expect more generally that the entire **G** matrix would also vary around an expectation as a result of drift. Because each of the components of **G** is a function of allele frequencies, and because these frequencies change unpredictably as a result of drift, it is clear that drift should lead to heterogeneity

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in the changes in **G** as well. If so, then the shape of the **G** matrix in any particular population could change considerably but unpredictably.

The expectation of overall proportional change in the elements of **G** is a change in matrix "size." Variation in the orientation of the covariances among the traits, or element-specific changes, can then be referred to as changes in matrix "shape." Matrix shape is frequently described using the principal component or eigen structure of the matrix (Flury 1988). If in fact the shape of the genetic covariance matrix was different in populations with varying histories of drift, then even with uniform selection pressure the response to selection in these populations could be very different (Lande 1979; Schluter 2000; although see Zeng 1988). Hence it is important to understand how inbreeding alters the genetic associations among morphological traits.

Various lines of empirical evidence suggest that the **G** matrix is not constant, but how much does it change over time? For example, Arnold and Phillips (1999) showed that the **G** matrix of Thamnophis garter snakes diverged among populations while maintaining underlying similarities in matrix structure. Other studies found differences in **G** measured in different populations or related species, while some found relative constancy of **G** (see reviews in Roff 1997; Arnold and Phillips 1999; Roff *et al.* 1999). There is less experimental work than strict observation in this field, but Bryant and Meffert (1988) showed that the average shape of a small number of **G** matrices estimated for the housefly *Musca domestica* changes as a result of population bottlenecks. Camara and Pigliucci (1999) and Camara *et al.* (2000) showed that induced mutations could change the structure of **G**.

In addition to causing an average reduction in genetic variance within populations, genetic drift also tends to increase the genetic variance among populations. Under a strictly additive model of genetic variation, the amount of genetic variance among populations is expected to be $2F$ times the genetic variance of the base population (Wright 1951). Genetic drift should affect the whole **G** matrix in a similar way such that the matrix of variances and covariances among population means has the expectation $2F\mathbf{G}$ (Lande 1979, 1980a). This provides a way to predict the extent and pattern of divergence among populations under genetic drift. Schluter (1996) showed that species pairs are much more likely to diverge along the "genetic lines of least resistance," *i.e.*, along the major axes of the **G** matrix, which he interprets as evidence of the influence of **G** on response to selection. However, if the main source of population divergence were genetic drift, we would also expect most divergence along these major axes (Lande 1979).

In a large study of the changes in variance due to population bottlenecks in *Drosophila melanogaster*, we found that on average the additive genetic variance within lines for a set of six wing characters declined quantitatively as expected by the additive theory. However, changes in genetic variance varied considerably among the lines (Whitlock and Fowler 1999). Within-line phenotypic variance for the characters also decreased, and this change was also extremely variable (Fowler and Whitlock 1999a), including the phenotypic variation in fitness (Fowler and Whitlock 1999b). The changes in phenotypic variance were not as extreme as in genetic variance because the environmental variance on average increased (Whitlock and Fowler 1999). In total, these results indicated that classical theory was correct and sufficient to predict the *expected* change in variance components but not to predict what might happen in any given population.

In this article we investigate the changes in genetic and environmental covariance matrices that may result from the inbreeding during a population bottleneck. We use a recent innovation in matrix comparison that allows simultaneous investigation of the changes in both the size and shape of these matrices (Flury 1988; Phillips and Arnold 1999). We show that while the mean change in the genetic covariance matrix follows additive expectations almost exactly, the matrices from different inbred lines are extremely variable. Distinct populations could evolve quite differently even under uniform selection pressure.

MATERIALS AND METHODS

Strains and derivation of inbred lines: The stocks, culture maintenance, and measurement procedures are described in Whitlock and Fowler (1999). Experiments were conducted in three batches separated in time by ~3 months. At each time point two independent sets of ~400 randomly mated pairs were used to form outbred control populations, totaling 1945 families over the six control lines. Over this same time period, 52 inbred lines were derived from the outbred base population via one generation of brother-sister mating, with ~90 families per inbred line being analyzed (4680 total families in the inbred lines). For each control and inbred line family, the wings of eight daughters were mounted on microscope slides and measured using a digitizing tablet attached to a computer. Ten landmarks on each wing were determined and used to measure one size and five shape characters on the basis of the angles made by the intersection of the wing veins (Figure 1).

Calculation of phenotypic, genetic, and environmental matrices: Values for the outbred controls were normalized to their replicate mean before calculations. Before calculating the pooled estimates for the inbred lines, values for each line were normalized to the line mean to keep the among-line variance from biasing the average within-line estimates. The among-line variances and covariances were calculated from the line means after they had been normalized to the mean value of the control line in their batch to eliminate inclusion of any among-batch variance in these estimates.

Quantitative genetic parameters were estimated from the regression of the mean trait value of all measured offspring in a family on the midparent value. Additive genetic variances were estimated as twice the parent-offspring covariance (Falconer and Mackay 1996). Additive genetic covariances were

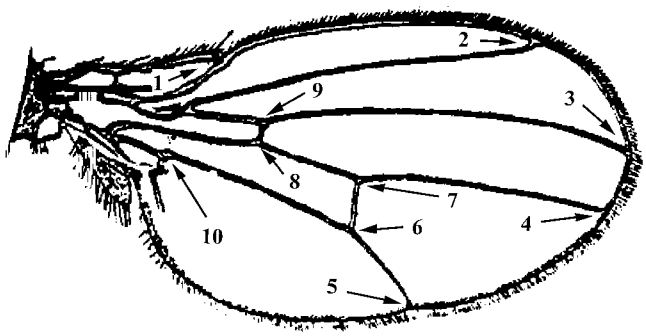

FIGURE 1.—*Drosophila melanogaster* wing characteristics measured in this study. Ten landmarks were measured for each wing, labeled from 1 to 10. The measurements were consistently made from the same point of the junction of the wing veins. The characters used were (A) wing area (the area of the polygon defined by vertices at points 1–5 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 mm^2 and the angles are measured in radians.

estimated as the sum of the covariance of trait one in the parents with trait two in the offspring and the covariance of trait two in the parents with trait one in the offspring. Phenotypic variances and covariances were estimated as twice the midparent values. Environmental variances and covariances were calculated from the difference of the phenotypic and additive genetic values; therefore, they include residual environmental variance as well as possibly some nonadditive genetic effects. Estimates of pooled parameters (*i.e.*, those that had been normalized prior to analysis) were adjusted by the appropriate value to correct for the loss in degrees of freedom caused by this procedure. Standard errors on each of these estimates were calculated using a bootstrap procedure in which families were repeatedly resampled from a given line with replacement followed by recalculation of the genetic parameters. The standard error was then calculated as the variance among these estimates (EFRON and TIBSHIRANI 1993). The mean of the bootstrap distribution provides an unbiased estimate of the underlying parameter (EFRON and TIBSHIRANI 1993). These analyses and estimates were calculated using the *h2boot* software program (PHILLIPS 1998a).

Comparison of matrices: Matrix comparisons were conducted using the approach outlined by FLURY (1988) and applied to quantitative genetic data by PHILLIPS and ARNOLD (1999). In brief, the Flury approach allows matrices to be compared along an entire hierarchy of possible hypotheses depending on their overall pattern of similarity. Two matrices that have the same values at each element are said to be equal, while matrices that differ by a single constant at each element are proportional. Alternatively, the matrices may have the same orientation in multivariate space (*i.e.*, have common principal components or eigenvectors) but differ in the amount of variation displayed along each axis. Finally, some of the axes of the matrices can be shared in common while others are oriented differently (the partial common principal component model). Any number of matrices can be compared simultaneously using this approach. Each hypothesis in this hierarchy is evaluated by testing its likelihood over the alternative of no shared structure. Including the partial common principal component models, there are eight hypotheses that can be tested for each comparison. Hypothesis testing begins at the bottom (*i.e.*, one common principal component) and is built up toward the endpoint of matrix equality (FLURY 1988). Any time a significant departure from the lower model is determined, the progression up the hierarchy is halted at that step (PHILLIPS and ARNOLD 1999). We tested the significance of each test using a randomization procedure in which the families from each line were randomly reassigned to different lines and retested for matrix similarity (PHILLIPS and ARNOLD 1999). Repeating this procedure many times allows the distribution of the test statistic under the null hypothesis of shared structure to be estimated. The significance of the actual test was then calculated as the percentage of runs that the randomization samples exceeded the likelihood statistic of the actual sample. This analysis was conducted using the *CPCrand* program (PHILLIPS 1998b) with 10,000 randomization runs per test.

Patterns of two-trait covariance were visualized by constructing 95% confidence ellipses of the bivariate variance-covariance matrix under the assumption of normality. Principal components of the matrix were calculated and used to orient the ellipse in the plane. Distance along each principal axis was calculated as 1.96 times the eigenvalue associated with that particular axis. These ellipses are useful for comparing the covariance patterns of two matrices (PHILLIPS and ARNOLD 1999) but are fairly simplified two-dimensional projections of a complex multidimensional space. The absolute orientation of these plots is also influenced by the scale of measurement, which is unnormalized in this case.

RESULTS

Patterns of genetic covariance: The average **G** matrices for the control and inbred lines are shown in Table 1. In the control lines, phenotypic correlations varied from −0.44 to 0.54, while genetic correlations varied from −0.27 to 0.60. Environmental correlations tended to be smaller and more variable (Table 2). As was found in WHITLOCK and FOWLER (1999), there is substantial variation among inbred lines in both their variance and covariance estimates (see values in brackets in Tables 1 and 2).

Comparison of inbred and outbred genetic covariance matrices: First, let us consider the changes in the average (*i.e.*, pooled) **G** matrix in the inbred lines. Using the randomization test of the Flury hierarchy, comparison of the outbred and pooled inbred matrices suggests a great deal of shared structure. In particular, the hypothesis of proportionality could not be ruled out ($P = 0.1450$) whereas equality was clearly rejected ($P < 0.0001$). As can be seen in Figure 2, the average **G** matrix is proportional to that of the outbred population. All of the genetic covariances maintained their relative orientation in multivariate space following genetic drift, with the entire matrix simply shrinking by a nearly constant proportion. Bootstrapping across families (PHILLIPS 1998a), the proportionality constant for the genetic covariance matrix was estimated to be 0.680 (standard error = 0.016). This decrease is comparable to that reported in WHITLOCK and FOWLER (1999) for just the variances. The brother-sister mating used here is expected to generate an $F = 0.25$. However, effective population sizes in laboratory populations are usually less than their census sizes (BRISCOE *et al.* 1992), which leads

TABLE 1

Additive genetic variance-covariance matrices (G) for the outbred control and average of the inbred populations

Trait	Wing area	Angle 5-7-4	Angle 8-7-6	Angle 2-9-3	Angle 2-1-5	Angle 2-3-5
A. Wing area	12.10 (0.57)[a]					
	7.12 [3.71][b]					
B. Angle 5-7-4	3.51 (0.33)	9.07 (0.36)				
	2.09 [1.83]	6.25 [2.60]				
C. Angle 8-7-6	1.50 (0.53)	−3.85 (0.45)	21.84 (1.03)			
	0.77 [3.55]	−3.60 [2.86]	15.37 [6.63]			
D. Angle 2-9-3	1.03 (0.10)	0.95 (0.08)	−0.46 (0.14)	0.90 (0.04)		
	0.63 [0.62]	0.62 [0.53]	−0.26 [0.75]	0.63 [0.24]		
E. Angle 2-1-5	1.79 (0.15)	2.44 (0.14)	−1.31 (0.19)	0.80 (0.04)	1.96 (0.07)	
	1.14 [1.13]	1.51 [0.98]	−0.91 [1.50]	0.54 [0.28]	1.32 [0.55]	
F. Angle 2-3-5	1.61 (0.26)	−1.48 (0.21)	2.71 (3.24)	0.09 (0.06)	0.02 (0.09)	5.79 (0.21)
	0.60 [1.62]	−1.10 [1.61]	2.07 [1.85]	0.11 [0.44]	0.05 [0.60]	3.78 [1.36]

For the elements in the matrix, the estimate for the outbred control population is given on the top line and the estimate for the average value over all inbred lines is given on the bottom line. All variances, covariance, and standard errors were $\times 10^4$ for ease of viewing. Variance units are mm^4 for area and $radians^2$ for the angles.

[a] Values in parentheses give the standard error of the estimate for the control population.

[b] Average estimates from the 52 inbred lines. Values in brackets give the standard deviation of the (co)variance across lines, not the standard error of the estimate.

to an increase in the realized F to ~0.3 (Whitlock and Fowler 1999). Under an additive model, the expected proportionality constant between the inbred and control matrices is $(1 - F)$, which in this case would be ~0.7. The estimated value is not significantly different from this expectation. The change in average genetic covariance structure observed here is therefore consistent with the theoretical prediction of the change expected for drift of a set of correlated additive traits.

Variance among inbred genetic covariance matrices: Although, on average, genetic drift maintained proportionality between the outbred and bottlenecked **G** matrices, when one looks at the variation around this average, a very different picture emerges. Figure 3 highlights the covariance pattern estimated within the 52 inbred lines for one of the two-trait combinations. Although most matrices are seen to wobble around the expected orientation (as represented by the control population), some of the covariance patterns can be extremely divergent, with the genetic correlation occasionally even changing sign (see also Fowler and Whitlock 1999a, Figure 5). Figure 4 summarizes the distribution of covariance patterns for all of the traits by displaying the divergence of the orientation of the covariance matrix in terms of

TABLE 2

Environmental variance-covariance matrices (E) for the outbred control and average of the inbred populations

Trait	Wing area	Angle 5-7-4	Angle 8-7-6	Angle 2-9-3	Angle 2-1-5	Angle 2-3-5
A. Wing area	5.26 (0.47)[a]					
	7.24 [3.22][b]					
B. Angle 5-7-4	0.43 (0.28)	5.40 (0.28)				
	0.62 [1.47]	5.16 [1.65]				
C. Angle 8-7-6	−0.08 (0.50)	−7.41 (0.43)	22.59 (1.05)			
	0.43 [2.18]	−6.88 [2.42]	21.98 [5.95]			
D. Angle 2-9-3	−0.30 (0.07)	−0.03 (0.06)	0.03 (0.11)	0.37 (0.02)		
	−0.18 [0.36]	0.08 [0.29]	−0.13 [0.57]	0.42 [0.14]		
E. Angle 2-1-5	−0.19 (0.10)	0.79 (0.09)	−0.35 (0.14)	1.67 (0.03)	0.56 (0.04)	
	−0.32 [0.57]	0.76 [0.39]	−0.42 [0.13]	0.21 [0.13]	0.60 [0.18]	
F. Angle 2-3-5	−0.10 (0.17)	−0.44 (0.14)	0.47 (0.27)	0.01 (0.04)	−0.12 (0.05)	1.59 (0.13)
	0.17 [0.84]	−0.47 [0.73]	0.50 [1.13]	0.07 [0.19]	−0.08 [0.25]	1.75 [0.54]

For the elements in the matrix, the estimate for the outbred control population is given on the top line and the estimate for the average value over all inbred lines is given on the bottom line. The phenotypic variance-covariance matrix (**P**) is the sum of the elements in this matrix (**E**) with those in Table 1 (**G**). Scale as in Table 1.

[a] Values in parentheses give the standard error of the estimate.

[b] Average estimates from the 52 inbred lines. Values in brackets give the standard deviation of the (co)variance across lines, not the standard error of the estimate.

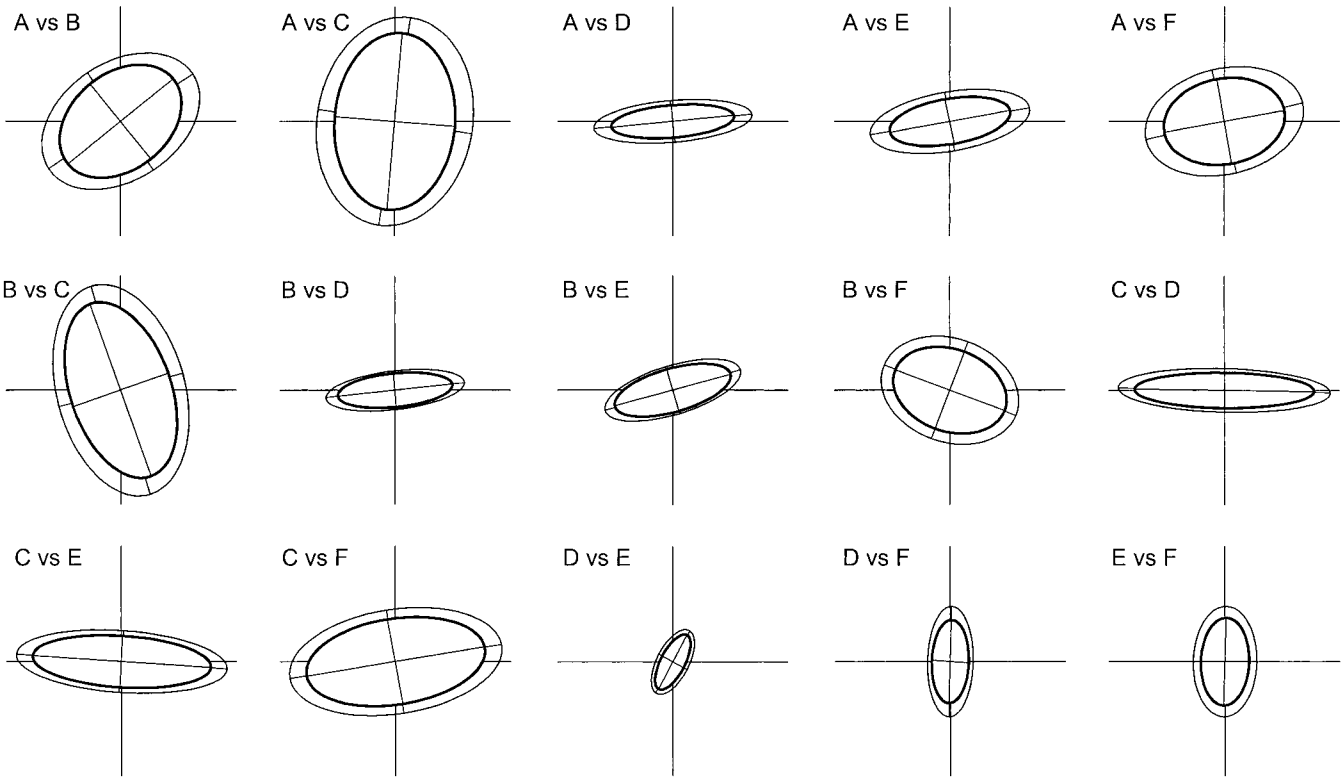

FIGURE 2.—Genetic covariance structure of the outbred control and average inbred lines. Graphs illustrate the 95% confidence ellipse calculated from the genetic variance-covariance (**G**) matrix. Principal axes of variation are shown on top of each ellipse. Each graph is for a different combination of traits. The outer ellipse gives the covariance pattern for the outbred control, while the ellipse layered on top of this gives the covariance for the average of the 52 inbred-line **G** matrices. Note that the averages of the inbred matrices are smaller, but in the same orientation as the outbred matrix, yielding proportionality. Traits are as in Figure 1 with A, area; B, angle 5-7-4; C, angle 8-7-6; D, angle 2-9-3; E, angle 2-1-5; and F, angle 2-3-5. Total length along each axis is 0.1 units, where the units are mm^2 for area and radians for the angles.

the angle of the major principal component relative to the outbred control. Some trait combinations demonstrate a much wider distribution of orientations than others (Figure 4). There is a weak tendency for the trait combinations showing the least amount of variation in orientation to be those with the smallest covariances between the traits (Figure 2), although this is not significant (Spearman's $r = 0.38$, $P = 0.1641$). Some trait combinations with similar covariances, such as D-F and E-F, have substantially different variances in orientation after inbreeding ($F_{max} = 4.4$, $P < 0.01$). Comparing the genetic covariance structure for all traits across the entire set of inbred lines shows that no principal components are shared in common across all lines ($P < 0.0001$).

Phenotypic and environmental covariance matrices: The pooled environmental covariance matrices (**E**) of the inbred lines were somewhat less similar to the outbred controls than were the genetic covariance matrices (Table 2). The hypothesis of shared common principal components was not ruled out ($P = 0.1948$), the hypothesis of proportionality was marginally rejected ($P = 0.0508$), and equality was more clearly rejected ($P = 0.0155$). Although this analysis suggests that it is probably not the appropriate model, if the environmental covariance matrices are constrained to be proportional, their estimated proportionality constant is 1.10 ± 0.04. This indicates that the environmental covariance matrix increased slightly in overall variance. This increase in environmental variance is comparable to that found in WHITLOCK and FOWLER (1999).

Interestingly, the similarities observed in the outbred and average inbred genetic and environmental covariance do not hold with the phenotypic covariance matrices (**P**). Here, the average inbred **P** matrices do not share more than one principal component in common with the controls (test of common principal component CPC[1], $P = 0.2901$; while for CPC[2], $P = 0.0020$). Since **P** is the sum of the **G** and **E** matrices, if each of these latter matrices is affected by drift in different ways, then **P** will necessarily be more divergent than the other matrices. As was found for the **G** matrices, when the inbred lines are compared to one another, there is no indication that any principal components are shared in common for either the **P** or **E** matrices ($P < 0.0001$).

Covariance among line means: The among-line covar-

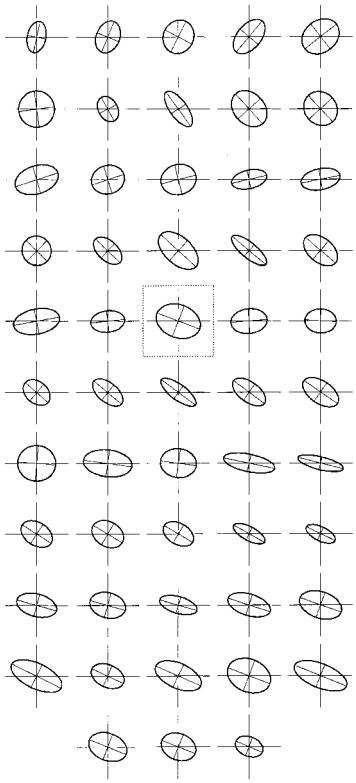

FIGURE 3.—Genetic variances and covariances illustrated as 95% confidence ellipses for the 52 inbred lines for angles (B) 5-7-4 and (F) 2-3-5. The covariance pattern for the outbred control is given in the center box. The total length of each axis is 0.086 radians. Note the extreme divergence in covariance patterns of the inbred lines.

iance matrix cannot be statistically compared to the within-line **G** matrix because they are sampled from different classes of covariance estimates (Table 3). The among-line covariances are estimated using a product-moment covariance among the population means, while the elements of the **G** matrix are estimated as the covariance components derived from the parent-offspring regression. These two types of covariances have very different sampling properties (LYNCH and WALSH 1998). Fortunately, using family means to calculate the **G** matrix (VIA 1984) provides a very good estimate in this case (as the number of families is very large) and allows a direct comparison since these are also product-moment covariances (PHILLIPS and ARNOLD 1999). Using this approach, the among-line covariance pattern is seen to be very similar to the within-line estimates (Tables 1–3). In particular, proportionality among these matrices cannot be rejected ($P = 0.4209$), while equality is strongly rejected ($P = 0.0028$). The proportionality constant in this case is estimated to be 0.64 ± 0.05. Using an F of 0.3 as above, this constant is not significantly different from the theoretical expectation of $2F$ (LANDE 1979) and is exactly equal to two times one minus the proportionality constant estimated for the within-population **G** matrix, as expected.

DISCUSSION

Genetic drift affects genetic variances and covariances, but in complex ways. With traits determined by additively interacting genetic effects, on average the additive genetic variance for each trait will decrease in proportion to the inbreeding coefficient of the population. LANDE (1980a) showed that the same was true of the whole genetic covariance matrix; on average **G** would decrease proportionally, multiplied by the scalar term $(1 - F)$. Roff interpreted this to mean that the shape of **G** would be unchanged by inbreeding or population bottlenecks, but overall genetic variance and covariance would decline uniformly (see ROFF and MOUSSEAU 1999; ROFF *et al.* 1999; ROFF 2000). Under this interpretation, changes in **G** due to genetic drift would slow the *rate* at which evolution proceeds, but not the *direction*.

These theoretical predictions are *expectations*, the mean over all possible outcomes. Any particular population need not be at this expectation; in fact, under certain circumstances the variance around the expectation can be very large (AVERY and HILL 1977; ZENG and COCKERHAM 1991; WHITLOCK 1995; WHITLOCK and FOWLER 1999). As a result, different inbred populations isolated from the same ancestral population can have very different evolutionary trajectories. In this experiment, drift affected the average pattern of genetic covariance among wing size and shape characteristics in close accord with the theoretical expectation: a proportional shrinking of the **G** matrix across all traits (Figure 2). When each line is examined separately, however, there is wide variance among lines in the orientation and magnitude of genetic variance and covariance (Figure 4). Thus, inbreeding leads to significant divergence in **G** matrix structure among lines even as it maintains structure when all lines are considered jointly.

Divergence among populations: Drift has two separate but important consequences for the divergence of isolated populations. First, drift generates variance among the means of each population so that they each start at different points in phenotypic space. The results of this study are consistent with the theoretical expectations about the pattern of divergence among means by drift (REEVE 1950; LANDE 1979). Under this model, population means are expected to diverge along the underlying genetic covariance structure shared across populations. There does appear to be some evidence that this is the case (SCHLUTER 1996, 2000), although Schluter interpreted this pattern as the influence of **G** on the response to selection rather than the possible influence of **G** on drift. The trouble is that both drift and selection often predict the same pattern of divergence, so it can be difficult to distinguish between them (LANDE 1979; EMERSON and ARNOLD 1989). Two recent studies in birds (MERILÄ and BJÖRKLUND 1999; BADYAEV and HILL 2000) interpreted the disparity between the within- and between-population patterns of covariance as a sign of

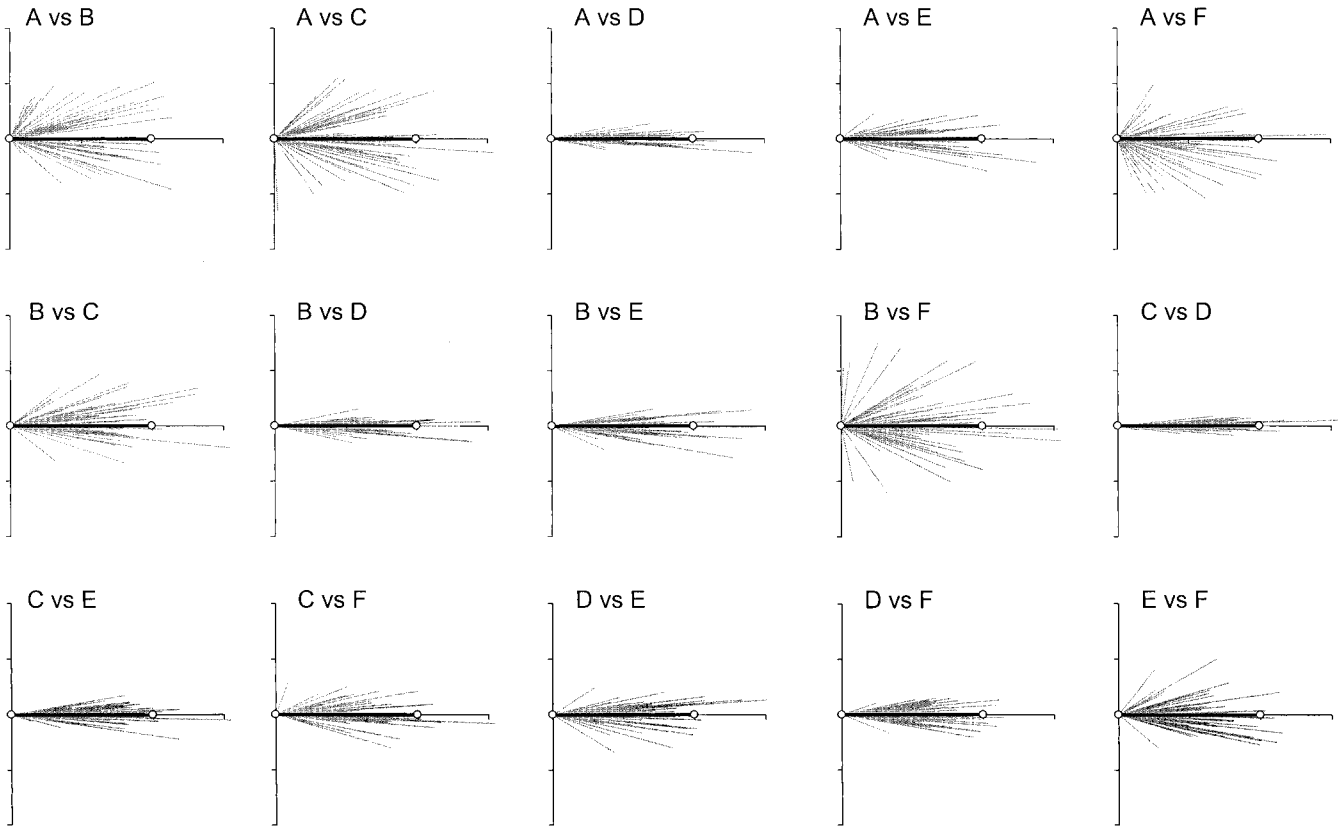

FIGURE 4.—Comparison of the orientations of the genetic covariance in the outbred control and 52 inbred lines. Each line in the graphs shows the alignment of a particular inbred line relative to the outbred population. The principal axis of the outbred population is given by the line segment bounded by the open circles. The angle of the other lines relative to the segment gives the angle between the principal axes of variation for the inbred and outbred covariances (*e.g.*, Figure 3). The length of each line gives the amount of variance along that axis in the inbred line relative to the outbred control. Each hash mark represents 0.5 variance units, up to 1.5 total on the *x*-axis. Dispersion away from the *x*-axis provides a representation of the distribution of change in matrix orientation generated by inbreeding. Lines longer than 1 unit indicate bottlenecked lines in which the variance along the major axis exceeds the variance in the outbred population. Note the variation in the pattern of covariance induced by genetic drift. Traits are as in Figure 1 with A, area; B, angle 5-7-4; C, angle 8-7-6; D, angle 2-9-3; E, angle 2-1-5; and F, angle 2-3-5.

localized response to selection. This interpretation is consistent with the results obtained here, although this study clearly shows that one must be careful not to overinterpret the within-population pattern of covariance for any given population. Instead one needs to pool over a potentially large number of different populations to adequately address the drift hypothesis (as was indeed done in both of these studies).

Second, drift causes idiosyncratic changes in the variance-covariance structure of particular populations. If similar patterns of directional selection were to act on all populations following isolation, the response to selection within some populations could be quite variable. For example, if selection were to act on just one trait (say wing size) in a uniform manner, the correlated response to selection on the other traits would follow the lines of divergence shown in the top row of Figure 4. Again, the expected change under selection will follow the path predicted by the average **G**, but individual populations could be evolving in very different ways—even in the opposite direction. Drift-induced variation in **G**, even with samples derived from the same base population, may explain variation in correlated responses to selection frequently observed in replicated selection experiments (reviewed in HARSHMAN and HOFFMANN 2000). Further, the greater sensitivity of **P** to drift found here is yet another reason to be cautious about using phenotypic estimates as a substitute for genetic estimates in selection analyses (CHEVERUD 1988; WILLIS *et al.* 1991).

Under more complex patterns of selection, the consequences of drift in covariance structure become more complicated. If selection leads to the existence of two separate evolutionary equilibria (WRIGHT 1932), then it is possible for drift-induced variation in the structure of **G** to cause evolution to alternate adaptive peaks (LANDE 1979; SCHLUTER 2000).

Trait-specific variation in covariance structure: The covariance between different traits is variable to different extents, and that variability is weakly correlated with the magnitude of the covariance in the outbred population. To some extent it takes initial covariance to gener-

TABLE 3

Among-population variance-covariance matrix for the inbred lines

Trait	Wing area	Angle 5-7-4	Angle 8-7-6	Angle 2-9-3	Angle 2-1-5	Angle 2-3-5
A. Wing area	6.21 (0.99)					
B. Angle 5-7-4	2.66 (0.79)	4.48 (0.70)				
C. Angle 8-7-6	0.06 (1.25)	−1.28 (1.00)	11.71 (1.90)			
D. Angle 2-9-3	0.45 (0.22)	0.51 (0.19)	−0.49 (0.29)	0.54 (0.15)		
E. Angle 2-1-5	1.24 (0.34)	1.40 (0.28)	−0.88 (0.53)	0.47 (0.17)	1.01 (0.18)	
F. Angle 2-3-5	0.71 (0.66)	−0.89 (0.44)	1.40 (0.75)	0.12 (0.19)	0.05 (0.26)	2.60 (0.48)

Product-moment (co)variances estimated from the line means. Scale as in Table 1.

ate divergence in covariance structure. However, it is possible for traits that are influenced by pleiotropic alleles to not display any genetic covariance (if, for instance, positive and negative effects balance one another; Houle 1991; Gromko 1995). For example, the D-F and E-F ellipses shown in Figure 2 show a very similar structure of essentially no covariance between the traits. Inbreeding appears to generate more divergence in orientation in the E-F comparison than in the D-F comparison, however (Figure 4). It is difficult to know how important sampling error is in explaining this difference, but it is likely that the underlying details of the genetic architecture are going to have a large influence on the distribution of change in **G** matrix structure. For example, existing models for the variance in additive genetic variance already display a strong dependence on the number of loci and distribution of effects underlying the traits (Lynch and Hill 1986; Zeng and Cockerham 1991; Whitlock 1995). Whether or not an analysis of the distribution of the shape of the **G** matrix after inbreeding can therefore be used to infer something about this underlying architecture remains to be seen. There is some evidence for independent genetic effects on the components of wing shape (Zimmerman *et al.* 2000).

Long-term evolution of G: Over longer periods of time, the structure of **G** will be determined by some combination of mutation, genetic drift, migration, and natural selection (Lande 1979, 1980b, 1984; Barton and Turelli 1987; Turelli 1988; Deng *et al.* 1999). There are an increasing number of studies that examine how **G** changes in natural populations from a comparative standpoint (see reviews in Roff 1997; Arnold and Phillips 1999; Roff *et al.* 1999). Most of this work focused on the stability of **G** over time in terms of its potential usefulness in reconstructing historical patterns of selection (Lande 1979). Thus far, little attention has been paid to the nature of the changes in **G** itself. This study demonstrates both that the basic theory is successful in predicting the average effects of drift on multivariate traits and that there can be substantial variation around this expectation.

Roff (2000; also Roff and Mousseau 1999; Roff *et al.* 1999) recommended using the expectation of proportionality as the primary indicator of whether drift is responsible for divergence in **G**. Nonproportional change is then interpreted as the result of selection. The among-population variance in **G** caused by drift alone as seen in this study suggests that reliance solely on the expectation is likely to underestimate the potential role of drift in explaining divergence among populations. Extended over long periods of evolutionary time, many possible patterns of **G** matrix structure are likely to be compatible with the hypothesis of drift. We need a theory that describes the distribution of **G** generated by drift to provide a null hypothesis against which alternative hypotheses of **G** matrix evolution can be tested, as was already done for changes in means (*e.g.*, Lande 1977; Turelli *et al.* 1988). As demonstrated in this study, a more comprehensive theory of the evolution of genetic covariance structure needs to include the variance induced by drift, in addition to its expectation, to establish a solid foundation upon which comparative quantitative genetic studies can be based.

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