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RESISTANCE OF GENETIC CORRELATION STRUCTURE TO DIRECTIONAL SELECTION IN *DROSOPHILA MELANOGASTER*

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Abstract.—The genetic covariance and correlation matrices for five morphological traits were estimated from four populations of fruit flies, *Drosophila melanogaster,* to measure the extent of change in genetic covariances as a result of directional selection. Two of the populations were derived from lines that had undergone selection for large or small thorax length over the preceding 23 generations. A third population was constituted using flies from control lines that were maintained with equivalent population sizes as the selected lines. The fourth population contained flies from the original cage population from which the selected and control lines had been started. Tests of the homogeneity of covariance matrices using maximum likelihood techniques revealed significant changes in covariance structure among the selected lines. Prediction of base population trait means from selected line means under the assumption of constant genetic covariances indicated that genetic covariances for the small population differed more from the base population than did the covariances for the large population. The predicted small population means diverged farther from the expected means because the additive genetic variance associated with several traits increased in value and most of the genetic covariances associated with one trait changed in sign. These results illustrate that genetic covariances may remain nearly constant in some situations while changing markedly in others. Possible developmental reasons for the genetic changes are discussed.

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Lande (1979) suggested that micro-evolution should be modeled as a hill-climbing process in which multivariate phenotypes are ascending adaptive topographies to maximize the average level of adaptation, $\bar{W}$, in the population. The vector of mean phenotypes, $\bar{z}_{t+1}$, in the generation after selection can be predicted from

$$\bar{z}_{t+1} = \mathbf{G}\nabla \ln \bar{W} + \bar{z}_t, \qquad (1)$$

where $\nabla \ln \bar{W}$ is a vector in which each element, $\partial \ln \bar{W}/\partial z_i$, equals the change in Malthusian mean fitness due to a small change in z_i, holding all other z_j fixed. As Lande and Arnold (1983) have shown, equation (1) can be utilized in retrospective or prospective studies by calculating the vector, **B**, which contains the partial regression coefficients for each trait on relative fitness, i.e.,

$$\bar{z}_{t+1} = \mathbf{GB} + \bar{z}_t. \qquad (2)$$

To use equation (2) for micro-evolutionary studies a number of controversial assumptions must be made. For example, as Endler (1986 pp. 190–192), Mitchell-Olds and Shaw (1987), and Crespi and Bookstein (1989) have pointed out, to estimate **B** one must assume that all traits that affect fitness are included in the regression if the traits are not statistically independent. An equally problematic assumption is that the genetic covariance matrix, **G**, must remain constant in the face of selection. There are several reasons for doubting that this latter assumption is valid.

Genetic covariances can change when selection alters the gene frequencies of pleiotropic alleles or causes linkage disequilibrium between loci. Turelli (1988) reviews three models that outline conditions under which genetic covariances can remain constant during selection under the assumption of no linkage disequilibrium. For Lande's (1976, 1980) model, which assumes that the joint distribution of allelic effects across loci is multivariate Gaussian, three conditions must be met. First, **G** must not change in new environments, i.e., there can be no genotypes by environment interaction (but see Via and Lande, 1987) or evolution of **G**. Second, the shape of the fitness surface must remain constant as the phenotypic optimum changes, and third, the covariances among the effects due to new pleiotropic mutations must remain constant. When the distribution of allelic effects is permitted to be non-Gaussian, then any change in the

mean will generally be accompanied by a change in any associated covariance (Bohren et al., 1966; Barton and Turelli, 1987). The response of the genetic variance (and any associated covariance) to a change in the mean depends on the number of loci, the distribution of allelic effects at individual loci, and the number of alleles per locus (Barton and Turelli, 1987). Whereas these theoretical studies indicate that the conditions for constancy of the genetic covariance structure are stringent and probably invalid for most cases of evolution in the wild, they do not permit estimation of the magnitude of change in the genetic covariance matrix which might occur in response to selection. This empirical question is the focus of this paper.

Because all previous studies that have examined the constancy of covariance structure assumption used species with unknown selection histories (e.g., Lofsvold, 1986; Kohn and Atchley, 1988), we decided to estimate the genetic correlation matrix, $\mathbf{R}_g$, and **G** from large samples of several populations that had undergone selection in the lab. Although artificial selection studies can be criticized as being unrepresentative of natural selection because of the intensity and specificity of selection, continual directional selection on a few characters in small populations, such as that realized in many artificial selection studies, is expected to occur in populations experiencing sexual selection by a Fisherian runaway process (Lande, 1981), in populations which are derived from few fertilized foundresses colonizing a new habitat (Carson and Templeton, 1984), and in populations exposed to human-imposed selective agents such as toxic wastes, pesticides, or habitat disruption (Bishop and Cook, 1981). Inasmuch as these situations may be important for micro-evolutionary change, speciation, or extinction, artificial selection studies may provide important insight into the nature of change in genetic architecture associated with such events.

Materials and Methods

Laboratory Procedures

The study involved four generations from four populations of the fruit fly, *Drosophila melanogaster*. Throughout the experiment flies were maintained at 25 ± 1.5°C with a fixed illumination cycle of 12 hr dark followed by 12 hr light. Standard Edinburgh food medium (see Fowler and Partridge, 1986, for details) was used for both the maintenance of populations and experiments. All handling and measuring of live flies was performed at room temperature using CO_2 anesthesia.

The first experimental population was produced from samples of adults taken from an outbred wild-type stock that was collected in Dahomey in 1970 and has been maintained since then in population cage culture. This base population sample was produced from three unyeasted culture bottles in each of which 40 adults (20 males and 20 females) were left for 48 hr. The progeny were collected as virgin males and females and pooled across replicates to constitute generation two for the base (P) population.

The three remaining experimental populations were produced from a set of 12 lines that had been artificially selected for thorax length for 23 generations. These lines derived originally from the Dahomey population cage. Their history is given in Partridge and Fowler (in preparation). Briefly, four large and four small lines were maintained by selecting the largest and smallest 10 of the first 25 pairs to emerge in each generation. Four unselected control lines were maintained by taking a random sample of 10 of the first 25 pairs to emerge. To reduce inbreeding effects, in generation one of this experiment we made four-way crosses among lines within each selection regime as follows. Five pairs of virgin males and females were taken from each of the four lines within each size-category and placed in one-third pint culture bottles containing 65 ml of medium. After 48 hr, these 40 adults were discarded and the bottles retained for progeny collection. For each of the three size categories there were three replicates of this four-way cross. The resulting progeny were collected as virgins and pooled across replicates to constitute the large (L), small (S), and control (C) populations for generation two.

Because variation in pre-imaginal density can have large effects on the values of adult metric characters (e.g., Spiers, 1974; Cali-

gari and Baban, 1981), we raised both the parental flies (generation three) and the offspring flies (generation four) from first instar larvae under standardized conditions of low larval density.

For each population the generation two flies were allocated to mating groups of 10 pairs of virgin females and males and kept in standard food vials: 75 × 24-mm shell vials containing seven ml of medium. There were 20 mating groups for each population. After 24 hr, all mating groups were transferred to egg-laying vials. These 65 × 35-mm vials had plastic lids that contained an egg-laying medium (15 g agar, 75 ml 95% ethanol, 15 ml glacial acetic acid in 1,500 ml water), which encourages egg-laying and facilitates larval removal. To ensure that females were well-fed, a small dab of baker's yeast was smeared onto the side of each vial. After 24 hr the adults were discarded, and after 36–48 hr, two samples of 30 larvae were collected from the medium surface and transferred on paint brushes to two standard food vials. This procedure yielded 40 larval cultures per population. For each population, the progeny from these cultures were collected as virgin females and males and pooled to form a sample of 300 females and 300 males. These parental flies were scored for thorax length and mated assortatively in pairs to increase the precision of our heritability and genetic correlation estimates (Hill, 1977).

The procedure for generation four was similar to that for generation three. The mating pairs were kept in standard food vials for 24 hr prior to transfer to egg-laying vials. The mating pairs were removed after 24 hr and scored subsequently for five metric characters (Fig. 1): thorax length (TX), sternopleural bristle number (BB), wing length (WL), wing width (WW), and tibia length (TB). After 36–48 hr, one sample of 30 larvae was collected from each egg-laying vial in which sufficient larvae were present and transferred to a standard food vial. This procedure yielded 181 families for the L population, 153 families for the S population, 177 families for the C population and 190 families for the P population. To balance the design we chose a random sample of three male and three female offspring from each family for measuring. After imposing this constraint our data set contained 145 families for the L population, 127 families for the S population, 143 families for the C population and 124 families for the P population.

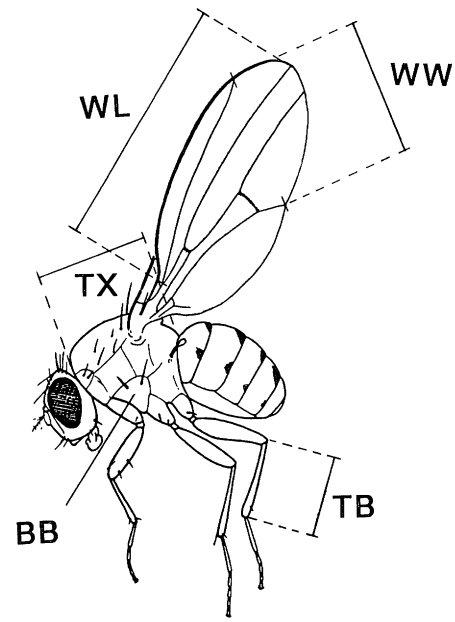

FIG. 1. Description and abbreviation for each of the five traits—bristle number (BB), thorax width (TX), wing length (WL), wing width (WW), and tibia length (TB)—measured.

To measure the wing and leg characters we removed both wings and both hind legs from flies, mounted them on glass slides, and scored them using a compound microscope at 50× (WL) or 100× (WW and TB) magnification. Left leg and wing measurements were used unless damage precluded accurate measurement. TX was measured by laying flies on their sides under a binocular microscope at 25× magnification and recording the distance between the base of the most anterior humeral bristle and the tip of the scutellum with an eyepiece graticule. Individuals were assayed for BB by adding together the number of sternopleural bristles counted on their left and right sides at 50× magnification. Because trait distributions within populations did not depart significantly from normality, no measurements were transformed. Offspring were numbered arbitrarily when measured for subsequent covariance analysis.

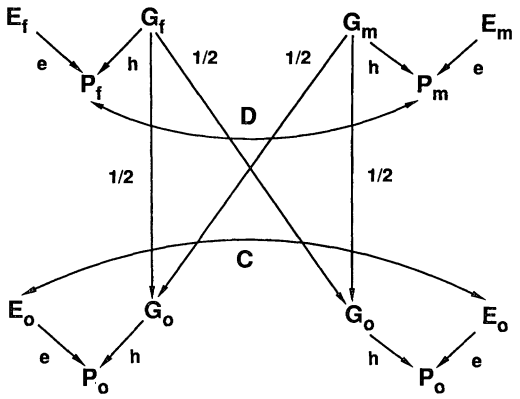

FIG. 2. Path diagram indicating path coefficients and correlations used to determine the expected correlations listed in Table 2. **E** and **G** are latent variables indicating the environmental and additive genetic components of the observed phenotype, **P**, for the male parent, m, female parent, f, and offspring, o.

Estimation of Genetic Parameters

Because our goal was to obtain precise estimates of **G** and $\mathbf{R}_g$ and to evaluate the similarity in content of pairs of matrices, we used path analysis to estimate genetic parameters (Vogler, 1985; Carey, 1986). The methods we employed are multivariate extensions of path analytic models developed by Wright (1931) and more recently used by human geneticists to separate biological from environmental causes of trait variation (e.g., Rao et al., 1974; Eaves et al., 1978). These techniques permit combining data from multiple relationship types, e.g., parent-offspring with full or half-sib data, into a single nonlinear model that then can be used to estimate the relevant genetic and environmental parameters. Furthermore, because the estimates are obtained using a log-likelihood loss function, the effect of setting individual parameters to zero on the overall fit of the model can be statistically evaluated using the difference between twice each of the resulting loss functions. This difference follows a chi-squared distribution and is tested using the difference in degrees of freedom used in the two models (Bollen, 1989 pp. 289–292). If the data are multivariate normal and large samples are used, the inverse of the information matrix, the matrix containing the second derivatives of the loss function with respect to each of the parameters, provides an estimate of the standard errors associated with each parameter estimate. Because the behavior of these error estimates under departures from multivariate normality is not well characterized, we have not presented those estimates here. Instead, we have computed standard errors using the formulae in Klein et al. (1973) and Falconer (1981 pp. 166–167) for parent-offspring regression studies with assortative mating. These estimates should overestimate the true error in our data because the additional information provided by the full-sib correlations is not included. One drawback in using these model-fitting techniques is that substantial numerical analysis is required particularly if many traits are measured for large families. Shaw (1987) has detailed additional pros and cons of these procedures and has emphasized that restricted maximum likelihood estimates are unbiased in contrast to maximum likelihood estimates. However, because maximum likelihood is nearly unbiased when the number of fitted fixed effects are small relative to sample size (Searle, 1987 pp. 502–506), we chose a maximum likelihood estimator.

Figure 2 illustrates the multivariate path model used to estimate the genetic and environmental parameters. In contrast to conventional path diagrams, the letters and numbers associated with each path are not simple partial regression coefficients but are matrices of path coefficients. The correlation between any two phenotypes in the diagram is a phenotypic correlation matrix, **R**, which can be equated to a sum of component matrices obtained using rules for analyzing multivariate path diagrams (Vogler, 1985) that are analogous to those for univariate path diagrams (Li, 1975 p. 294). This model includes a path labeled **C** to measure the extent to which full-siblings resemble each other more than parents resemble offspring. Although **C** connects environments and can, therefore, be thought of as measuring a common environmental component of variation, one should realize that other nonadditive effects that increase sibling similarity, such as dominance variation, would enter into these parameters. This path was included after analyzing a series of sequential univariate models for possible maternal effects, common environment ef-

fects, sex effects, heritability and assortative mating. For all five traits, the maternal effect parameter could be set to zero and sex-specific heritability estimates could be equated without loss of fit, but overall heritability, common environment and assortative mating parameters were required to obtain an adequate fit for each of the five traits in each of the four populations tested. We acknowledge, however, that with this design we have little statistical power to detect differences between the sexes in heritabilities. Although females are larger than males for almost every metric trait measured, other studies (Cowley et al., 1986; Cowley and Atchley, 1988) have failed to document many differences between the sexes in the heritabilities of metric traits similar to those measured in this study.

Numerical estimates of variance components were obtained using the MINUIT (1977) optimization program (Fulker and DeFries, 1983) by minimizing the log likelihood ratio

$$L = (N - 1)\{\ln|\mathbf{E}| - \ln|\mathbf{S}| + \mathrm{tr}[\mathbf{S}\mathbf{E}^{-1}] - \phi\} \quad (3)$$

in which N is the number of families, $|\mathbf{E}|$ indicates the determinant of $\mathbf{E}$, and ϕ is the order of the observed and expected covariance matrices. $\mathbf{S}$ is a 40 by 40 observed phenotypic covariance matrix composed of 64 five by five symmetric covariance submatrices. Each submatrix contains the variances and covariances for the five traits and is obtained by pairing each of the eight individuals, i.e., six progeny and two parents, per family together. $\mathbf{E}$, the 40 by 40 expected covariance matrix, contains the expected covariance components for each submatrix of $\mathbf{S}$. The covariance components in $\mathbf{E}$ are derived from the correlation components presented in Table 1 by pre- and post-multiplying the expected correlation matrix by a matrix containing the square root of the diagonal of $\mathbf{S}$, i.e., the observed standard deviation for all of the traits measured in each individual, along the diagonal and zeros elsewhere. In Table 1 $\mathbf{R}_p$ is the phenotypic correlation matrix, $\mathbf{R}_g$ is the additive genetic correlation matrix and $\mathbf{R}_e$ is the matrix containing environmental correlations. The diagonal matrices, $\mathbf{h}$, $\mathbf{e}$, and $\mathbf{1/2}$, contain the square-root of the heritability, h^2, the square root of what is sometimes referred to as the environmentality, $e^2 = 1 - h^2$, or 1/2, respectively, on the diagonal and zeros off the diagonal. $\mathbf{D}$ is a matrix that measures the assortment among mates after partialling out the effects of phenotypic correlations (Carey, 1986). Because we imposed assortative mating, $\mathbf{D}$ is defined as a matrix containing zeros everywhere except at position 2,2 which contains the imposed mate correlation for thorax length. Thus, these structural equations define a nonlinear model with 40 parameters. Expressed in standardized form these parameters include 5 h's, 10 r_g's, 10 r_e's and 15 c's.

TABLE 1. Components of expected correlation submatrices.

Observed	Expected
$\mathbf{R}_p$	$\mathbf{hR_gh} + \mathbf{eR_ee}$
$\mathbf{R}_{mf}$	$\mathbf{R_pDR_p}$
$\mathbf{R}_{fo}$	$[\mathbf{1/2}][\mathbf{hR_g} + \mathbf{R_pDhR_g}]\mathbf{h}$
$\mathbf{R}_{mo}$	$[\mathbf{1/2}][\mathbf{hR_g} + \mathbf{R_pDhR_g}]\mathbf{h}$
$\mathbf{R}_{oo}$	$[\mathbf{1/2}]\mathbf{h}[\mathbf{R_g} + [\mathbf{1/2}]\mathbf{R_gh}(\mathbf{D} + \mathbf{D})\mathbf{hR_g}]\mathbf{h} + \mathbf{eCe}$

Tests of Matrix Homogeneity

To determine how well the estimates of the genetic and environmental parameters for the base population fit the data for the large and small populations, we conducted two analyses. A likelihood test under the null hypothesis that these parameters have not changed was performed by initializing the 25 genetic and environmental parameters at the final estimates for the base population, setting the observed covariance matrix equal to either the 40 by 40 phenotypic covariance matrix computed from the large or the small population after selection, and then calculating the log-likelihood loss function value after one pass through the optimization routine. Twice the value of the loss function was tested using the difference in degrees of freedom to indicate how well the base population parameter estimates fit the selected population data.

To quantify the consequences of any change in the covariance structure we used equation (2) to perform two retrospective simulation analyses—one for each of the two

TABLE 2. Results of ANOVA and Scheffé multiple comparisons for heterogeneity of population means for parental flies. All F tests are significant at $P < 0.0001$. Homogeneous means are connected by a continuous underline. BB is a count of sternopleural bristles; all other measurements are in mm.

Sex	Variable	F	Population means			
			Large	Base	Control	Small
Male	BB	14.89	18.536	18.995	18.921	17.405
	TX	929.11	0.979	0.919	0.912	0.801
	WL	801.57	1.640	1.590	1.587	1.440
	WW	1,080.36	0.941	0.898	0.906	0.792
	TB	847.07	0.686	0.657	0.646	0.585
Female	BB	12.67	19.431	19.763	20.017	18.484
	TX	1,204.45	1.124	1.059	1.025	0.901
	WL	708.31	1.907	1.852	1.854	1.68
	WW	981.77	1.066	1.017	1.026	0.897
	TB	708.60	0.741	0.710	0.701	0.636

selected populations. For each population, average sex-specific selection differentials for thorax length were calculated for all four large and small lines. Selection differentials on the four other traits unmeasured during the course of selection were estimated each generation by multiplying the thorax selection differential by the appropriate base population phenotypic correlation between thorax and trait. Separate **G**'s for males and females were obtained by pre- and post-multiplication of the base population $\mathbf{R}_g$ by **h** and a matrix containing sex-specific parental standard deviations for each trait on the diagonal and zeros off the diagonal. The effects of sampling error on our estimates of **G** were incorporated into these predictions by iterating each retrospective simulation 1,000 times. At the beginning of each iteration a random standard normal deviate was chosen and multiplied by the standard error for each element of $\mathbf{R}_g$ and **h** before computing **G**. The observed mean values for each of the five traits in the base population were then compared to the resulting distributions of predicted values.

In this retrospective analysis we assume that all genetic correlations between the sexes are unity. While we have evidence that heritabilities do not differ between the sexes (see above), precise estimation of genetic correlations between sexes requires a half-sib design (e.g., Cowley and Atchley, 1988). Violation of this assumption due, for example, to x-linked variance could account for sex-specific difficulty in predicting response trajectories across populations, but it is unlikely to account for any population-specific effects. While an analogous prospective analysis also could be conducted, a retrospective analysis is more accurate and, therefore, more appropriate because mean trait values were measured under constant environmental conditions after, but not prior to, selection.

RESULTS

Selection Outcome

The response to selection on thorax length was asymmetrical (Table 2). Lines selected for small thoraxes responded more rapidly to selection than lines selected for large thoraxes. This difference is not due to any difference in selection intensities between populations. The average selection intensity each generation for flies in the small lines ($I_{male} = 0.86$, $I_{female} = 0.88$) was not significantly different from that in the large lines ($I_{male} = 0.87$, $I_{female} = 0.85$). The difference in response is consistent with the change in the heritability for thorax length as described below. In addition to this direct response to selection on thorax lengths, both male and female flies in lines selected for larger and smaller thoraxes showed significant correlated responses for the wing and leg characters (Table 2). Sternopleural bristle number decreased in the small lines but did not change in the large lines. For some of these traits the control lines also differed significantly from the base population flies. These results suggest that the control lines

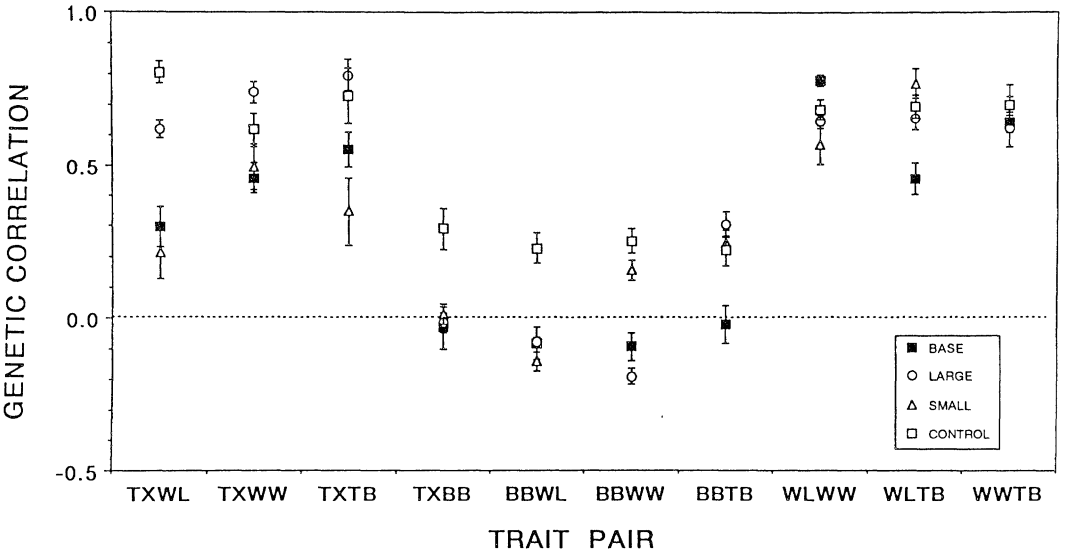

FIG. 3. Genetic correlations with error bars indicating one standard error for each pair of traits in each of the four populations.

may also have experienced inadvertent selection. Selection for rapid development may have occurred in the control lines due to the two week generation time imposed on all of the selection and control lines but not on the base population. Thus, changes in genetic covariances and correlations between base population and control line flies may be due to both drift and selection. Note, however, that genetic drift alone cannot explain differences between the selected lines.

Effects on Genetic Correlations and Covariances

Figure 3 illustrates our estimates of all 10 genetic correlations for each of the four populations. While the overall pattern among these correlations is similar across populations, there are numerous trait pair combinations where the genetic correlation differs significantly between the base population and one or more of the other populations. The control line population differs conspicuously in that all correlations involving sternopleural bristle number are significantly positive in this population while they are either not significantly different from zero or are negative in the base population. Heritabilities of each of the five traits show somewhat less variation among populations than do genetic correlations (Fig. 4). Notably, the heritability of thorax length has not changed in response to directional selection although heritability of thorax length in the control line was significantly lower than it was in the base population. On the other hand, 11 of the 15 heritability estimates were less than the base population estimates suggesting a reduction in overall genetic variation

The causes of these changes in the genetic correlations can be deduced by inspecting the additive genetic and phenotypic variances and covariances (Appendix). The increase in heritabilities for sternopleural bristle number in the selected lines relative to the base population was due to a substantial increase in additive genetic variation in the selected lines. Additive genetic variance for thorax length decreased in the large and control lines but increased in the small line population. This extra genetic variation for thorax length accounts for the asymmetry in selection response noted above. Additive genetic variation for wing width and tibia length decreased in the selected and control lines while no consistent change occurred for wing length. The most obvious change among the genetic covariances was the change in sign associated with the sternopleural bristle covariances in the small and control populations.

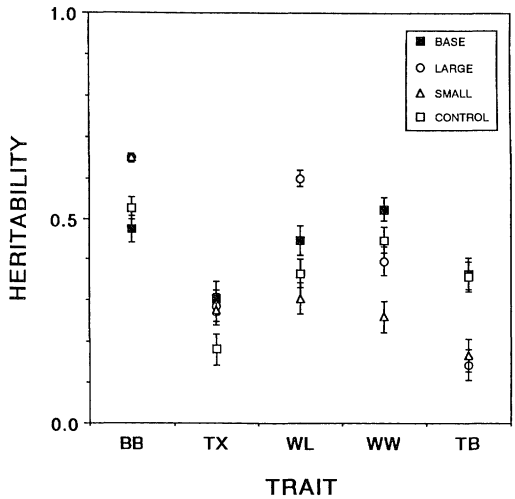

FIG. 4. Heritabilities with error bars indicating one standard error for each of the five traits in each of the four populations.

Constancy of **G** after selection was evaluated by constraining the expected covariance matrix, **E**. The 25 **h**, $\mathbf{R}_g$ and $\mathbf{R}_e$ parameters for the small, large, and control population **E** matrices were equated to their estimates for the base population before minimizing equation (3) for each observed covariance matrix, **S**. Twice the difference in the log-likelihood from the overall fit to the base population and the constrained fit to one of the selected or control populations was tested with 25 degrees of freedom to determine the goodness of fit under the assumption of no change in **G** and **P**. In these analyses **C**, the matrix of common environment parameters, can vary. The results of these tests show (Table 3) that the genetic and environmental parameters of the base population do not adequately fit the observed covariances for any of the three other populations. Furthermore, the magnitude of the difference chi-squared values indicate that the large and control line observed covariances fitted the base population genetic parameters about three times more closely than did the small population covariances. Thus, greater genetic changes have occurred in the small than in the large and control populations. This conclusion is corroborated by the following retrospective analysis in which **G** is assumed to remain constant.

TABLE 3. Goodness-of-fit tests using the **h**, $\mathbf{R}_g$ and $\mathbf{R}_e$ parameter estimates obtained from the base population and the observed covariance matrices from the selected and control populations.

Population	Free parameters	*df*	χ^2	$\Delta\chi^2$	*P*
Base	40	500	930		
Large	15	525	1,026	96	<0.001
Small	15	525	1,254	324	<0.001
Control	15	525	1,028	98	<0.001

Comparison of Predicted to Observed Responses

If the genetic covariance structure was unaffected by selection, then the mean values of the base population traits should be predictable from the selected line means by retrospective application of equation (2). Deviations between observed and predicted trait means must be due to changes in covariance structure. The distributions of predicted sex-specific trait means for each selected population are compared to the observed trait means in the base population in Figure 5.

Figure 5 illustrates that selection for large thoraxes had relatively little effect on **G**. Eight of the ten observed trait means for the base population fell well within the distribution of 1,000 predicted trait means and five of the observed means closely matched the distribution medians. The two observed means that were above the distribution of predicted means were for thorax length. These results are consistent with our observation that the additive genetic variance in thorax length declined in the large lines relative to the base population (Appendix). This decline apparently constrained the large lines from responding as much as predicted. In contrast, for the small population only 1 of 10 observed trait means landed within the 50 percentile range of the predicted trait means. In every other case the observed small population mean exceeded most if not all of the 1,000 predicted mean values indicating that greater response to selection occurred than was predicted by the assumption of constant covariance structure. Although most of the off-diagonal elements of the small population **G** changed only a small amount from corresponding elements

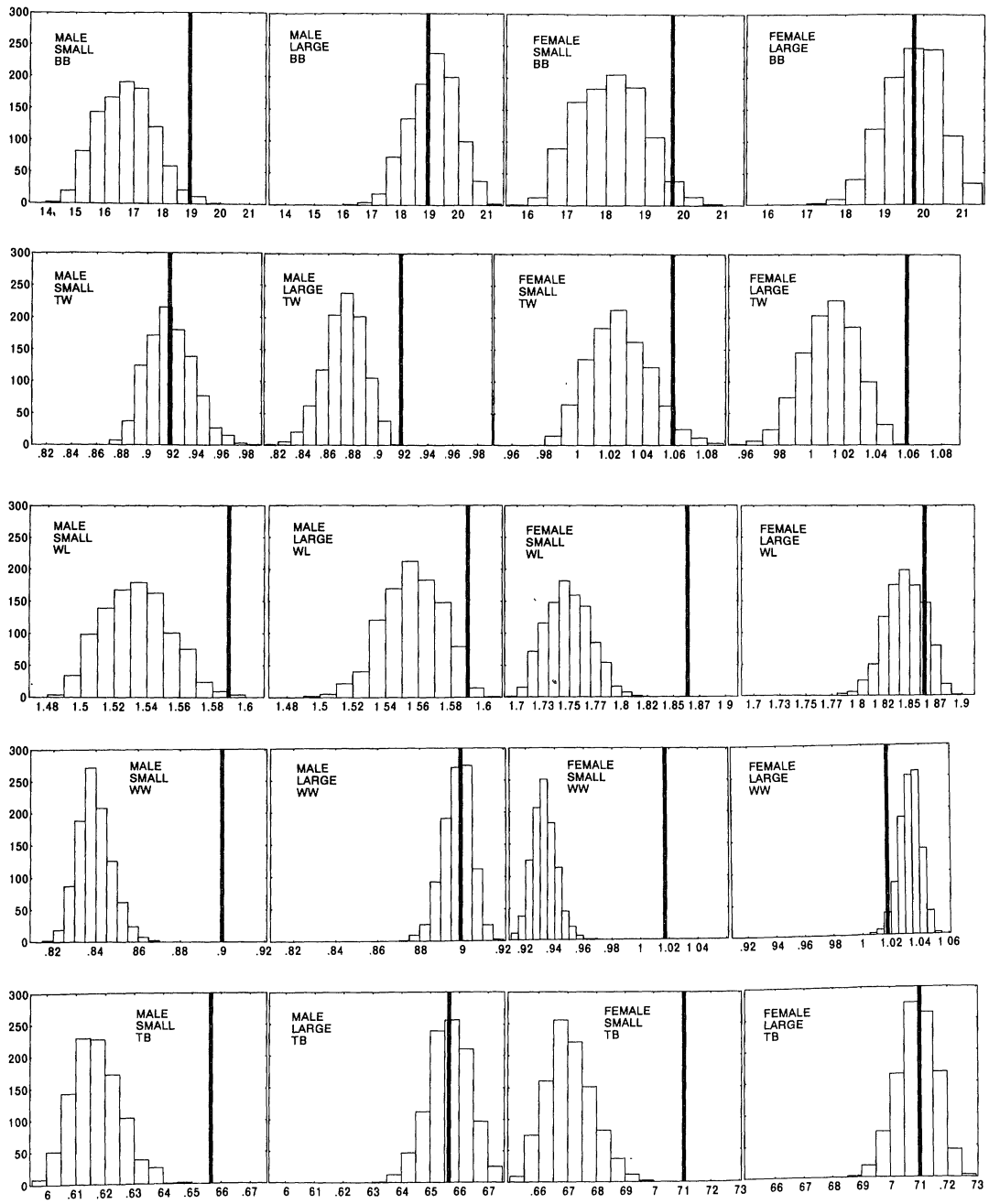

FIG. 5. Open bars indicate frequency histograms from 1,000 retrospective simulations that used observed selection differentials in each population to predict the mean of each trait for each sex in the base population. The dark vertical bar in each panel indicates the observed mean trait value in the base population. Variation in the predicted means is caused by incorporating the sampling error associated with the genetic correlations and heritabilities as described in the text.

of **G** in the base population (Appendix), the additive genetic variance for 3 of 5 traits increased markedly in the small population (Appendix). The heritabilities for these same traits decreased (Fig. 4), on the other hand, because total phenotypic variation increased more than the additive genetic variation. Furthermore, the sign changed for 4 of 5 covariances involving sternopleural bristle number. Thus, instead of bristle number increasing with a decrease in thorax length as predicted, it decreased in the small lines (Table 2).

Discussion

In this study we imposed a perturbation on **G**, the genetic covariance matrix relating five morphological traits, by conducting bidirectional selection on one trait for 23 generations. Resistance to this perturbation, i.e., the degree to which a state variable is changed following a perturbation (Pimm, 1984), was measured directly by estimating **G** in the base, large and small populations. Goodness-of-fit tests and prediction of base population trait means from selected line means under the assumption of constant genetic covariance structure indicated that **G** for the small population changed significantly from the base, control and large population estimates of **G**. The predicted small population mean values diverged farther from expected values because the additive genetic variance associated with several traits increased in value and most of the genetic variances associated with several traits increased in value and most of the genetic covariances associated with bristle number changed in sign. Thus, these results demonstrate an asymmetry in resistance to perturbation in that selection for large thorax length had much less of an effect on **G** than did selection for small thorax length. Similar findings have been observed in several other, although not all, artificial selection studies in which genetic correlations have been measured.

For example, Sen and Robertson (1964) selected simultaneously for increased sternopleural bristle number and fifth abdominal sternite bristle number in *Drosophila melanogaster* using three different selection protocols. After 12 generations of selection, neither the heritabilities nor the genetic correlations had changed significantly. In a similar experiment, Sheridan and Barker (1974) selected *Drosophila melanogaster* coxal and sternopleural bristle number simultaneously up, down, and divergently in both combinations for 22 generations and measured realized genetic correlations at 10 and 22 generations. While large differences in correlations between replicate lines were observed, average correlations and heritabilities remained remarkably constant across selection regimes. Bell and Burris (1973) monitored five traits in *Tribolium castaneum* beetles that were selected in all four directions for larval and pupal weight for eight generations. Although both genetic variances and covariances were disrupted by selection, the genetic correlation between the selected traits remained surprisingly constant across lines.

In contrast to these results, Berger (1977) found substantial changes in the realized genetic correlation for pupal weight and family size in lines of *T. castaneum* selected for one or the other of these two traits for 16 generations. The genetic correlation for these traits in the base population was -0.17 but became -0.43 in the line selected for high pupal weight and 0.03 in the line selected for high family size. Similar changes in the realized genetic correlations have been observed by Bell and McNary (1963) who selected *T. castaneum* for increased pupal weight in both a wet and dry environment.

Changes in genetic correlations also have been observed in the absence of selection as a consequence of reduced effective population size. Bryant and Meffert (1988) recently showed that the genetic correlations among eight morphological traits in houseflies changed more if the populations were subjected to intermediate (populations reduced to 4 and 16 pairs) bottleneck levels rather than extreme (2 pairs) or no bottlenecks. The genetic correlations and covariances of the intermediate bottleneck replicates increased relative to the controls which, Bryant and Meffert (1988) argue, is consistent with epistatic gene action.

Fewer selection studies have been performed on vertebrates, but those that have indicate that genetic correlations are frequently unstable. Falconer (1960) selected lines of mice for growth on high and low

planes of nutrition and observed the correlated response on the alternative nutritional level. The realized genetic correlations were equal for the first four generations of selection (0.67, 0.65 for high and low planes, respectively) but changed dramatically between generations 5 to 13 (1.25, −0.02). These changes were accompanied by large changes in the phenotypic standard deviations.

In addition to these artificial selection studies, several recent studies have examined the constancy of covariance or correlation assumption by comparing estimates of **G** or the genetic correlation matrix, $\mathbf{R}_g$, obtained from either different populations or closely related species. In one of the first studies of this kind, Arnold (1981) estimated $\mathbf{R}_g$ for chemoreceptive traits measured on two geographic races of garter snakes and reported no change in the correlation structure using factor analysis. Similarly, Atchley et al. (1981) compared $\mathbf{R}_g$ for skeletal traits in rats and mice using factor analysis and found no obvious differences. In contrast, Berven (1987) found dramatically significant differences in the genetic correlation between development rate and larval size in mountain ($r_g = -0.86$) and lowland ($r_g = 0.65$) populations of wood frogs.

These three studies did not, however, statistically compare genetic covariances. Lofsvold (1986) computed **G** for a set of 15 cranial traits in two subspecies of *Peromyscus maniculatus* and in *P. leucopus*. He compared the pattern in **G** using vector correlations and a Mantel test. His results led him to conclude that **G** had not changed perceptibly between the two subspecies but had changed between the species although the genetic correlation matrices were similar between subspecies and species. Kohn and Atchley (1988) performed a similar analysis of pelvic measurements in random-bred ICR mice and lab rats and found differences in **G** between these species, but on reanalyzing Lofsvold's (1986) data using more randomizations and additional association statistics, argued that the two *Peromyscus* species do have significantly similar **G** structures.

In all of these studies $\mathbf{R}_g$ typically changes much less than estimates of **G**. In each of the comparisons of **G** just discussed, the statistical test measured degree of similarity in structure, not content, under the null hypothesis that the two matrices are different. For example, the Mantel tests evaluate if the rank order, not value, of the matrix elements are similar, while vector correlations indicate the degree of collinearity among ranked eigenvectors, i.e., the proportionality of two matrices. In many cases the absolute value of individual elements has changed as much as those in the studies that indicated changes in correlations from lines selected in opposite directions. For this reason, these similarity tests can be misleading if the purpose of the comparison is to validate a retrospective or prospective study rather than measure the proportionality of two matrices. If equation (2) is applied recursively, small differences in the size of **G** can result in large phenotypic differences over many generations even though matrix randomization tests, such as the Mantel test, indicate that the covariance matrices are more similar than one would expect by chance.

Although some of the genetic changes we observed could be due to linkage caused by selection and drift (Avery and Hill, 1977), linkage alone should not have altered genetic covariances greater in the small lines than in the large lines. Such asymmetry in covariance change could occur for at least two reasons: alleles that decrease body size have greater phenotypic effects than those that increase body size or more loci influence small than large body size. While we have no way of evaluating the phenotypic effects of allelic changes between small and large flies, greater genetic variation among small population flies could result if there are more developmental paths for decreasing body size than for increasing body size. Adult size is determined by three factors—larval growth rate (Bakker, 1969), larval weight at pupation (Bakker, 1959, 1969), and target cell number in the imaginal discs (Bryant and Simpson, 1984). Whereas both larval feeding rate (Burnet et al., 1977) and development time (Clarke et al., 1961; Sang, 1962) can be readily lowered by artificial selection, neither can be increased very much. Presumably, prior directional selection has exhausted most additive genetic

variation for rapid growth. In contrast, any decrease in larval feeding rate, mobility, or digestive efficiency can decrease adult body size. Although we have not measured these variables on the flies in this study, we do know from other work that small line flies have a lower growth rate than the large and control line flies (Partridge and Fowler, in preparation). Inasmuch as these developmental patterns are typical of holometabolous insects that undergo rapid development, we expect **G** among morphological traits to change more readily as body size decreases than when selection favors an increase in body size. If this prediction is correct, then the rate of morphological change may be greater when selection favors small rather than large body sizes.

Our results, in conjunction with those reviewed above, suggest that the constancy of **G** assumption must be applied cautiously to natural populations and, preferentially, tested when possible. Further experiments of this kind are needed to determine if **G** remains constant under selection for increased body size but changes under selection for smaller body size. We suggest that the most revealing comparison of two genetic covariance structures is obtained by iterating selection episodes. While the best comparison is made when observed selection differentials are used, even hypothetical selection regimes may indicate the potential magnitude of error involved in an evolutionary prediction.

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APPENDIX

Additive genetic (below diagonal) and phenotypic (above diagonal) covariance matrices for each of the four populations.

Base	BB	TX	WL	WW	TB	
	0.8028	0.0279	0.0104	−0.0086	0.0364	BB
		0.5373	0.2836	0.4601	0.3136	TX
BB	0.3812		0.3881	0.4617	0.3136	WL
TX	−0.0079	0.1640		1.1449	0.4378	WW
WL	−0.0214	0.0500	0.1730		0.6432	TB
WW	−0.0444	0.1446	0.2513	0.6003		
TB	−0.0066	0.1088	0.0924	0.2420	0.2353	

Large	BB	TX	WL	WW	TB	
	0.9370	0.0042	−0.0119	−0.0022	0.0492	BB
		0.4844	0.2367	0.3713	0.1868	TX
BB	0.6078		0.3238	0.3696	0.1564	WL
TX	−0.0045	0.1392		1.0241	0.2511	WW
WL	−0.0272	0.1021	0.1943		0.3733	TB
WW	−0.0947	0.1764	0.1821	0.4058		
TB	0.0554	0.0689	0.0672	0.0925	0.0537	

Small	BB	TX	WL	WW	TB	
	0.9663	0.0302	0.0594	0.1655	0.1466	BB
		0.8263	0.5622	0.7023	0.5385	TX
BB	0.6285		0.6872	0.7380	0.5289	WL
TX	0.0481	0.2286		1.4019	0.6722	WW
WL	−0.0510	0.0468	0.2093		0.7413	TB
WW	0.0753	0.1443	0.1582	0.3662		
TB	0.0690	0.0587	0.1244	0.1494	0.1240	

Control	BB	TX	WL	WW	TB	
	0.9722	0.1287	0.0994	0.2127	0.1191	BB
		0.6806	0.2831	0.3729	0.2725	TX
BB	0.5104		0.4409	0.4077	0.2643	WL
TX	0.0731	0.1231		1.0161	0.3500	WW
WL	0.0654	0.1139	0.1615		0.3906	TB
WW	0.1213	0.1463	0.1862	0.4556		
TB	0.0588	0.0956	0.1046	0.1768	0.1400	