# Selection on Wing Allometry in *Drosophila melanogaster*

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ABSTRACT

Five bivariate distributions of wing dimensions of *Drosophila melanogaster* were measured, in flies 1) subjected to four defined environmental regimes during development, 2) taken directly from nature in seven U.S. states, 3) selected in ten populations for change in wing form, and 4) sampled from 21 long inbred wild-type lines. Environmental stresses during development altered both wing size and the ratios of wing dimensions, but regardless of treatment all wing dimensions fell near a common allometric baseline in each bivariate distribution. The wings of wild-caught flies from seven widely separated localities, and of their laboratory-reared offspring, also fell along the same baselines. However, when flies were selected divergently for lateral offset from these developmental baselines, response to selection was rapid in every case. The mean divergence in offset between oppositely selected lines was 14.68 SD of the base population offset, after only 15 generations of selection at 20%. Measurements of 21 isofemale lines, founded from wild-caught flies and maintained in small populations for at least 22 years, showed large reductions in phenotypic variance of offsets within lines, but a large increase in the variance among lines. The variance of means of isofemale lines within collection localities was ten times the variance of means among localities of newly established wild lines. These observations show that much additive genetic variance exists for individual dimensions within the wing, such that bivariate developmental patterns can be changed in any direction by selection or by drift. The relative invariance of the allometric baselines of wing morphology in nature is most easily explained as the result of continuous natural selection around a local optimum of functional design.

SIZE variations in the parts of organisms form allometric patterns of covariation, which often reflect contiguity and homology, and which may roughly predict underlying patterns of genetic covariance (LOFSVOLD 1986; CHEVERUD 1988). In one view, these allometries sometimes represent persistent developmental constraints, able to resist adaptive optimization by mass selection, and evolving mainly by large quantum transitions (HUXLEY 1932; GOULD 1982a, 1986; but see GOULD 1966). Another view regards such allometries only as constellations of continuously varying characters, molded by selection and drift, along routes permitted by the genetic correlations among component characters (LANDE 1979; ZENG 1988; see also LEVINTON 1986).

Evolution in all growth patterns may be severely constrained (ALBERCH 1980, 1982; GOULD 1980, 1982b), either because the internally balanced, homeostatic organization of genetic systems (LERNER 1954; CARSON 1975) normally resists disruption, or because developmental laws simply prohibit some outcomes. In this view, selection experiments are practically irrelevant to the study of large-scale evolution, because the response is limited to minor readjustment. But in the view at the opposite extreme, the response to a few generations of artificial selection, or even correlations between relatives in a single generation, can be extrapolated to interpret patterns of evolution spanning millions of years and divergent taxa (LANDE 1979).

It is true that genetic homeostasis (or the opposition of natural selection) almost always curtails response in selection experiments and even tends to cause regression after selection is relaxed (FALCONER 1981). However, these limits seem to represent neither the boundaries of forbidden developmental outcomes nor the last frontiers of genetic reorganization, since they increase with the size of the selected population (JONES, FRANKHAM and BARKER 1968; EISEN 1975; ENFIELD 1977; YOO 1980; WEBER 1990; WEBER and DIGGINS 1990). This is due to the increased efficiency of selection in larger populations and their greater variability, and can be accommodated within the conceptual framework of LERNER's (1954) homeostasis: the greater variety of recombinants in larger populations allows more efficient construction of new balanced combinations enriched for alleles of the selected trait. Moreover, regression under relaxed selection does not routinely return a population to its original state. Repeated episodes of directional selection can even build a new homeostatic equilibrium (RICKER and HIRSCH 1988). Thus it is plausible that the stand-

ing, selectable genetic variation of populations is representative of the material of major evolutionary change (MAYNARD SMITH 1983).

On the other hand, there is some limit to the precision of projections based on heritabilities and genetic correlations determined by a few generations of selection. If these quantities are constant under a given selective regime (*cf.* TURELLI 1988), then one can predict a theoretical trajectory of suboptimal stages along an indirect route to the new adaptive optimum (LANDE 1979; ZENG 1988), or perhaps not quite to the new optimum (CHARLESWORTH 1990). In any case, trajectories may be less interesting than 1) the ultimate precision of optimization, in terms of the number of separate characters that can simultaneously conform to individual optima, and 2) the maximum speed with which new optima can be approached.

FISHER (1930, pp. 42–44) provided a clear rationale for the primary importance of *small* individual gene effects, particularly in multivariate adaptive evolution based on pleiotropic genes. It would follow from this argument that systems with larger numbers of loci, and correspondingly smaller per-locus effects, are more likely to provide existing variants and new mutations with immediately useful effects. Thus the capacity for multivariate precision and overall speed, in major adaptive change under the microevolutionary model, should require not only some initial genetic variance and genetic correlations of absolute value less than one, but also large numbers of loci with small, independent, and phenotypically localized effects.

A sensitive assay of the genetic variability unique to localized regions of morphology is to select antagonistically on two adjacent dimensions. Neighboring traits will usually have high phenotypic and genetic covariance, but can respond independently to selection. The limits of their divergence, under the rapid change typical of experimental mass selection, are not predictable from initial genetic variances and covariances, but will reflect the numbers of locally acting, additive genes affecting each trait. In the present study divergent antagonistic selection, based on an allometric index of paired wing dimensions, was used to investigate the extent and nature of genetic variation for form within localized morphogenetic fields.

## MATERIALS AND METHODS

**The system of measurement:** All measurements were performed using the "planomorphometer" system, which has been described in detail elsewhere (WEBER 1988). The system allows rapid measurement of live, $CO_2$-anesthetized flies, by projection of greatly enlarged images of body parts onto a computer-linked digitizing pad. Suction from a vacuum pump is used to draw one wing into a narrow gap between two parallel sheets of clear plastic, which form a window in the tip of a hand-manipulated aluminum holder. This holder fits into a slot above the lens of a modified microprojector, leaving both hands free for focusing and digitizing. After measurement, each fly can be ejected from the holder into its appropriate phenotypic class among a set of numbered test tubes, according to results appearing on the computer monitor.

**The scale of measurement:** The standard equation for allometric relations expresses one measurement as a power function of another:

$$D_2 = \mathrm{A}\, D_1^{\mathrm{b}}.$$

After evaluation of various alternatives, it seemed that for the present purposes a more convenient expression of allometric relationship could be based on a curve using polar coordinates. Such a curve can be derived, for example, by the linear regression of the logs of the angles ($\theta$) on the logs of the radii ($r$) of points with coordinates $D_1$ and $D_2$, where

$$\theta = \arctan (D_2/D_1)$$

and

$$r = (D_1^2 + D_2^2)^{1/2},$$

so that the fitted curve is a polar equation of the form

$$\theta = \beta r^{\alpha}$$

describing a curve through the center of the bivariate distribution. The coefficient $\beta$ depends on the units of $D_1$ and $D_2$, while the exponent $\alpha$ is independent of the units. Using this curve as a baseline, lateral deviations from the average allometric relation of variates can be expressed conveniently as angular offsets. That is, the angular deviation or offset ($\phi$) of any point can be calculated from its true values of $r$ and $\theta$ as

$$\phi = \beta r^{\alpha} - \theta.$$

Deviations clockwise or counterclockwise from the reference baseline are thus quantified in positive or negative radians, respectively. In the present material this baseline is hardly different from the curve of the standard allometric equation given above, within the range of the data. Variation in $\phi$ is analogous to variation in the exponent of the conventional allometric equation.

The long axes of the bivariate distributions of wing dimensions mainly represent the genetic and environmental variation in wing size, corresponding to variation in the parameter $r$. The variation of the angular offset ($\phi$) is expected to be largely independent of genetic or environmental effects on size, because the arc subtended by any constant angular offset from the central baseline increases with $r$ just as the standard deviations of component dimensions increase proportionally to their means (an empirically demonstrable property of wing dimensions). The method assumes positively correlated variables, and works best for morphological dimensions or other variables that scale closely with size.

This metric was chosen strictly for convenience: no assumption is implied about the natural shape and curvature of these bivariate distributions, except that points farther from the origin are more dispersed. In actual samples, these baselines pass very close to the centroids and lie very near the major axes; the sign of $\phi$ is approximately independent of $r$; and the sum of $\phi$ is approximately zero. (By these criteria, various other ways of deriving baselines and of quantifying deviations worked as well when tested with the present data.) The distribution of $\phi$ occupies a scale limited to one quadrant or $\pi/2$ radians. Therefore at extreme angular offsets (either $D_1$ or $D_2$ disproportionately small) the distribution will be compressed. For the general case an infinite linear scale occupying one quadrant can be created using hyperbolic radians, but in the present data $D_1$ and $D_2$

are always of comparable size, and the more intuitive circular radian scale is adequate for the small range of offsets considered here.

Extension to multivariate distributions is straightforward. For any number n of measurements, a point representing an individual with coordinates $D_1, D_2, D_3, \ldots, D_n$ in $n$-dimensional space defines a vector of length

$$r = \left( \sum_{i=1}^{n} D_i^2 \right)^{1/2}$$

which forms an angle of arccos $(D_i/r)$ to each respective axis in $n$-space. Regressions of log $\theta$ on log $r$ in each dimension yield an array of $n$ parametric equations

$$\theta = \beta_i r^{\alpha_i}$$

which describe the multivariate angular centerline as a function of $r$. The angle $Y$ between two points $a$ and $b$ at the same $r$ in $n$-space, which could represent the deviation between an individual and a multivariate baseline, or the deviation between two arrays at $r$, can be derived from the relation

$$\cos Y = \sum_{i=1}^{n} \cos \theta_{a,i} \cos \theta_{b,i}$$

**Definition of traits:** This study primarily employs five bivariate distributions of wing dimensions, defined as shown in Figure 1. Each of the five pairs of dimensions is designated by a single letter (M, S, F, G or R), and can either be treated as a single trait (angular offset), or decomposed into its subdimensions. Within each pair of dimensions the longer is designated $D_1$ and the shorter $D_2$.

All measurements in this study are from right or left wings at random of live, male flies. Flies were always grown on the same formula of cornmeal-yeast-molasses medium, at various temperatures as noted. All cultures were in 8-dram vials founded with two pairs, except in one environmental treatment. All measurements were made by the author, except for line means in some of the selection lines in earlier generations.

**Derivation of base population:** The laboratory base population for these experiments ("LF350") was derived from 350 mated female *D. melanogaster* captured near a cider press in Lincoln, Massachusetts, in September 1981, and has been maintained on cornmeal medium in half-pint bottles at large population size since then. This stock was the source of all selection lines and was the only control for the selection experiments. It was also used to derive all reference baselines.

**Environmental treatments:** Base population flies were cultured in vials at temperatures of 18°, 24° and 30°, producing three nearly nonoverlapping ranges of body dimensions. A fourth range of even smaller body size was produced by keeping a set of old bottle cultures for almost 1 month. Old bottle cultures retain adequate moisture, but the medium becomes exhausted and toxic, producing progressively smaller flies.

**Selection lines:** For each of the five bivariate traits defined above, one line was selected for positive and one for negative angular offset from the original allometric baseline. In each selection line approximately 100 males and 100 virgin females were measured each generation and the most extreme 20% selected. The selected flies were usually cultured at about 20°, but at 24° in generation 16 when extra measurements were taken.

**Wild populations:** Seven samples of wild-caught *D. melanogaster* flies were obtained during September and October of 1988, from the vicinities of Tucson, Arizona; Middletown, Connecticut; Amherst, Massachusetts; White Bear Lake, Minnesota; Eugene, Oregon; Oxford, Pennsylvania; and Edgewood and Orwell, Vermont. Each sample represents a single collection site except for the sample from Vermont, which was obtained from two sites, about 15 miles apart. All samples were supplied by others (see ACKNOWLEDGEMENTS) except the sample from Minnesota. Every fly was inspected in this laboratory to make sure that no individuals of the sibling species *D. simulans* were included. Two samples of 50 wild-caught males were measured from each locality, and later two samples of 50 laboratory-reared descendant males of the same strains were measured, except for the Arizona sample, from which females had been removed.

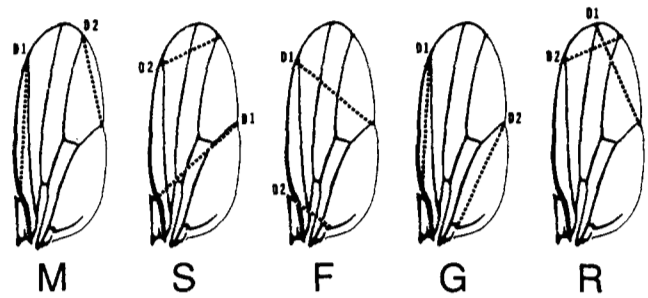



FIGURE 1.—The five pairs of dimensions. Each pair defines a distribution of allometric offsets designated by an arbitrary letter.

**Isofemale lines:** The isofemale lines used in these experiments are among the lines collected from many locations by BRUCE WALLACE and now maintained by the Mid-America Drosophila Stock Center in Bowling Green, Ohio. Each line was founded with a single wild-caught mated female and has since been maintained in vial cultures. For this study 21 isofemale lines were measured, from seven collection sites in six states, with each site represented by three lines. The locations and years of collection, and the individual line designations, are given in Table 1.

## RESULTS

**Deviation of unselected means from allometric baselines:** The mean effects of environmental and geographic variation on wing development are summarized in Figure 2. In each graph, 17 bivariate distributions of 100 male flies apiece are represented by their centroids. In each of the four environmental regimes (asterisks), the wings of base population flies acquire a different size and a different shape. The distinctive wing shape produced by each treatment is shown by the different angle each centroid makes with the axes. The environmental effect on wing size corresponds to the effect on overall body size and is reflected in the relative distance of each centroid from the origin. The linear dimensions of 18° giants are 40–50% larger than those of old-culture dwarfs. The wild-caught flies are consistently a little larger than their descendants which were cultured at 24° in the laboratory, but the centroids of both groups are all clustered tightly along the same baseline, inside the range of size defined by the extreme environmental treatments.

Each polar equation plotted in Figure 2 was derived by the regression of log $\theta$ on log $r$, from a single sample of approximately 100 flies from the laboratory base population (LF350), grown at about 20°. These

TABLE 1

**Isofemale lines measured in this study**

| Collection site | Year collected | Line designations |
|---|---|---|
| Monroe County, New York | 1961 | MO1; MO12; MO18 |
| Commack, New York | 1961 | CO3; CO4; CO7 |
| Riverside, California | 1963 | RVC2; RVC3; RVC4 |
| Red Top Mt., Georgia | 1966 | Wild5a; Wild5b; Wild5c |
| Oxford, North Carolina | 1966 | Wild11a; Wild11c; Wild11d |
| Painesville, Ohio | 1966 | Wild2a; Wild2c; Wild 2d |
| Manning, South Carolina | 1966 | Wild10a; Wild10d; Wild10e |

curves were actually computed before any of the samples plotted in Figure 2 had been measured, to serve as baselines for selection. Only a slight improvement in the fit of the curves could have been obtained by using all the later, more dispersed measurements. As will be seen below, these baselines approximate not only the centroids but also the slopes of the individual samples from all environments and populations.

**Selection to change the allometric baselines:** Bidirectional selection for displacement from each of the five baselines was performed on pairs of populations derived from the base population. The displacement of each fly was quantified as the difference between the actual angle, given by $\arctan(D_2/D_1)$, and the predicted angle, given by $\beta r^{\alpha}$. This difference ($\phi$) is expressed in radians and is positive for clockwise offsets, negative for counterclockwise offsets.

Figure 3 shows the displacements of bivariate distributions from the old allometric baselines in generation 16, after 15 generations of selection. Each graph shows the distributions of one pair of divergently selected lines, with the baseline from Figure 2 between them, and with rotations of the same curve through angles equal to the mean angular offsets of the selected populations. Each baseline passes through the approximate centroid for control flies grown under similar conditions. Through each control centroid is drawn an arc of a circle centered on the origin. This arc represents the original directions of selection, and the paths which would have been followed by the centroids of the populations if they had responded in a perfectly plastic way to selection.

Angular offset, as an antagonistic index of two traits, can be increased in either direction by enlarging one trait or by reducing the other. Hence each of the responses in Figure 3 can be decomposed into components from both dimensions. Table 2 shows the changes in the component dimensions for each trait. In general both dimensions participate in producing each response, but not equally. In one line ($S^-$) the response is only significant in dimension $D_2$. In all other lines, significant changes are found in both dimensions. In line $R^+$ both dimensions change in the same direction, but one decreases more than the other so that a net change in the offset is still produced in the direction of selection. (Trait $R$, uniquely, is defined by crossing rather than by parallel dimensions.)

It might be supposed that decreases in local wing dimensions, through deficiencies in growth, could occur more easily than increases. Then the offsets would usually owe more to unilateral decreases in length than to increases, and the radii would tend to decrease for both clockwise and counterclockwise offsets. Table 3 shows that this is not the case. Out of ten selection lines, nine have significant changes in radius: six of these increase in radius, and three decrease, so that no rule emerges.

The angular offsets can be treated as univariate quantitative characters. Figure 4 shows the distributions of angular offset, in radians, for each pair of divergent selection lines in generation 16. In each case zero represents the mean offset of the base population wing, corresponding to a point anywhere on the allometric baseline. The offset distributions of the selected populations have remained nearly normal but most have diverged in variance. In each case the variance is greater in the line selected for counterclockwise or negative offsets. A trend in this direction is to be expected since the unselected centroids of these traits are all situated clockwise of the quadrant centerline (see MATERIALS AND METHODS). Figure 5 shows the course of selection response in each line, again in radians. No controls were measured during selection, but the angular offsets appear to be rather insensitive to intergenerational environmental variation. There are few simultaneous fluctuations between divergent lines. The response curves in several lines have an almost artificially smoothed appearance. Certain irregularities in Figure 5 require explanation, however.In line $M^-$ a distinct and extreme phenotype suddenly appeared in generation 11, in 18 out of 100 measured males (Figure 6), and in 17 out of 100 measured females. Within about three generations the line contained only flies of this type. This phenotype continued to segregate in a subline (not shown) from which it was culled each generation.Similarly, in line $S^-$ a few flies with an equally distinct, extreme phenotype appeared in generation 6. Although an increasing number of these appeared in subsequent generations, males of this new type had very low

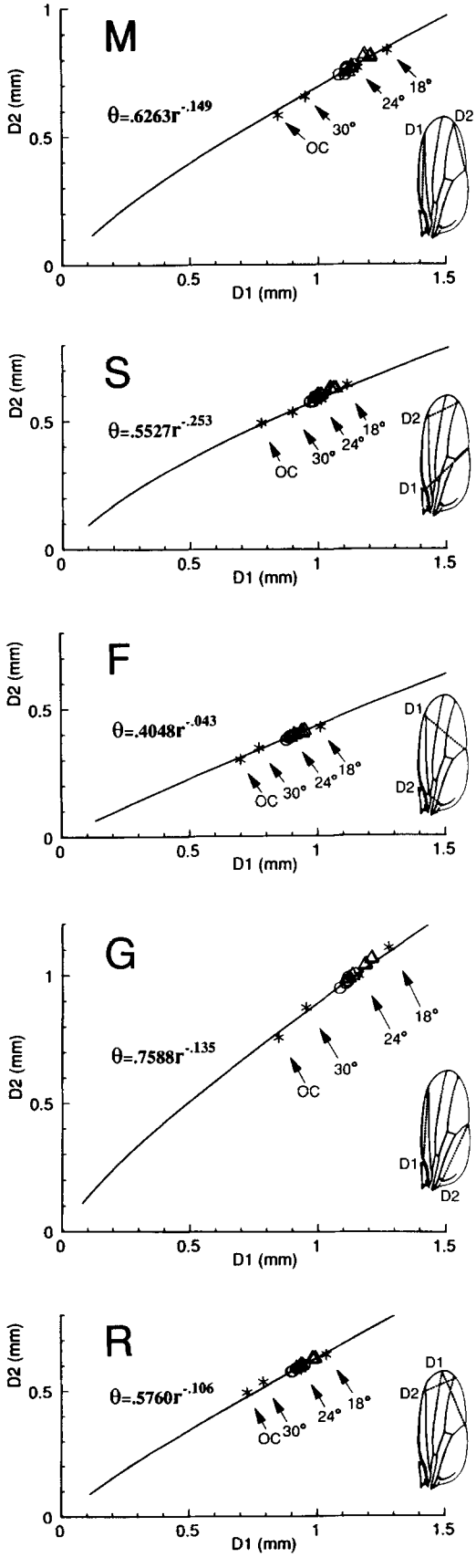


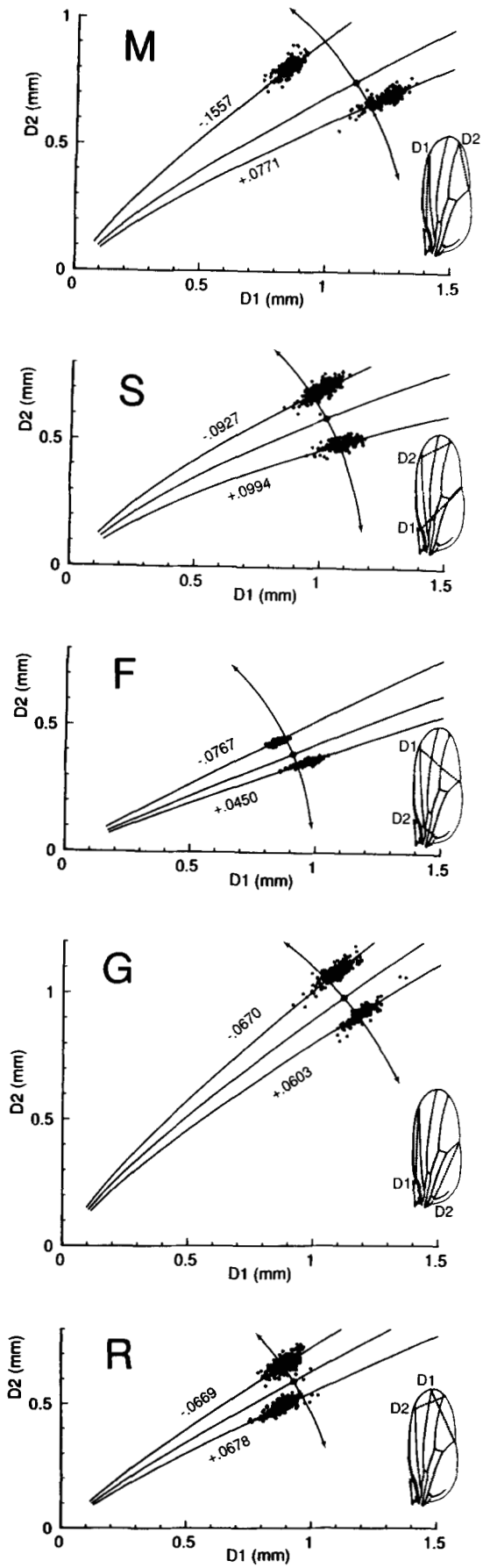

FIGURE 2.—The effects of environmental and geographic variation. Each symbol represents the centroid of 100 male flies. Asterisks represent the base population (LF350) under four environmental regimes (old culture, 30°, 24°, 18°). Seven triangles show wild-caught samples from OR, MN, PA, MA, CN, VT, and AZ. Six circles show laboratory-reared descendants from all states except AZ. Baseline curves were derived from single samples of about 100 LF350 males, cultured at 20°. The whole range of form at all sizes is reasonably approximated by these curves.

fertility while females were completely sterile or would not mate. A subline was begun in generation 9, in which all flies of this type were weeded out, with continued selection on the remainder. The original line was ultimately abandoned because of reproductive failure. These two cases show that some initially uncommon alleles of large homozygous effect contribute to the variance, as selection proceeds.

In line $R^+$ (Figure 5), the large fluctuations in generations 7 and 8 are attributable to technical error in measurement, as are the occasional single-generation bursts in phenotypic variance. It is necessary to draw each wing completely into the window and release the suction during measurement to prevent slight pleating of the wing. As the $R$ measurements are at the tip of the wing, this was sometimes handled carelessly by a technician who assisted only with the $R$ lines during selection.

Table 4 shows realized heritabilities, response ranges, and estimates of the effective number of genes for each of the five allometric-offset traits. Realized heritabilities were calculated simply as the ratio of cumulative response to cumulative selection differential in generation 6. The heritability can be derived in this way once for each direction of selection so that a mean and standard error of the two estimates can be obtained for each of the five offset traits. The responses are rather symmetrical in the early generations (Figure 5), so that the standard errors of the estimates are not large. When the range of response in each trait is expressed as a multiple ($R_p$) of the standard deviation of the base population, an estimate of the Castle-Wright effective number of genes for each trait is given by $n_e = R_p^2/8h^2$ (see FALCONER 1981). This estimate assumes all genes have equal additive effect, had initial frequencies of 0.5, and were fixed by selection in both directions. The estimate also depends on the method used to estimate initial heritabilities. Genes affecting the same bivariate trait probably include an independent subset for each subdimension, since the subdimensions usually responded in opposite directions. But the sets of genes affecting different bivariate traits probably overlap extensively.

Superimposed tracings of selected wings show that response is achieved not only by changes in local contours of the wing, but also by movement of vein intersections along the wing margins. Such displacement of vein intersection landmarks can cause two adjoining subdimensions to vary inversely, giving rise to correlations among bivariate offsets which have a




purely geometric basis. Correlations are also imposed where two bivariate offset traits share one subdimension, which happens in two cases (Figure 1). Figure 7 shows the magnitudes of correlated responses for each index of form. Although there are large overlapping effects, the pattern of correlated response is unique for each trait, and the range of response is always greatest in the trait under selection. Thus each of these traits adds different information about the genetics of wing morphology.

**Comparison of variation among all lines and treatments:** In Figure 8 the final (generation 16) distributions of the selected lines from Figure 3 are superimposed on full plots of the combined distributions of wild-caught flies from all seven localities, plus the base-population flies from all four environmental regimes. In each graph the bivariate distributions of all unselected flies have nearly the same slope and form a single long, narrow developmental zone, which must nearly span the natural range of genetic and environmental variation for wing form and size, at least for the northern United States. The selected lines have been shifted outward to occupy new developmental zones, hardly overlapping the range of wild type, in 15 generations of selection.

The same comparison is illustrated in Figure 9, in terms of the means of individual lines and populations. Many of the wild population offsets are significantly different from the allometric baselines of the laboratory base population (*i.e.*, significantly different from zero), but the full range of wild-population mean offsets occupies only a tiny part of the potential range demonstrated by the selected lines, also shown in Figure 9. Within the narrow range of offsets of the wild-caught populations and their laboratory-reared descendants, much of the variation is apparently environmental, or perhaps genotype-by-environmental. The regression of laboratory-reared on wild-caught population means gives a weakly positive slope (0.171) which is not significantly different from zero. However, the variance among localities is significant for both wild-caught and laboratory-reared flies, except for trait *R*.

Figure 10 shows the angular offsets of 21 isofemale lines, three from each of seven localities, using the same baselines as in Figure 9. These lines were collected by BRUCE WALLACE 22 to 27 years before these measurements were made, and have been maintained at small population size. The dispersion of offsets

FIGURE 3.—The effects of divergent selection on wing form. One line was selected in each direction for increased displacement from the baselines of Figure 2. Graphs show all males measured in generation 16. Mean angular offsets from baselines are given in radians and indicated by rotations of the baselines passing through the new centroids. In each graph a circular arc through the old centroid shows the original directions of selection.

**TABLE 2**

**Changes in dimensions $D_1$ and $D_2$; generation 16**

| Line | $N$ | $D_1$ (mm) | $\Delta D$ (mm) | $\Delta D_1$ (%) | $D_2$ (mm) | $\Delta D_2$ (mm) | $\Delta D_2$ (%) |
|---|---|---|---|---|---|---|---|
| $M^0$ | 100 | 1.105 ± 0.004 | | | 0.751 ± 0.003 | | |
| $M^+$ | 180 | 1.220 ± 0.004 | +0.115 ± 0.006 | +10.4 | 0.696 ± 0.002 | −0.056 ± 0.004 | −7.4 |
| $M^-$ | 180 | 0.837 ± 0.003 | −0.268 ± 0.005 | −24.2 | 0.809 ± 0.002 | +0.058 ± 0.004 | +7.7 |
| $S^0$ | 100 | 0.995 ± 0.003 | | | 0.598 ± 0.002 | | |
| $S^+$ | 206 | 1.078 ± 0.003 | +0.082 ± 0.004 | +8.3 | 0.494 ± 0.001 | −0.104 ± 0.002 | −17.4 |
| $S^-$ | 223 | 0.994 ± 0.003 | −0.001 ± 0.004 | −0.1 | 0.707 ± 0.002 | +0.109 ± 0.003 | +18.2 |
| $F^0$ | 100 | 0.903 ± 0.003 | | | 0.382 ± 0.001 | | |
| $F^+$ | 220 | 0.959 ± 0.002 | +0.056 ± 0.004 | +6.2 | 0.360 ± 0.001 | −0.021 ± 0.002 | −5.6 |
| $F^-$ | 215 | 0.846 ± 0.001 | −0.057 ± 0.003 | −6.3 | 0.443 ± 0.001 | +0.061 ± 0.001 | +16.1 |
| $G^0$ | 100 | 1.105 ± 0.004 | | | 0.978 ± 0.003 | | |
| $G^+$ | 171 | 1.189 ± 0.003 | +0.084 ± 0.005 | +7.6 | 0.919 ± 0.002 | −0.059 ± 0.004 | −6.1 |
| $G^-$ | 220 | 1.088 ± 0.003 | −0.017 ± 0.005 | −1.6 | 1.083 ± 0.002 | +0.104 ± 0.004 | +10.7 |
| $R^0$ | 100 | 0.920 ± 0.003 | | | 0.598 ± 0.002 | | |
| $R^+$ | 342 | 0.874 ± 0.002 | −0.046 ± 0.004 | −5.0 | 0.488 ± 0.001 | −0.110 ± 0.002 | −18.4 |
| $R^-$ | 275 | 0.883 ± 0.002 | −0.036 ± 0.004 | −4.0 | 0.655 ± 0.002 | +0.057 ± 0.003 | +9.5 |

Subdimensions of selected and unselected wings, grown under identical conditions. Note that $D_1$ in M and G, and $D_2$ in S and R, are identical.

**TABLE 3**

**Changes in mean radial distance; generation 16**

| Line | $N$ | $r$ (mm) | $\Delta r$ (mm) | $\Delta r$ (%) |
|---|---|---|---|---|
| $M^0$ | 100 | 1.336 ± 0.004 | | |
| $M^+$ | 180 | 1.404 ± 0.004 | +0.068 ± 0.006 | +5.1 |
| $M^-$ | 180 | 1.164 ± 0.003 | −0.172 ± 0.005 | −12.9 |
| $S^0$ | 100 | 1.161 ± 0.003 | | |
| $S^+$ | 206 | 1.185 ± 0.003 | +0.024 ± 0.004 | +2.1 |
| $S^-$ | 223 | 1.220 ± 0.003 | +0.059 ± 0.004 | +5.1 |
| $F^0$ | 100 | 0.981 ± 0.003 | | |
| $F^+$ | 220 | 1.025 ± 0.002 | +0.044 ± 0.004 | +4.5 |
| $F^-$ | 215 | 0.955 ± 0.001 | −0.026 ± 0.003 | −2.7 |
| $G^0$ | 100 | 1.476 ± 0.004 | | |
| $G^+$ | 171 | 1.503 ± 0.004 | +0.027 ± 0.006 | +1.8 |
| $G^-$ | 220 | 1.535 ± 0.004 | +0.059 ± 0.006 | +4.0 |
| $R^0$ | 100 | 1.097 ± 0.003 | | |
| $R^+$ | 342 | 1.001 ± 0.002 | −0.096 ± 0.004 | −8.8 |
| $R^-$ | 275 | 1.100 ± 0.002 | +0.003 ± 0.004 | +0.3 |

Distances from origin of bivariate centroids of selected and unselected wings grown under similar conditions.

illustrates the action of founder effect and drift on wing morphology, perhaps including the effects of new mutations. The variance among isofemale lines within localities is highly significant, with $P \ll 0.001$ for all traits. The variance among localities has been obscured by the divergence among lines, and is not significant for any trait.

The five-trait average of the variance among localities of laboratory-reared wild population means (the only significant component of their variance) is 0.000014, while the five-trait average of the variance of isofemale line means *within* localities (the only significant component of their variance) is 0.000148. Thus the random effects of drift and founding on interline variance can eventually contribute more than ten times the effect of current geographic differences among localities. This ratio is biased because the isofemale line means are estimated with greater precision due to their much lower within-line variance (see below), and would be larger if both sets of line means were estimated with equal precision.

Table 5 gives the mean phenotypic variances of the angular offset from base population baselines, for all traits in all unselected lines and populations. The mean phenotypic variance of all traits is almost 50% higher in samples of wild-caught flies than in their laboratory-reared descendants. Since only two or three generations had passed in laboratory culture and the populations were founded from many individuals, the reduction in variance is due only to the uniformity of culture conditions in the laboratory. The mean phenotypic variance of the base population is lower than the mean variance of the newly established wild populations, although the base population has been maintained at large size to minimize drift loss of genetic variance. The mean variance of the old isofemale lines is in turn much lower than that of the base population, again under identical culture conditions. This reflects the attrition through inbreeding of the original genetic variance, which now appears in the increased variance among isofemale line means within localities (Figure 10).

Since the mean realized heritability in the first 6 generations (Table 4) is 35.2%, the mean variance of the base population excluding the additive genetic component could be estimated at 0.00008 (64.8% of 0.00013). This equals the mean phenotypic variance of the isofemale lines (Table 5), suggesting that they have retained virtually no additive genetic variance

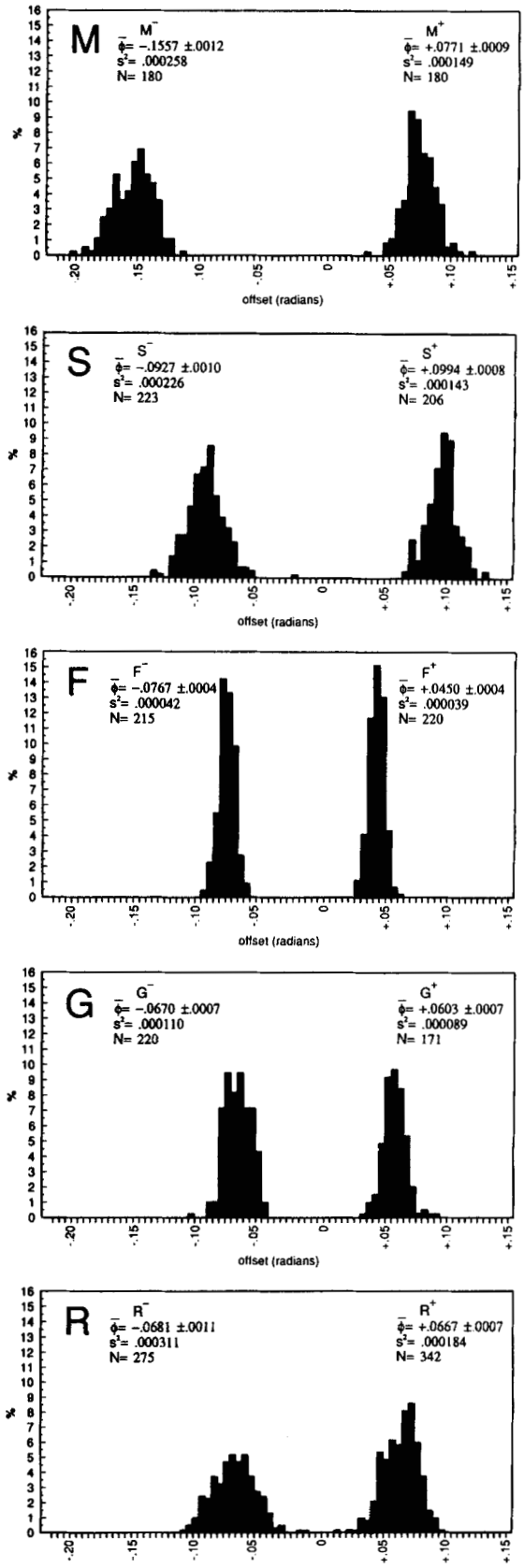



FIGURE 4.—The data of Figure 3 converted to univariate distributions of angular offset from the allometric baselines. Scales are in radians and all occupy the same range. The offsets can be treated as typical quantitative characters.

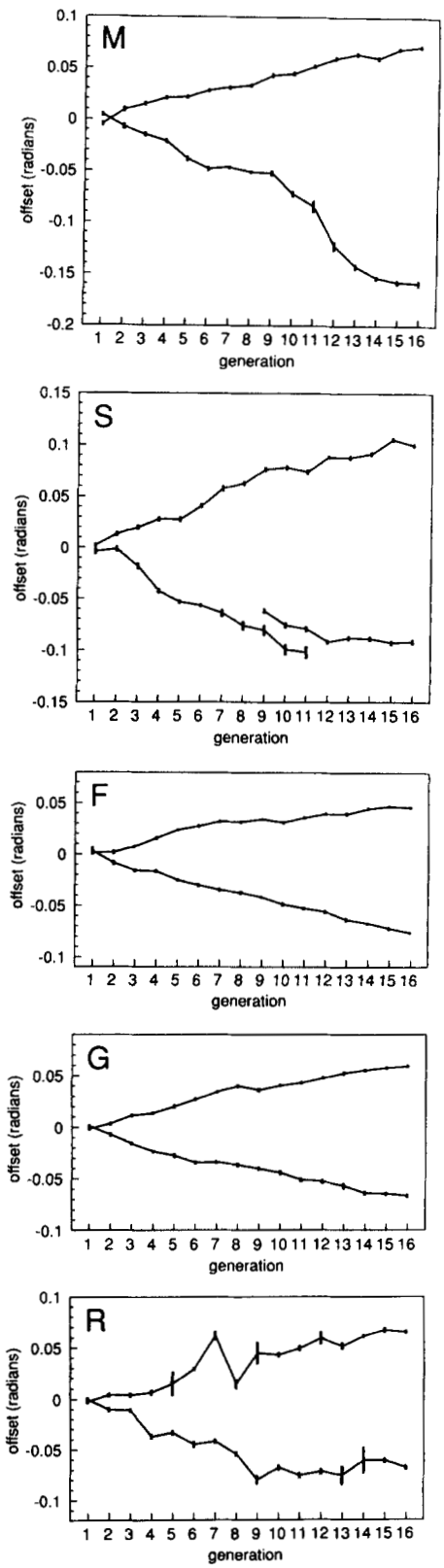



FIGURE 5.—The course of response to divergent selection on offsets. Data are for males; females were almost identical. Bars represent generation means ± 2 SE. Most means are based on samples of 100 flies. Several anomalies are discussed in the text.

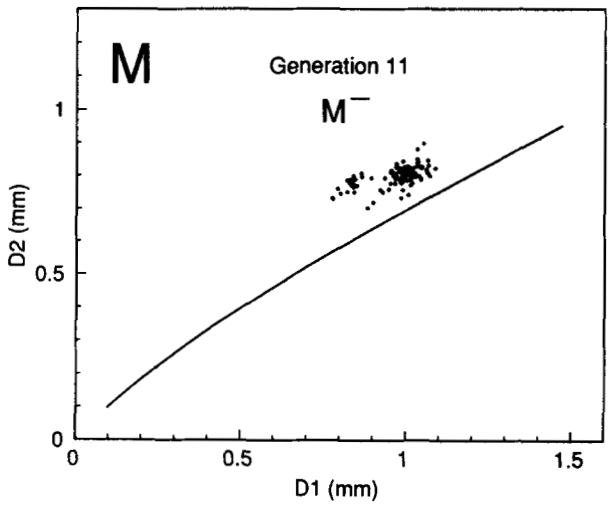



FIGURE 6.—Plot of all 100 males measured in generation 11 of line M⁻. A distinct new phenotype suddenly appeared, with equal frequencies in both sexes. A similar case (but with associated sterility) occurred in line S⁻ in generation 6.

for wing form. The last column of Table 5 gives the mean phenotypic variances between left and right wings among 50 flies measured for all traits on both wings. This indicates that about 38% of the environmental variance in wing form is due to internal "developmental noise" (and measurement error) rather than to external influences.

## DISCUSSION

Angular offset from an axial baseline is a convenient way to quantify variation in form. It provides a metric which is largely unconfounded with genetic variance for overall size, and is also relatively insensitive to various large environmental effects on size. When this metric is applied to selection on wing form in *D. melanogaster*, the environmental variation between generations is unusually low compared to the change in mean from selection. The resulting smooth patterns of response allow certain inferences about the genetic variation underlying form within the wing.

A salient fact about this genetic variation is that the estimates of effective gene numbers are high (Table 4). These estimates depend on a number of assumptions, however (FALCONER 1981). It is assumed, for example, that the contributory alleles in the base population all had frequencies of 0.5. This would be quite unlikely in such a large base population, with its large number of founders. It cannot be true of the alleles of large effect which appeared in lines M⁻ and S⁻. These were rare initially, or even arose *de novo* during selection. It is significant, however, that the initial responses are symmetric, in every trait (Figure 5). Also, the pattern of later response shows no increase of genetic variance in either direction, aside from line M⁻, where an initially uncommon allele goes to fixation. These observations tend to exclude a primary role for initially rare alleles in the other lines,

TABLE 4

**Realized heritabilities through generation 6 and response ranges in generation 16, with estimates of effective numbers of genes**

| Trait | $h^2 \pm$ SE | Range (radians) | $R_p$ = range/$S_p$ | $n_e = R_p^2/8h^2$ |
|---|---|---|---|---|
| M | 0.36 ± 0.07 | 0.2328 | 17.85 | 111 |
| S | 0.39 ± 0.04 | 0.1924 | 14.34 | 66 |
| F | 0.33 ± 0.01 | 0.1217 | 19.26 | 141 |
| G | 0.37 ± 0.02 | 0.1273 | 11.63 | 46 |
| R | 0.31 ± 0.08 | 0.1347 | 10.33 | 43 |

Realized heritability estimates are bidirectional means of response/cumulative selection differential. Ranges are given in radians and as multiples of base population phenotypic standard deviations for each trait.

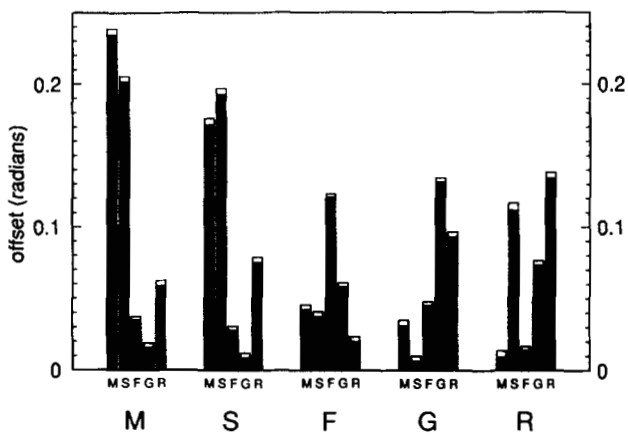



FIGURE 7.—The ranges of correlated response in each trait. Samples of 100 male flies from each selection line were measured for all traits on each individual. Each bar represents the absolute value of the difference in mean offset between divergently selected lines, plus 2 SE of the difference in means.

unless so many loci are involved that the total frequency changes are small.

It may therefore be reasonable to assume that many of the alleles contributing to the initial variance were at something approaching intermediate frequencies. Using the more general formula $n_e = pqR_p^2/2h^2$, one can show that even if initial frequencies of all less common alleles were as low as 0.1, gene number estimates would still be one third as high, and therefore still rather large. The additional assumptions required in the estimates of gene number are, similarly, bound to be wrong but perhaps not entirely misleading. For example, Figure 5 shows that the response did not reach a limit in most cases, so that in this regard the effective gene numbers are actually minimum estimates. The assumption of equal gene effects yields the smallest number of factors that could account for the response given the initial genetic variance. A distribution of unequal effects would increase the estimated number of loci. The last assumption required (perhaps a large one) is that the additive genetic variance in the base population is not partially hidden by linkage disequilibrium, possibly involving a systematic internal balancing of effects (MATHER 1941; 1953).

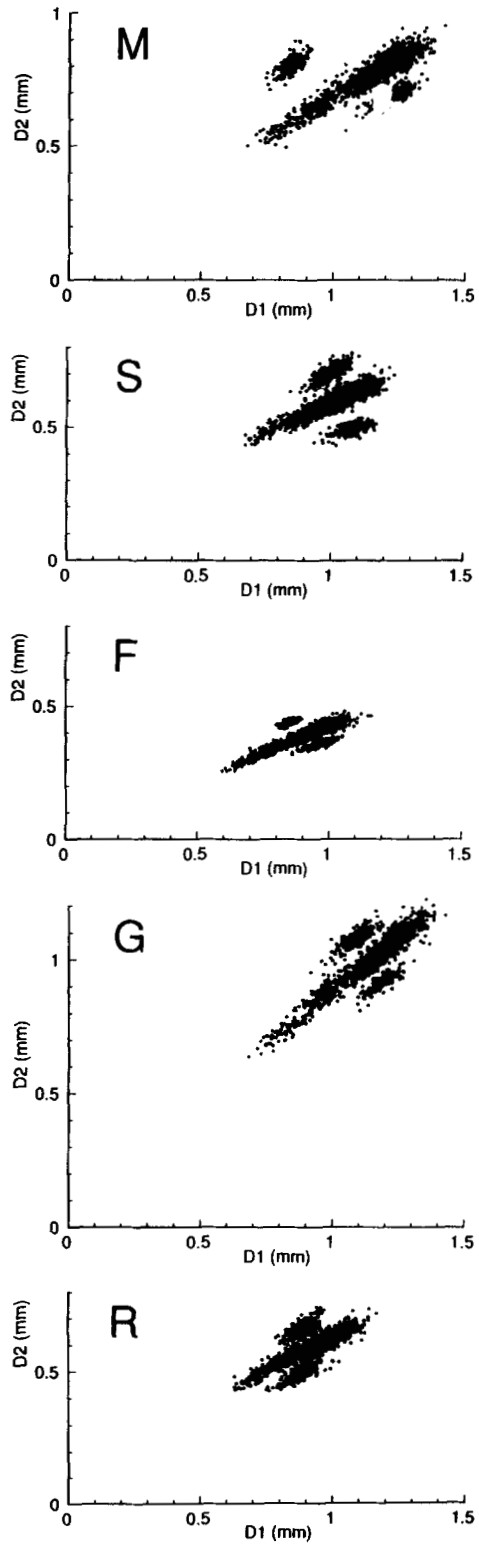



FIGURE 8.—Scatter plots combining all individual flies from environmental treatments, wild-caught samples, and selected lines. All unselected flies fall along a single narrow zone for each trait. Selected flies lie almost completely outside this zone, after 15 generations of selection at 20%.

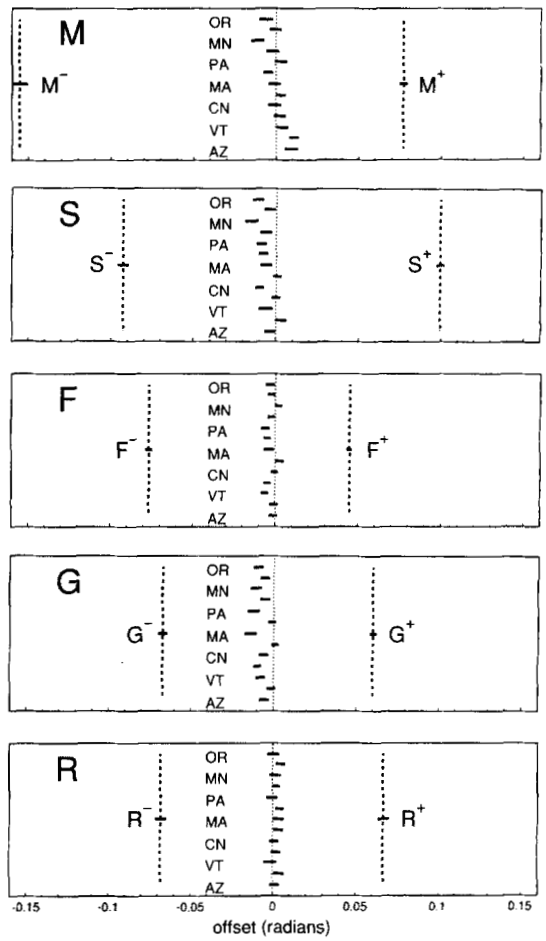



FIGURE 9.—Distribution of mean offsets (± 2 SE) of wild-caught flies and their laboratory-reared descendants. Beside each locality the wild-caught mean is above the laboratory-reared mean, except for AZ where only the wild-caught mean is shown. Each statistic is based on two pooled samples of 50 flies. Dashed vertical lines on either side pass through the means (± 2 SE) of selected lines in generation 16.

Given so many assumptions, the effective number of loci is mainly interesting as a currency for comparison with other selection experiments. The calculation relates the achieved change in the mean to the initial additive genetic variance (or heritability, in the present formulation). Initial genetic variances (or heritabilities) do not predict the limits to change by artificial selection, at typical selection intensities and population sizes (see ROBERTSON 1960; WEBER and DIGGINS 1990). The effective number of loci, which is derived from selection to the limits (or at least extended selection), is an index which must increase with the true number of loci. Allometric deviations of the wing are at least as selectable in relation to their initial heritabilities as most other quantitative traits, suggesting comparable numbers of genes. In terms of effective numbers, domains of the wing spanning 0.5–1.0 mm are affected by tens of segregating loci whose effects are separable from similar domains an equal distance away.

Some evidence for genetic variability in wing subdimensions was found by CAVICCHI, GIORGI and MOCHI (1978), who detected progressive differences in wing form between lines maintained at different temperatures. However, the changes were not significant in any single wing dimension, and significance ap-

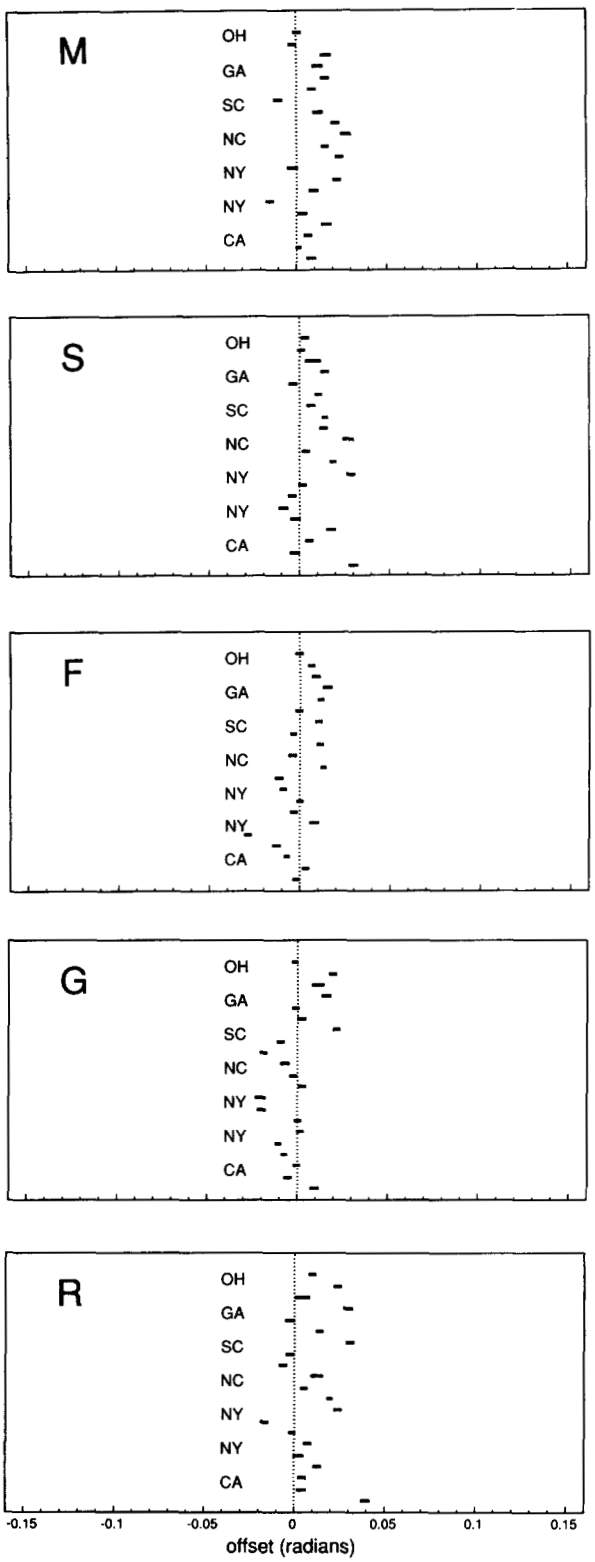



FIGURE 10.—Distribution of mean offsets (± 2 SE) of 21 isofemale lines measured for all traits. These lines had been maintained in small populations for 22–27 years. Each locality is represented by three coeval isofemale lines. Sample size for each isofemale line is 50 flies.

peared only in a multivariate analysis. CAVICCHI, PEZZOLI and GIORGI (1981) argued that the individual dimensions of the wing are largely inseparable genetically. They selected on one large wing dimension in one direction (shorter fourth longitudinal vein) and then measured correlated changes in seven other wing dimensions. There was not much change in the ratios of these seven dimensions to the selected dimension, in three selected lines compared to the control line. This was interpreted as evidence of an integrated genetic system determining wing shape, perhaps involving only a few genes. The contrary evidence reported here illustrates that correlated response to selection on the size of single dimensions is not a sensitive assay of genetic variability for localized effects on form, because most of the genetic variance for size in any single dimension is simply variation for total size, affecting all dimensions simultaneously. In another recent study, HAYNES (1988) selected divergently on the ratios of various individual wing dimensions to the third longitudinal vein. All ratios responded to selection, but because only ratios were recorded, it is not clear from these results how changes were distributed among individual areas of the wing, nor how much change occurred by allometric adjustment of overall wing size.

Selection using the present method of angular offsets allows resolution of localized effects. It is evident that the wing does not evolve as a uniformly elastic, evenly deformable field. It is easier for some dimensions to contract, and for others to expand. However, an equally important observation is that all subdimensions of the wing do reveal significant locally acting additive genetic variation (Table 2).

The morphological shifts in lines $M^-$ and $S^-$ resemble the quantum transitions postulated by ALBERCH (1980) and others, though possibly on a smaller scale. But sudden increments in response, immediately following increments in phenotypic variance, occur frequently in selection experiments, and randomly among replicate lines (*e.g.*, YOO 1980). These larger transitions are not saltations across forbidden regions of developmental space, but merely represent the high end of the spectrum of additive effects. Various mutations are known which have major effects on the contours of the wing or on the placement of veins on the wing (WADDINGTON 1950; LINDSLEY and GRELL 1968). The present results appear to show that the other end of the spectrum of gene effects provides the larger component of initial genetic variance in wing form, in the standing variability of many additive genes of small effect. This is the variability which allows the developmental outcome of wing growth to be selected 5–9 SD in any direction from its original mean axis, in 15 generations of selection.

Evidence for large numbers of genes with small localized effects on morphology of the mouse mandible has been found by BAILEY (1985; 1986). These "morphogenes" were detected in chromosomal regions near various introgressed histocompatibility system loci, assumed to be a random probe of the genome. Similarly, COYNE (1983) found that genes on

TABLE 5

**Mean phenotypic variances within samples, in radian² offset**

| Trait | $S^2$ (wild) | $S^2$ (wild laboratory-reared) | $S^2$ (base) | $S^2$ (isofemale) | $S^2$ (developmental) |
|---|---|---|---|---|---|
| M | 0.00029 | 0.00019 | 0.00017 | 0.00010 | 0.00004 |
| S | 0.00025 | 0.00020 | 0.00018 | 0.00010 | 0.00003 |
| F | 0.00012 | 0.00009 | 0.00004 | 0.00005 | 0.00002 |
| G | 0.00021 | 0.00012 | 0.00012 | 0.00007 | 0.00002 |
| R | 0.00023 | 0.00015 | 0.00016 | 0.00010 | 0.00003 |
| Means | 0.00022 | 0.00015 | 0.00013 | 0.00008 | 0.00003 |
| No. samples | 14 | 12 | 2 | 21 | 1 |

Mean phenotypic variances of samples from unselected lines, including wild-caught and laboratory-reared individuals of natural populations; the base population (LF350); and long established inbred isofemale lines. Last column gives the variance between wings within flies in the base population. Means at bottom are the grand mean variances of all traits. The bottom line gives the number of 50-fly samples on which each mean sample variance in each trait is based. All but the wild-caught flies were cultured under identical conditions.

every chromosome affect the morphology of the tiny male genital arches in Drosophila. BAILEY (1986) proposed a model of region-specific morphogenes under a bifurcating system of control. This recalls the binary model of progressive compartmentalization of cell clone growth in Drosophila (GARCIA-BELLIDO 1975). These ideas may be partially inadequate for the present data, since the moveable patterns of the wing veins are laid down secondarily upon the developing blade of the wing (WADDINGTON 1940; GARCIA-BELLIDO 1977). EDELMAN (1988) has elaborated a model of differentiation involving local cell-to-cell interaction in pattern formation, in addition to cascading control. All these models allow narrowly localized additive genetic effects.

What holds the zone of natural wing form within its existing genotypic range? From seven states, wild-caught and laboratory-reared flies conform to the same multidimensional zone of growth, which occupies a narrow sector of genetically accessible morphological space. The means of long established isofemale lines are ten times as dispersed, within localities, as the wild-caught lines are among localities. This is what would be expected from random sampling, with founder effect and drift, if the initial (and mutational) genetic variation for the trait includes many additive genes without important pleiotropic effects on fitness. This somewhat discredits the idea that a simple internal or genetic constraint preserves the clustering of wild population means.

On the other hand, the maximum dispersion among isofemale lines is still below the range attained by selected lines. This is consistent with the interpretation that many genes affect the traits, but could also indicate some influence of natural selection on wing form, either through fitness correlations (LINNEY, BARNES and KEARSEY 1971) or by direct selection on wing form even within the confines of culture vials. In Figure 10 there is clearly a nonrandom scattering of isofemale line means in trait *R*, at the tip of the wing. A multidimensional analysis of all possible angular offsets (see MATERIALS AND METHODS) among these landmarks confirmed that most of the 21 isofemale lines have blunter wing tips than any of the newly established wild lines.

The standard explanation for phenotypic uniformity is canalization. As operationally defined for metric traits, this simply means developmental resistance to environmental and genetic perturbations (WADDINGTON 1960; RENDEL 1962; see also MILKMAN 1970). It is demonstrated here that for five cases the bivariate baselines of wing form are indeed largely insensitive to environmental stress, but are immediately responsive to selection. A canalized character should initially resist selection, and its response should accelerate as it escapes the zone of canalization. The patterns of response in Figure 5 show that these allometries are not canalized against genetic change.

The data suggest a partial hierarchy of growth control, extending from simultaneous whole-wing effects down to local fields and patterns. It is already known that the size of the whole wing can be changed by selection (references in ROFF and MOUSSEAU 1987), with minimal effect on its internal proportions (CAVICCHI, PEZZOLI and GIORGI 1981). The present results show that when selection is focused on shape, the heritability of allometric deviations is as large as the heritability of wing size. This implies that the proportion of environmental variation is also approximately equal at both levels. However, in wing size only about 16% of the environmental variance is due to "developmental noise" plus measurement error (REEVE and ROBERTSON 1953), whereas these comprise about 38% of the environmental variance in wing shape. If external stresses tend to affect growth more evenly at the local level, then wing allometries may appear to be buffered or canalized against environmental stress, though responding compliantly to genetic change caused by selection.

However, the system will behave in this way only when the external stresses remain constant throughout development. It is known that brief, carefully

timed heat shocks during pupal development can generate a variety of abnormalities in wing shape (WADDINGTON 1950), which mimic the effects of known mutations and cause departures from the allometric baselines described here.

Although angular offsets seem largely unaffected by environmental stress in laboratory culture, the higher phenotypic variances of wild-caught flies compared to their laboratory-reared descendants (Table 5) must in some way reflect the higher diversity of natural environments. In light of the facts just discussed, the additional variance in nature may be due to fluctuations in temperature during pupal development. In any case, the heritabilities of these traits in nature must be reduced by this extra variance below the value of *ca.* 35% measured in the laboratory (see COYNE and BEECHAM 1987; PROUT and BARKER 1989; RISKA, PROUT and TURELLI 1989). The higher phenotypic variance of laboratory-reared wild compared to base population flies suggests that genetic variance may be higher in the newly established wild lines than in the older laboratory base population which was the source of the selection lines. Since the means of wild-caught and laboratory-reared offsets are nearly the same (Figure 9), and since the genotype-by-environmental variance would appear to be small compared to the additive genetic variance, one might use these simple interpretations of the variance differences in Table 5, to estimate heritabilities in nature at around 30%. If it is assumed that wild-caught flies have the same genetic variance as the base population, then natural heritabilities are still around 20%.

In nature, any net selection for a different wing form must be entirely absent, at least in terms of these individual bivariate distributions (see CHARLESWORTH 1990). But more than an absence of directional selection is required to account for the clustering of geographically diverse wild-population means. One might assume that wing form is a neutral character, and then invoke massive migration to keep alleles at many loci near average frequencies, everywhere locally from Oregon to Vermont. However, many comparable species exhibit genetic differentiation over similar ranges (GOULD and JOHNSTON 1972), including other Drosophila species (STALKER and CARSON 1947, 1948, 1949; REED and REED 1948; PREVOSTI 1955; MISRA and REEVE 1964; HYYTIA *et al.* 1985), and *D. melanogaster* itself (DAVID, BOCQUET and DE SCHEEMAEKER-LOUIS 1977; STANLEY and PARSONS 1981; LOUIS *et al.* 1982; COHAN and GRAF 1985; COYNE and BEECHAM 1987; RIM, LEE and LEE 1988; and references in HENDERSON and LAMBERT 1982), and also other vagile and recently introduced species (JOHNSTON and SELANDER 1971; BRYANT 1977; BRYANT and TURNER 1978; BAKER and MOEED 1979; BAKER 1980). The evidence fits the hypothesis of contemporary optimizing selection on wing form in nature.

Wing allometry may actually vary more in nature than is demonstrated in this limited survey. Except for the Arizona sample, the wild-caught populations here are all from northern states and from the same time of year. Most clines in Drosophila have been related to latitudinal, altitudinal or seasonal gradients. There is significant geographic heterogeneity among the localities tested in this study, but to characterize ecological correlates will require a wider sampling. The focus here is on the quantification of genetic variation for wing allometry. It remains to be seen whether the variation in form produced in 15 generations of selection exceeds the limits of natural variation worldwide.

Genetic variation has been quantified in many traits, but only rarely in the small and separate details of growth patterns. Drastic effects on form have been studied in chemically induced developmental abnormalities of amphibian limbs (ALBERCH and GALE 1985), in the wings of heat-shocked flies (WADDINGTON 1950), and in the rather coarse genetic remodeling of cranial morphology in dogs and pigs, caused by selection for juvenilization (WAYNE 1986). Sometimes such phenomena have been interpreted as evidence that evolution is constrained or limited to these major routes of change (*e.g.*, GOULD 1986). But in these cases the ability of selection to produce more localized morphological changes of the type demonstrated here has not been explored.

If additive genes with small localized effects are plentiful, morphology may not be greatly constrained by interactions between functionally unrelated parts of organisms. This is a view in which morphological change is predominantly adaptive and stasis represents optimality (see MAYNARD SMITH 1983; MAYNARD SMITH *et al.* 1985). Many antioptimality arguments reduce to skepticism about the general availability of additive genetic variance for adaptive fine-tuning (GOULD and LEWONTIN 1979; KITCHER 1987). Skepticism is still in order, and the present study does not demonstrate that the fly has the best wing possible, only perhaps the best available at a certain level of morphological subdivision. However, the investigation of genetic variability is still incomplete, especially in the details of developmental patterns.

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