How Small Are the Smallest Selectable Domains of Form?

K. E. Weber[1]

Department of Ecology, Evolution and Behavior, University of Minnesota, Minneapolis, Minnesota 55455

Manuscript received January 14, 1991
Accepted for publication October 14, 1991

ABSTRACT

Two lines of *Drosophila melanogaster* from the same base population were selected in opposite directions to produce simultaneous antagonistic changes in two very small (<0.2 mm) and closely adjacent (<0.3 mm) dimensions within the base of the wing. Wing dimensions near the targeted area became differentiated by large positive and negative percentage differences, while only small homogeneous percentage changes occurred in the remainder of the wing. If very small regions of morphology (less than 100 cells across) can respond to selection almost independently, even in small population samples, then the control of developmental detail must involve many genes, and the diversity of possible outcomes in development and adaptation must be large.

INSECT wings undergo complex deformations during flight, which are passively controlled by the locations of the wing veins. In wings at rest, the veins maintain a topography of panels and corrugations in the wing membrane and are often hinged and reinforced at various points, all in such a way as to suggest that the deformations occurring in flight may be advantageous. Functional studies of wing morphology in diverse taxa, using high speed photography and principles of aerodynamics, confirm that many details in the way wings pleat, buckle and warp during flight can be interpreted provisionally in terms of optimal design (NORBERG 1972; NACHTIGALL 1981; BRODSKIY and IVANOV 1983; ENNOS 1988; WOOTTON 1981, 1990). Indeed, in some of these studies the idea of optimality is so pervasive that nearly every feature of contour and venation is automatically referred to as an adaptation, even where authors disagree or have no theory regarding the feature's function.

In a broad and membranaceous structure such as the insect wing, only two cells thick, a network of stiffening veins has obvious utility. But that every detail in such an irregular pattern represents the realization of mechanical perfection via natural selection, just as a cast form reflects its mold, is an interpretation that should attract the interest of geneticists, because of the high density of genetic information this would demand. Some developmental biologists already favor the view that morphological differentiation may be controlled by a relatively small number of genes (GARCÍA-BELLIDO 1983; RAFF and KAUFMAN 1983), or by a limited number of developmental outcomes (GOODWIN 1984), with much final detail supplied automatically by epigenetic mechanisms (HORDER 1981). Thus one could not, as a rule, change small individual parts autonomously (ALBERCH 1980), nor achieve fine-grained adaptive optimization. Yet this seems incompatible with the view of functional morphologists, that natural selection is able to fashion detailed wing morphologies which satisfy strict local design requirements at almost every point.

Studies of natural variation in wing form sometimes suggest a nonfunctional component. A purely descriptive study of wing morphology in several butterfly taxa suggested strong developmental constraints on some aspects of form (STRAUSS 1990). A study of wing form variation in cicadas (SIMON 1983) found divergences among isolated broods that could be due to genetic correlations or to drift. A related observation is that mimicry in butterflies often involves not only coloration but also some convergence in wing outline, indicating that the outer contours of the wing may not be under the strict control of aerodynamic requirements. But these are only interpretations and cannot clearly refute the idea that most details of contour and venation reflect precise mechanical demands.

A key question, then, is whether the necessary genetic potential exists for dense, fine-grained, autonomous and *localized* adaptive change all over the insect wing; or whether the potential for localized remodeling is only coarse-grained and scattered here and there. Common sense dictates that there is some lower limit to the size of the smallest morphological domains that can typically be altered independently by mass selection. However, very few experiments have explored this lower limit.

Several selection experiments have demonstrated localized polygenic effects on specific bristles in Drosophila (MAYNARD SMITH and SONDHI 1960; REEVE 1961; SPICKETT 1963); and other experiments have

[1] Present address: Department of Biological Sciences, University of Southern Maine, Portland, Maine 04103.

demonstrated the existence of modifiers of small gaps in wing veins caused by several mutations (MILKMAN 1970; THOMPSON 1974a; COHAN 1984; SCHARLOO 1987). But all these cases show variation only in a structure's presence or absence at a fixed location, not in the location itself. This was already familiar, at a larger scale, in major mutations (most described in LINDSLEY and GRELL 1968) that add or subtract bristles (PLUNKETT 1926; ASHBURNER 1982) or wing veins (THOMPSON 1974b; DIAZ-BENJUMEA and GARCÍA-BELLIDO 1990) in predetermined regions. A distinction has often been made between such variation, and variations in *form* (BATESON 1894; COCK 1966; CHERRY *et al.* 1979; GARCÍA-BELLIDO 1983): for example, the necks of giraffes and dolphins differ in form but not in plan, both having seven vertebrae. Most differences in wing venation among related insects involve considerable changes in form, *i.e.*, in the relative locations of homologous landmarks. Most of the fine adjustments presumably necessary to achieve optimization consist of shifts in form (WOOTTON 1990).

Interspecific crosses have shown that sibling species of Drosophila, differing in the form of individual parts, are fixed for multiple factors affecting the form of those parts (TEMPLETON 1977, analyzed in LANDE 1981; COYNE 1983; COYNE, RUX and DAVID 1991; SPICER 1991). A few selection experiments have also demonstrated within-population genetic variation affecting the proportions of inter-landmark distances in Drosophila. SCHARLOO (1987) changed the locations of dorsocentral bristles, and HAYNES (1988) changed the ratios of various wing dimensions to wing length. WEBER (1990) selected on pairs of major wing dimensions antagonistically, and produced rapid change in every tested dimension, with paired dimensions responding in opposite directions simultaneously. Well defined allometries of the wing were easily broken. This plasticity of wing form in response to selection on major dimensions raised the question of how small the lower limits of localized selectable effects would be.

The selection experiment reported here aimed to find such a limit. The selected trait involves the smallest region of external insect morphology that has been tested for the presence of segregating genes with localized effects on form. The region was chosen because it presented the tightest cluster of good landmarks that could be identified on the wing of *Drosophila melanogaster*, but it was otherwise chosen at random. A metric was devised to distinguish localized variations inside this region from variations of the surrounding wing.

MATERIALS AND METHODS

At high magnification, many landmarks on the wing are grainy and hard to pinpoint, but the four points in Figure 1 are reasonably distinct. Two small adjacent dimensions (D_1 and D_2), derived from these points, were the objects of selection. Both dimensions are transverse to the long axis of the wing. Dimension D_1 extends from the junction of vein L1 and the inner costal margin, to the junction of veins L2 and L3; D_2 extends from the inner proximal edge of the costal gap at the base of the costal cell, to the junction of L1 with L2. These two dimensions are around 0.15 mm (D_1) and 0.11 mm (D_2) in control flies cultured at 24° with uncrowded larvae. The number of cell diameters in each dimension can be estimated at about 35 (D_1) and 20 (D_2), since each wing cell bears a single bristle; however, changes in shape are analyzed here in terms of distance only.

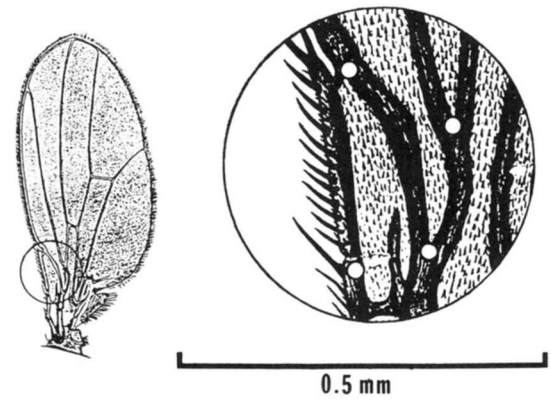

FIGURE 1.—Full wing and enlargement, showing the target area with four landmark points. D_1 is the distance between the upper pair of points; D_2 is the distance between the lower pair. Selection was for large D_1/small D_2 in population E^+ and for small D_1/large D_2 in population E^-.

To detect the segregation of genes which affect primarily a single local dimension, it is necessary to normalize the variations in that dimension against the variations common to the surrounding area. The simplest approximation of this idea is to normalize a pair of neighboring dimensions against each other, by applying selection perpendicularly to the major axis of the joint distribution of the two dimensions. If the two dimensions are isometrically related, this could be done approximately by selecting on their ratio. If not, then the ratio is correlated with size, and correction must be made to avoid selecting for differences which are merely the allometric effect of change in overall size. In pilot experiments (K. WEBER, unpublished results) with selection on the ratios of allometrically related wing dimensions, much of the response was attributable simply to change in overall wing size, confounding the high heritability of wing size with the autonomous heritabilities of subregions.

The bivariate distributions of Drosophila wing dimensions are usually allometric and the major axis can be conveniently approximated by a polar equation ($\theta = \beta r^{\alpha}$) derived by the regression of the log of θ on the log of r, where $\theta = \arctan(D_2/D_1)$ and $r = (D_1^2 + D_2^2)^{1/2}$ (WEBER 1990b). (The two dimensions are assumed to be near each other and positively correlated.) The angular offset of individual points from this curve, in radians, then provides an allometrically corrected antagonistic scale of selection. On this scale, flies with large D_1 or small D_2 have positive (clockwise) offsets, while flies with small D_1 or large D_2 have negative (counterclockwise) offsets. This method allows simultaneous antagonistic selection on both dimensions using a single scale. The method is more fully explained in WEBER (1990), as is the system used to measure live anesthetized flies at high magnification (see also WEBER 1988).

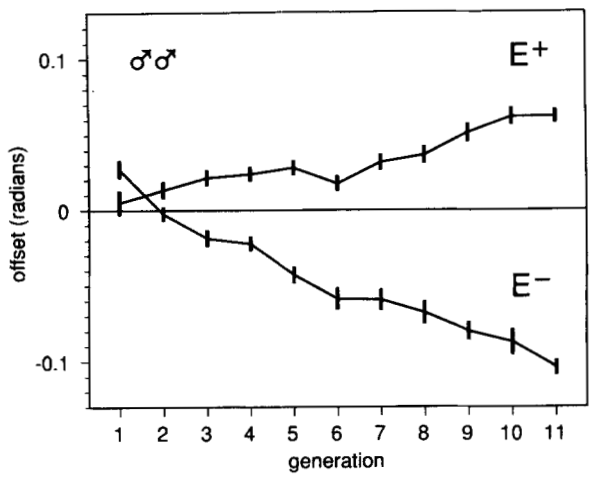

FIGURE 2.—Selection response in males, in radians of angular offset from the control baseline. Bars give two standard errors above and below generation means. Sample sizes were either 100 or 150.

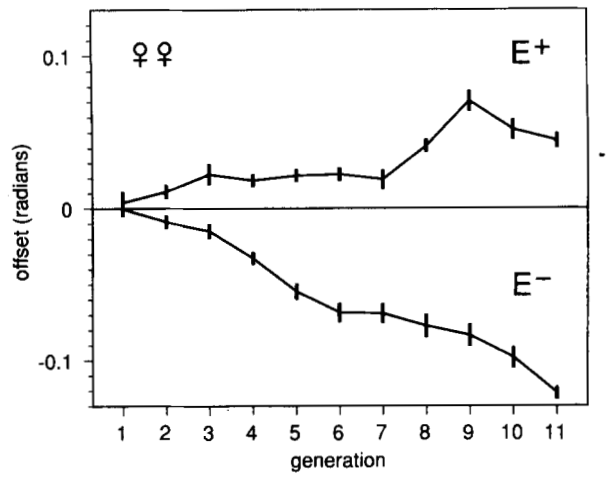

FIGURE 3.—Selection response in females, in radians of angular offset. As in Figure 2, with two standard errors of the means, and sample sizes of 100 or 150.

Two divergent selection lines (designated E^+ and E^-) were founded from a long established wild-type base population (LF350), which originated from a large sample of wild flies and has been maintained at large size. A baseline equation of $\theta = 0.527\ r^{-0.081}$ was derived for D_1 and D_2 in this population. Flies were selected for positive angular offset from this baseline in line E^+ and for negative offset in line E^-, with the offset (ϕ) of each fly calculated by subtracting its true angle from the angle predicted by the baseline for its r. In each selection line the most extreme 20% of 100–150 unmated flies of each sex were selected each generation for 10 generations. Controls were the large base population (designated here as CN1) and a subline of the base population (CN2) which has been maintained for more than 70 generations.

In generation 11 both selected lines were cultured under identical conditions and measured to determine proportional differences over the whole wing. The coordinates of 17 landmarks were digitized from left or right wings at random, from 100 live flies of each sex in each line. The landmarks included breaks, notches and intersections of veins, and were defined with detailed drawings. Each of the 17 points had been digitized by the author on several thousand flies, in other work, before final measurements in the present study were taken, so that interpretations had become habitual and fully standardized.

To evaluate the pattern of change, 26 inter-landmark dimensions were extracted and compared between lines E^+ and E^-, in each sex. All significance levels given here are corrected for the total number of comparisons (52) between the oppositely selected populations, by a modification of the "sequential Bonferroni" method (HOLM 1979; RICE 1989). Dimensional differences were ranked not by exact probabilities, but by their probability cutoff points ($P = 0.05, 0.01, \ldots, 0.000001$) up to the limits of Table 26.10 in ABRAMOWITZ and STEGUN (1972), using the t-distribution with d.f. = 198. Then these significance levels were corrected by the sequential Bonferroni method, as if all P values in each category were identical to the cutoff maximum. This modification gives lower significance levels than could be claimed with more exact methods.

RESULTS

Figures 2 and 3 show the course of selection response in both sexes, on the angular offset scale of

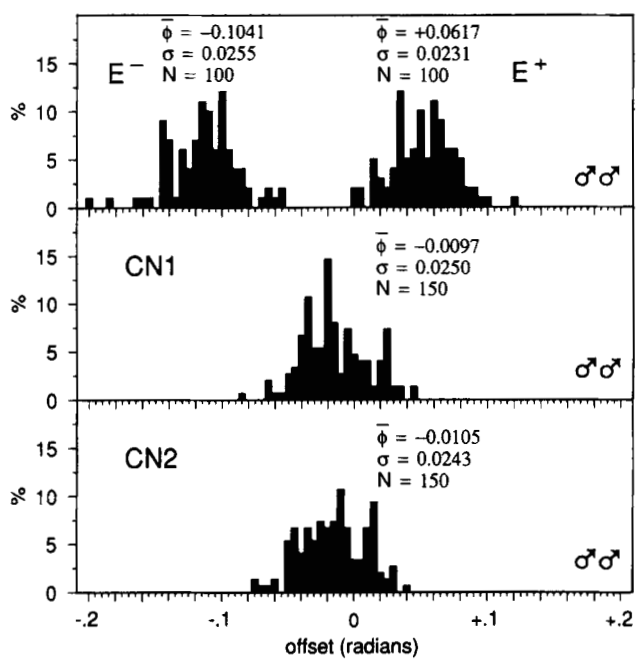

FIGURE 4.—Distribution of angular offsets. Males of both selected lines in generation 11, and of both control lines.

selection. After several generations, mean offsets already diverged significantly from the baseline in both directions. Selection was continued to the eleventh generation to intensify any correlated changes in the surrounding wing, and to determine the shape of the response curves. Response was continuous but asymmetrical, as might be caused for example by moderate asymmetries in allele frequencies (FALCONER 1981). The realized heritabilities of angular offset were 0.12 ± 0.02 in line E^+, and 0.24 ± 0.02 in line E^- (standard errors by the method of HILL 1972).

Figures 4 and 5 show the distributions of angular offset in males and females for both selected lines in generation 11, and for both unselected control populations. Each selected line is significantly different

from either control. The distributions of the selected lines are nonoverlapping, and the mean divergence between them in generation 11 is 6.7 times the mean standard deviation of controls. The variances have changed little.

During selection most measurements were made by an assistant, whose interpretations of the precise locations of landmarks underwent some readjustment during the first generation. This explains the significant first-generation difference between males of the two selection lines (Figure 2). All final measurements were made by the author. A small but consistent difference in interpretation of landmarks is detectable in the endpoint data: the distributions of the control populations (Figures 4 and 5) are not centered precisely on zero angular offset but are all slightly negative, and a similar negative shift appears in the selected lines between generations 10 and 11 in Figures 2 and 3.

The increased offset from the reference baseline, in both selected lines, shows that the bivariate distribution of D_1 and D_2 was relocated in both lines, in opposite directions. The divergence between the selected lines can be analyzed in terms of the changes in D_1 and D_2 and in other adjacent dimensions of the wing. Figure 6 shows nine landmarks (designated *A* through *I*) in the vicinity of the selection targets, connected by lines to form a solid framework of small dimensions. Dimensions *HG* and *AB* are the selected dimensions, D_1 and D_2. Dashed lines indicate dimensions which are larger in population E^+ than in E^-, and dotted lines show dimensions which are larger in population E^- than in E^+.

Table 1 gives the magnitudes of the most significant differences indicated in Figure 6. The differences in Table 1 are obtained by subtracting the mean size of each dimension in population E^- from the mean in population E^+. This gives a positive sign to the difference in those dimensions in which population E^+ exceeds population E^- (dashed lines in Figure 6), and a negative sign to those dimensions in which E^- exceeds E^+ (dotted lines in Figure 6). Table 1 also gives the standard error of the difference in means for each dimension, and the difference as a percent of the line E^- mean. The significance levels of the differences in means were corrected for the total number of comparisons in this study, as explained in MATERIALS AND METHODS.

The two selected populations are extremely differentiated in the target area. The differences in individual local dimensions are highly significant, and the pattern of percentage differences is similar in both sexes. The most important point is that many significant differences are opposite in sign, even between adjacent dimensions.

The complex reconstruction that would now be

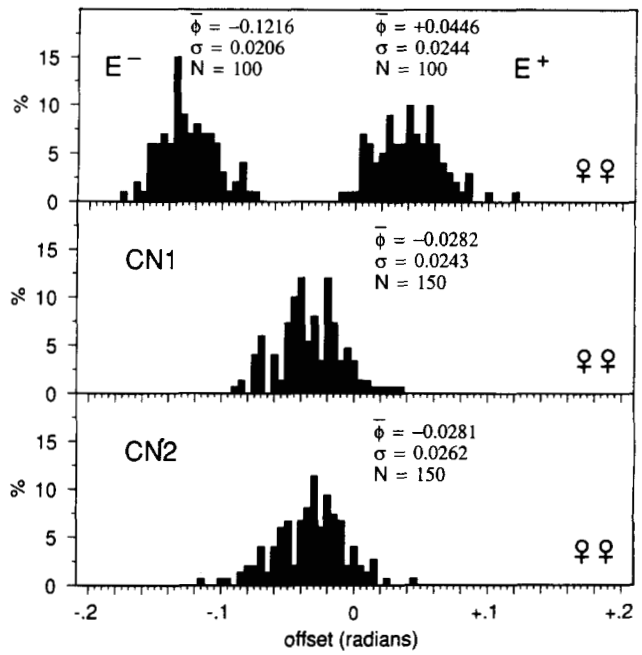

FIGURE 5.—Distribution of angular offsets. Females of both selected lines in generation 11, and of both control lines.

required to change the wing of either selected population into the wing of the other, can only be understood by visualizing both expansions *and* contractions of adjacent dimensions. Moving in the direction E^- to E^+, for example (as indicated in Figure 6), dimensions *IH* and *IE* decrease, while the dimension *IG* lying between them *increases* by 15%. This is accomplished by the independent lowering of point *G* relative to *I*, reducing both dimensions *GF* and *GB*. (For simplicity all directions are given with reference to the orientation of the wing in Figure 6.) The relative downward relocation of point *G* increases the selected dimension *HG* (D_1). At the same time, the dimensions *HA* and *HB* increase, so that point *H* moves upward while *G* moves downward, both relative to *I*. The net effect is a difference between populations E^- and E^+ of around 40% in dimension *HG* (D_1), and a corresponding difference in the angle *HBG*, which is about 10% larger in line E^+ in both sexes. Although D_1 is 40% larger in E^+ than in E^-, D_2 (*AB*) is 3–4% smaller (Table 1). Thus it is possible to produce significant differences in opposite directions simultaneously, by localized rearrangements, in these two neighboring parallel dimensions.

Table 2 and Figure 7 show differences between the selected populations over the remainder of the wing, in dimensions aligned with either length or width. All the percent changes in length and width are very small. They are also consistently positive ($E^+ > E^-$). Large percentage, reciprocal positive and negative changes occur only in the vicinity of the selected dimensions, while for the wing as a whole (ignoring the target-area rearrangements in Figure 6) one can

TABLE 1

Differences in local dimensions in the target area

Dimension[a]	Males (mm)	Percent	Females (mm)	Percent
IH	−0.0131 ± 0.0016	−5.6****	−0.0163 ± 0.0019	−5.9****
IG	+0.0329 ± 0.0021	+15.0****	+0.0432 ± 0.0022	+16.2****
GF	−0.0345 ± 0.0010	−24.4****	−0.0402 ± 0.0013	−25.1****
GB	−0.0383 ± 0.0014	−18.1****	−0.0444 ± 0.0017	−18.2****
HA	+0.0209 ± 0.0019	+7.8****	+0.0263 ± 0.0019	+8.7****
HB	+0.0313 ± 0.0018	+11.6****	+0.0375 ± 0.0019	+12.2****
$HG(D_1)$	+0.0484 ± 0.0012	+39.6****	+0.0560 ± 0.0013	+41.0****
$AB(D_2)$	−0.0041 ± 0.0006	−3.9****	−0.0034 ± 0.0006	−2.8****
BF	+0.0040 ± 0.0010	+3.4**	+0.0053 ± 0.0010	+3.7****
CD	+0.0056 ± 0.0010	+3.2****	+0.0038 ± 0.0011	+1.9*

[a] Dimensions as shown in Figure 6. * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $0.0001 < P < 0.001$; **** $P < 0.0001$.

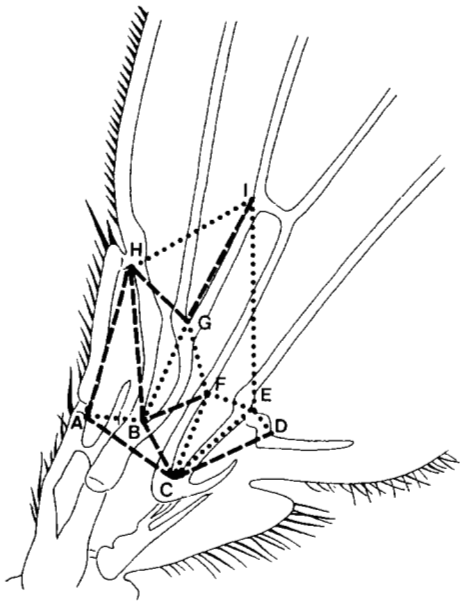

FIGURE 6.—Differences in generation 11 between oppositely selected populations, in distances separating 9 landmarks (*A–I*) in the target area. Dimensions marked with dashed lines are larger in population E^+ than in population E^-; dimensions marked with dotted lines are larger in population E^- than in population E^+. All measurements are from the same wings as in generation 11 in Figures 2, 3, 4 and 5. (See Table 1 for magnitudes of individual differences.)

simply say that the wings of population E^+ are everywhere slightly longer and slightly wider than the wings of E^-.

The small overall increase in wing width in E^+ relative to E^- cannot account for the disproportionately large percent increase in D_1. Figure 6 shows that the small width increase in dimension *9* (point *H* to point *D*) is actually the net of a large local expansion within *HG* (D_1), and compensating reductions in *GF*, *FE* and *ED*. The largest reduction is in *GF* (Table 1). Clearly point *G*, the junction of veins L2 and L3, has undergone an independent shift in its position on the wing in E^+ relative to E^-, moving downward and to the right in Figure 6. This relocation is a major source of the difference in D_1.

Especially to be noted is that although dimension *8* (Table 2 and Figure 7) is very near D_1, parallel to it, and within the same distal anterior wing compartment (GARCÍA-BELLIDO 1975), it does not change in the same proportion as D_1. In fact dimension *8* exhibits no greater percent width change than dimension *7*, which is farther away and in the posterior compartment. This shows that the large percent difference in D_1 cannot be explained by a homogeneous change in the width of one whole compartment, but must be limited to a more localized area.

The negative difference in D_2 is significant and in the direction predicted by the selection protocol, and is a localized difference in the opposite direction to the surrounding positive divergence. Thus line E^+, relative to E^-, achieves a reduction in D_2 in spite of expansion of the wing overall (Table 2 and Figure 7), and in spite of surrounding local expansion in *HA*, *HB*, *AC* and *BC* (Figure 6). These antagonistic changes must reduce the percentage magnitude of the simultaneous reduction that can be achieved independently in D_2 in line E^+.

The intrusion of some overall wing size divergence in these rearrangements is not surprising since other selection experiments have shown that wing size possesses much additive genetic variance (ROFF and MOUSSEAU 1987). By using a more constraining index, with D_1 selected antagonistically to both D_2 and overall size, the divergence in wing size might be reduced, thereby decreasing all dimensions in E^+ relative to E^- and making the difference less positive in D_1, more negative in D_2.

DISCUSSION

In this experiment, the strongly adaptationist standpoint of many functional analyses of insect wings has survived a stringent test of its genetic implications. A random bit of the wing-vein network was shown to

TABLE 2

Differences in length and width dimensions

Dimension[a]	Males (mm)	Percent	Females (mm)	Percent
1	+0.0194 ± 0.0053	+1.8*	+0.0285 ± 0.0058	+2.2***
2	+0.0262 ± 0.0071	+1.5*	+0.0470 ± 0.0078	+2.4****
3	+0.0218 ± 0.0069	+1.3 NS	+0.0352 ± 0.0075	+1.8***
4	+0.0058 ± 0.0042	+0.6 NS	+0.0080 ± 0.0049	+0.7 NS
5	+0.0017 ± 0.0021	+0.8 NS	+0.0067 ± 0.0022	+3.0 NS
6	+0.0415 ± 0.0043	+4.8****	+0.0477 ± 0.0044	+4.9****
7	+0.0100 ± 0.0012	+5.9****	+0.0068 ± 0.0013	+3.3****
8	+0.0026 ± 0.0006	+3.3***	+0.0035 ± 0.0006	+3.8****
9	+0.0274 ± 0.0020	+7.4****	+0.0293 ± 0.0021	+7.0****
10	+0.0025 ± 0.0009	+1.3 NS	+0.0017 ± 0.0011	+0.8 NS

[a] Dimensions as shown in Figure 7. * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $0.0001 < P < 0.001$; **** $P < 0.0001$.

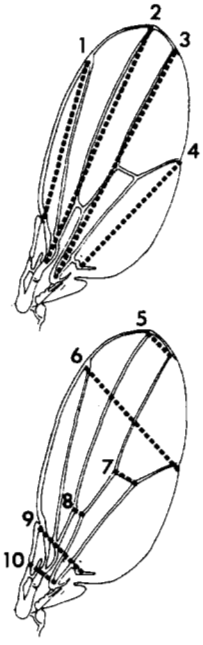

FIGURE 7.—Dimensions aligned with either the length (*1–4*) or the width (*5–10*) of the wing. See Table 2 for magnitudes of differences in these dimensions.

harbor its own differentiable genetic variability, so that its form is subject to selection. Small neighboring elements can shrink and expand inversely. There were certain constraints on this response, in that the antagonistic changes were not evenly distributed and the response was not entirely free of correlated effects; but response was certainly disproportionately localized in the targeted area of the phenotype. Given this result one might expect that even greater resolution and control of detail could be achieved in larger populations over longer times.

To the functional morphologist, or to anyone with a reflexive and literal belief that everything is selectable, such a result might seem too predictable to require any demonstration. The simplest adaptationist reasoning would assert that (1) any functional element (however minor) must be optimal, almost as a condition of life; (2) its optimality results from selection, presupposing the existence of additive genetic variance; and therefore (3) it is bound to display additive genetic variance again if subjected to artificial selection. This logical chain breaks down at all three points for the following corresponding reasons: (1) as the continual improvement of human inventions illustrates, many mechanisms already function extremely usefully even in an imperfect state; (2) in theory at least, even strongly adaptationist accounts of evolutionary change need not be based on mass-selectable, additive genetic variance (this is well stated in WRIGHT 1965); and (3) even adaptive changes arising from additive variability need not retain their original genetic polymorphism, and in traits involving few loci would often lose it all. (For that matter, an *absence* of immediate selectability in critical structures could be predicted just as reasonably on adaptationist grounds.) Thus no fair claim can be made that the present results were inevitable, as if this were a corollary of the theory of evolution itself. On the other hand these results, as far as they go, clearly support the assumption of fine-grained selectability that underlies strictly functionalistic interpretations of insect wing morphology.

The significance of this experiment for genetics is its demonstration of polymorphism in a category of presumably late-acting genes with extremely localized effects on the development of form. The result thus extends the range and variety of characters known to be selectable, in the tradition of many earlier selection experiments, such as CASTLE and PHILLIPS (1914), MACDOWELL (1917), TRYON (1940), ROBERTSON (1957, 1962), MAYNARD SMITH and SONDHI (1960), RENDEL and SHELDON (1960), CLARKE, MAYNARD SMITH and SONDHI (1961), SCHARLOO (1964), DOBZHANSKY and SPASSKY (1967), PATERNIANI (1969), CHINNICI (1971), PENNYCUIK and RENDEL (1977), MASRY and ROBERTSON (1978), CADE (1981) and JOHNSTON (1982). In each of the cited studies, the existence of additive genetic variability in some new or unusual trait (or traits) was satisfactorily demonstrated with a single pair of divergently selected lines

per trait, usually employing lines of 5–25 pairs selected for 10–15 generations, or about the same as in the present study.

The question of primary interest, however, is the extent to which the rest of the fly is also readily deformable by selection at the same small scale. Such experiments have been impossibly tedious by conventional methods. Can adjacent dimensions within leg, haltere or antenna be changed in opposite directions simultaneously? How fine-grained is the mosaic of the smallest selectable variations? In more general terms, as trait definitions approach identity within some constricting domain, how rapidly does their genetic differentiability tend to vanish? It would be useful to have more information about the lower limits of morphological detail that can be altered semi-independently by mass selection. Inferences about the genetic and developmental bases of morphology arise from such information. It is already apparent (MILKMAN 1970; THOMPSON 1980; SCHARLOO 1987; HAYNES 1988; WEBER 1990) that the system of pattern formation and cell determination that generates the Drosophila wing is capable of many localized adjustments by selectable genes. For the first time since WADDINGTON (1939, 1940) surveyed known wing mutants–all rare and with drastic effects–these more recent studies show that many genes with minor effects on wing development are common in all populations. Even in the tiny domain of form studied here, it appears that more loci than one contribute to the genetic variance, because (1) with a single locus and common alleles, line means would not evolve beyond the phenotypic range of the base population, and (2) with a single locus and rare alleles, bidirectional response would not continue at steady rates. It would seem improbable anyway that simultaneously opposed effects on growth would be due to a single locus. The total number of loci that could affect the region in the long term is likely to be even larger than the number that happened to be polymorphic in two selection lines with 60 founding parents apiece.

The existence of a large number of positionally responding loci is compatible with theories of field differentiation based on concentration thresholds in gradients of diffusing morphogens (FRENCH, BRYANT and BRYANT 1976; MEINHARDT 1983). Whatever the mechanism of pattern formation, there should be no obstacle to a belief in fine-grained evolutionary plasticity based on genes of localized effect. Cuticle grafts on large hemimetabolous insects have already revealed that cuticle cells retain fine-grained positional identity, and can intercalate small local structures (LOCKE 1959; BULLIÈRE 1971). Other evidence as well (reviewed in LEWIS and WOLPERT 1976) suggests a high density of positional information controlling localized developmental outcomes. In complex metazoans, late-acting genes with localized effects may form a large component of the genome (see BAILEY 1985, 1986). As the number of discrete selectable domains increases, so must the number of such loci.

Information about the smallest quanta of independent localized variability can also strongly influence ideas of adaptation. Only to the extent that the genetic system permits fluid regional subdivisibility of morphology, can the details of organisms adjust gradually and precisely to multidimensional selective pressures. At present, for example, it can be argued that if some fossil feature exhibits a prolonged interval of stasis as an apparent oddity, or within a larger pattern of directional change, the best explanation is a lack of genetic variance in the face of continued natural selection (*e.g.*, BAKKER 1983; BUERGIN *et al.* 1989). This presupposes an *integral* genetic system and a paucity of mutable sites with independent, fine-grained local effects.

The smallest details of morphology may reveal the most about the versatility of developmental mechanisms, and the corresponding scope of potential adaptation. For example, although natural selection on the beaks of birds has only been demonstrated in their most obvious aspects such as length and width (GRANT 1986; SMITH 1990), the same agency has clearly shaped much finer bill features with unambiguous functions. Thus in both the woodcock and the kiwi, with their convergent flexible earth-probing beaks, the tip of the upper mandible is extended and enlarged to occupy the same cross-sectional form as the combined upper and lower mandibles occupy more proximally. This small structural detail allows the bills of both species to be thrust into resistant substrates without being forced open. Such a feature could only evolve where the localized genetic variability of morphology is extremely fine-grained.

But occasional details whose optimality seems obvious do not prove that all details are easily selectable, especially not when the scale is much smaller as in the present case. The tips of woodcock and kiwi bills are three to four orders of magnitude larger in volume than the speck of tissue that was investigated in this study. Many biological systems undergo much simplification in the process of miniaturization (RENSCH 1948). Therefore it would have been easy to suppose that the edge of the base of a fruit fly's wing would be missing a few degrees of developmental freedom.

Without genetic evidence, claims of strong developmental constraints on form are as easy to make as claims of adaptive perfection. On developmental evidence, for example, a number of forbidden morphologies have been postulated (STOCK and BRYANT 1981; ALBERCH 1982) for the tetrapod limb. Yet these rules seem to be broken even by existing species. The feet of phocid seals are fishtail shaped, with long outer

digits and short central ones, while hoofs of equids have a single symmetrical toe (HOLDER 1983). In other striking examples, the hands of both the lemuriform genus *Daubentonia* and the phalangerid genus *Dactylopsila* possess one greatly attenuated finger (the third finger in the first case, the fourth finger in the second), which is highly differentiated from adjacent fingers on either side, as a convergent adaptation for the extraction of wood-boring insect larvae (CARTMILL 1974). These and many other cases argue that the detailed form of the tetrapod limb is not strongly constrained. The clustered distribution of morphologies is not patent proof of internal constraints that limit functional optimality: toolboxes also contain clustered distributions of shapes.

It is sometimes assumed that, if we knew the rules of development, we could explain organic forms in terms of limited developmental repertoires. But perhaps adaptive evolution rarely exhausts the morphogenetic potential of developmental systems. Investigation of the autonomous selectabilities of small domains addresses this issue.

Useful comments on this paper were given by J. BEATTY, F. COHAN, L. DIGGINS, H. FUKUI, R. LEWONTIN, G. MAYER, H. TORDOFF, D. TOWNSEND, M. TURELLI and five anonymous reviewers, and careful technical assistance was rendered by E. POST. This research was supported by National Institutes of Health grants 5-RO1.GM 21,179; KO4.HD 00638; and RO1.GM 40907.

LITERATURE CITED

ABRAMOWITZ, M., and I. A. STEGUN, 1972 *Handbook of Mathematical Functions*. U.S. Government Printing Office, Washington, D.C.

ALBERCH, P., 1980 Ontogenesis and morphological diversification. Am. Zool. **20:** 653–667.

ALBERCH, P., 1982 Developmental constraints in evolutionary processes, pp. 313–332 in *Evolution and Development*, edited by J. T. BONNER. Springer-Verlag, Berlin.

ASHBURNER, M., 1982 The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. III. Hypomorphic and hypermorphic mutations affecting the expression of *Hairless*. Genetics **101:** 447–459.

BAILEY, D. W., 1985 Genes that affect the shape of the murine mandible. J. Hered. **76:** 107–114.

BAILEY, D. W., 1986 Genes that affect morphogenesis of the murine mandible. J. Hered. **77:** 17–25.

BAKKER, R. T., 1983 The deer flees, the wolf pursues: incongruencies in predator-prey coevolution, pp. 350–382 in *Coevolution*, edited by D. J. FUTUYMA and M. SLATKIN. Sinauer, Sunderland, Mass.

BATESON, W., 1894 *Materials for the Study of Variation*. Macmillan, New York.

BRODSKIY, A. K., and V. D. IVANOV, 1983 Functional assessment of wing structure in insects. Entomol. Rev. **62:** 35–52.

BUERGIN, T., O. RIEPPEL, P. M. SANDER and K. TSCHANZ, 1989 The fossils of Monte San Giorgio. Sci. Am. **260:** 74–81.

BULLIÈRE, D., 1971 Utilisation de la régénération intercalaire pour l'étude de la détermination cellulaire au cours de la morphogenèse chez *Blabera craniifer*. Dev. Biol. **25:** 672–709.

CADE, W. H., 1981 Alternative male strategies: genetic differences in crickets. Science **212:** 563–564.

CARTMILL, M., 1974 *Daubentonia, Dactylopsila*, woodpeckers and klinorhynchy, pp. 655–670 in *Prosimian Biology*, edited by R. D. MARTIN, G. A. DOYLE and A. C. WALKER. Duckworth, London.

CASTLE, W. E., and J. C. PHILLIPS, 1914 *Piebald Rats and Selection: An Experimental Test of the Effectiveness of Selection and of the Theory of Gametic Purity in Mendelian Crosses*. Carnegie Inst. Wash. Publ. 195.

CHERRY, L. M., S. M. CASE, J. G. KUNKEL and A. C. WILSON, 1979 Comparisons of frogs, humans, and chimpanzees. Science **204:** 435.

CHINNICI, J. P., 1971 Modification of recombination frequency in *Drosophila*. I. Selection for increased and decreased crossing over. Genetics **69:** 71–83.

CLARKE, J. M., J. MAYNARD SMITH and K. C. SONDHI, 1961 Asymmetrical response to selection for rate of development in *Drosophila subobscura*. Genet. Res. **2:** 70–81.

COCK, A. G., 1966 Genetical aspects of metrical growth and form in animals. Q. Rev. Biol. **41:** 131–190.

COHAN, F. M., 1984 Genetic divergence under uniform selection. I. Similarity among populations of *Drosophila melanogaster* in their responses to artificial selection for modifiers of ci^D. Evolution **38:** 55–71.

COYNE, J. A., 1983 Genetic basis of differences in genital morphology among three sibling species of *Drosophila*. Evolution **37:** 1101–1118.

COYNE, J. A., J. RUX and J. R. DAVID, 1991 Genetics of morphological differences and hybrid sterility between *Drosophila sechellia* and its relatives. Genet. Res. **57:** 113–122.

DIAZ-BENJUMEA, F. J., and A. GARCÍA-BELLIDO, 1990 Genetic analysis of the wing vein pattern of *Drosophila*. Roux's Arch. Dev. Biol. **198:** 336–354.

DOBZHANSKY, T., and B. SPASSKY, 1967 Effects of selection and migration on geotactic and phototactic behaviour of *Drosophila*. I. Proc. R. Soc. Ser. B **168:** 27–47.

ENNOS, A. R., 1988 The importance of torsion in the design of insect wings. J. Exp. Biol. **140:** 137–160.

FALCONER, D. S., 1981 *Introduction to Quantitative Genetics*, Ed. 2. Longman, London.

FRENCH, V., P. J. BRYANT andS. V. BRYANT, 1976 Pattern regulation in epimorphic fields. Science **193:** 969–980.

GARCÍA-BELLIDO, A., 1975 Genetic control of wing disc development in *Drosophila*, pp. 161–182 in *Cell Patterning* (CIBA Foundation Symp. 29). Elsevier, Amsterdam.

GARCÍA-BELLIDO, A., 1983 Comparative anatomy of cuticular patterns in the genus *Drosophila*, pp. 227–255 in *Development and Evolution*, edited by B. C. GOODWIN, N. HOLDER and C. C. WYLIE. Cambridge University Press, Cambridge.

GOODWIN, B. C., 1984 Changing from an evolutionary to a generative paradigm in biology. pp. 99–120 in *Evolutionary Theory: Paths into the Future*, edited by J. W. POLLARD. Wylie, New York.

GRANT, P. R., 1986 *Ecology and Evolution of Darwin's Finches*. Princeton University Press, Princeton, N.J.

HAYNES, A. K., 1988 Developmental constraints in the *Drosophila* wing. Ph.D. thesis, Harvard University.

HILL, W. G., 1972 Estimation of realised heritabilities from selection experiments. II. Selection in one direction. Biometrics **28:** 767–780.

HOLDER, N., 1983 Developmental constraints and the evolution of vertebrate digit patterns. J. Theor. Biol. **104:** 451–471.

HOLM, S., 1979 A simple sequentially rejective multiple test procedure. Scand. J. Stat. **6:** 65–70.

HORDER, T. J., 1981 On not throwing the baby out with the bath water. Evol. Today **2:** 163–180.

JOHNSTON, J. S., 1982 Genetic variation for anemotaxis (wind-

directed movement) in laboratory and wild-caught populations of *Drosophila*. Behav. Genet. **12:** 281–293.

LANDE, R., 1981 The minimum number of genes contributing to quantitative variation between and within populations. Genetics **99:** 541–553.

LEWIS, J. H., and L. WOLPERT, 1976 The principle of non-equivalence in development. J. Theor. Biol. **62:** 479–490.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LOCKE, M., 1959 The cuticular pattern in an insect, *Rhodnius prolixus* Stal. J. Exp. Biol. **36:** 459–477.

MACDOWELL, E. C., 1917 Bristle inheritance in *Drosophila*. II. Selection. J. Exp. Zool. **23:** 109–146.

MASRY, A. M., and F. W. ROBERTSON, 1978 Cell size and number in the *Drosophila* wing. I. The use of selection to study the interrelations between cell size and number and body size. Egypt. J. Genet. Cytol. **7:** 137–165.

MAYNARD SMITH, J., and K. C. SONDHI, 1960 The genetics of a pattern. Genetics **45:** 1039–1050.

MEINHARDT, H., 1983 Cell determination boundaries as organizing regions for secondary embryonic fields. Dev. Biol. **96:** 375–385.

MILKMAN, R., 1970 The genetic basis of natural variation in *Drosophila melanogaster*. Adv. Genet. **15:** 55–114.

NACHTIGALL, W., 1981 Der Vorderfluegel grosser Heuschrecken als Luftkrafterzeuger. J. Comp. Physiol. **142:** 127–134.

NORBERG, R. A., 1972 The pterostigma of insect wings an inertial regulator of wing pitch. J. Comp. Physiol. **81:** 9–22.

PATERNIANI, E., 1969 Selection for reproductive isolation between two populations of maize, *Zea mays* L. Evolution **23:** 534–547.

PENNYCUIK, P. R., and J. M. RENDEL, 1977 Selection for constancy of score and pattern of secondary vibrissae in *Ta/Ta-Ta/Y* and *Ta/+* mice. Aust. J. Biol. Sci. **30:** 303–317.

PLUNKETT, C. R., 1926 The interaction of genetic and environmental factors in development. J. Exp. Zool. **46:** 181–244.

RAFF, R. A., and T. C. KAUFMAN, 1983 *Embryos, Genes, and Evolution*. Macmillan, New York.

REEVE, E. C. R., 1961 Modifying the sternopleural hair pattern in *Drosophila* by selection. Genet. Res. **2:** 158–160.

RENDEL, J. M., and B. L. SHELDON, 1960 Selection for canalization of the scute phenotype in *Drosophila melanogaster*. Aust. J. Biol. Sci. **13:** 36–47.

RENSCH, B., 1948 Histological changes correlated with evolutionary changes of body size. Evolution **2:** 218–230.

RICE, W. R., 1989 Analyzing tables of statistical tests. Evolution **43:** 223–225.

ROBERTSON, F. W., 1957 Studies in quantitative inheritance. X. Genetic variation of ovary size in *Drosophila*. J. Genet. **55:** 410–427.

ROBERTSON, F. W., 1962 Changing the relative size of the body parts of *Drosophila* by selection. Genet. Res. **3:** 169–180.

ROFF, D. A., and T. A. MOUSSEAU, 1987 Quantitative genetics and fitness: lessons from *Drosophila*. Heredity **58:** 103–118.

SCHARLOO, W., 1964 The effect of disruptive and stabilizing selection on the expression of a *cubitus interruptus* mutant in *Drosophila*. Genetics **50:** 553–562.

SCHARLOO, W., 1987 Constraints in selection response, pp. 125–149 in *Genetic Constraints on Adaptive Evolution*, edited by V. LOESCHKE. Springer-Verlag, Berlin.

SIMON, C., 1983 Morphological differentiation in wing venation among broods of 13- and 17-year periodical cicadas. Evolution **37:** 104–115.

SMITH, T. B., 1990 Natural selection on bill characters in the two bill morphs of the African finch *Pyrenestes ostrinus*. Evolution **44:** 832–842.

SPICER, G. S., 1991 The genetic basis of a species-specific character in the *Drosophila virilis* species group. Genetics **128:** 331–337.

SPICKETT, S. G., 1963 Genetic and developmental studies of a quantitative character. Nature **199:** 870–873.

STOCK, G. B., and S. V. BRYANT, 1981 Studies of digit regeneration and their implications for theories of development and evolution of vertebrate limbs. J. Exp. Zool. **216:** 423–433.

STRAUSS, R. E., 1990 Patterns of quantitative variation in Lepidopteran wing morphology: the convergent groups Heliconiinae and Ithomiinae (Papilionoidea: Nymphalidae). Evolution **44:** 86–103.

TEMPLETON, A. R., 1977 Analysis of head shape differences between two interfertile species of Hawaiian *Drosophila*. Evolution **31:** 630–641.

THOMPSON, J. N., 1974a Studies on the nature and function of polygenic loci in *Drosophila*. I. Comparison of genomes from selection lines. Heredity **33:** 373–387.

THOMPSON, J. N., 1974b Studies on the nature and function of polygenic loci in *Drosophila*. II. The subthreshold wing vein pattern revealed in selection experiments. Heredity **33:** 389–401.

THOMPSON, J. N., 1980 Pattern compensation in *Drosophila* wing vein development. Heredity **44:** 93–102.

TRYON, R. C., 1940 Genetic differences in maze-learning ability in rats. Yearb. Natl. Soc. Study Educ. **39:** 111–119.

WADDINGTON, C. H., 1939 Preliminary notes on the development of the wings in normal and mutant strains of *Drosophila* Natl. Acad. Sci. Proc. **25:** 299–307.

WADDINGTON, C. H., 1940 *Organisers and Genes*. Cambridge University Press, Cambridge.

WEBER, K. E., 1988 A system for rapid morphometry of whole, live flies. Drosopila Inform. Serv. **67:** 97–102.

WEBER, K. E., 1990 Artificial selection on wing allometry in *Drosophila melanogaster*. Genetics **126:** 975–989.

WOOTTON, R. J., 1981 Support and deformability in insect wings. J. Zool. **193:** 447–468.

WOOTTON, R. J., 1990 The mechanical design of insect wings. Sci. Am. **263:** 114–120.

WRIGHT, S., 1965 Factor interaction and linkage in evolution. Proc. R. Soc. Ser. B **162:** 80–104.

Communicating editor: M. TURELLI