TEMPERATURE-RELATED DIVERGENCE IN EXPERIMENTAL POPULATIONS OF *DROSOPHILA MELANOGASTER*. I. GENETIC AND DEVELOPMENTAL BASIS OF WING SIZE AND SHAPE VARIATION

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ABSTRACT

The effects of environmental temperature on wing size and shape of *Drosophila melanogaster* were analyzed in populations derived from an Oregon laboratory strain kept at three temperatures (18°, 25°, 28°) for 4 yr. Temperature-directed selection was identified for both wing size and shape. The length of the four longitudinal veins, used as a test for wing size variations in the different populations, appears to be affected by both genetic and maternal influences. Vein expression appears to be dependent upon developmental pattern of the wing: veins belonging to the same compartment are coordinated in their expression and relative position, whereas veins belonging to different compartments are not. Both wing and cell areas show genetic divergence, particularly in the posterior compartment. Cell number seems to compensate for cell size variations. Such compensation is carried out both at the level of single organisms and at the level of population as a whole. The two compartments behave as individual units of selection.

ENVIRONMENTAL temperature plays an important role in ecogeographical differentiation between populations, and much morphological variation between geographic races of several Drosophila species has been interpreted as an expression of genetic adaptation to temperature (STALKER and CARSON 1947; PREVOSTI 1955; MISRA and REEVE 1964; DAVID and BOCQUET 1975a,b; DAVID, BOCQUET and DE SCHEEMAEKER-LOUIS 1977).

Although temperature does not appear to be the only environmental factor involved in population differentiation (SOKOLOFF 1966; ANDERSON 1968), ANDERSON (1966) showed that temperature-directed selection for body (wing) size occurs in the laboratory, making it "plausible that similar selective forces are at work in the laboratory and in nature" (ANDERSON 1973). In addition, POWELL (1974) showed that tropical populations of *Drosophila willistoni* possess genetic mechanisms to adjust body size to temperature that are similar to those of temperate species.

On the other hand, attention has seldom been paid to shape variation (STALKER and CARSON 1947; MISRA and REEVE 1964), although it too may

well be an adaptive response to climatic differences over the species range. On the basis of multivariate analysis of nine metric traits, we observed (CAVICCHI, GIORGI and MOCHI 1978) that a laboratory population of *Drosophila melanogaster* reared at two different temperatures (25° and 28°) exhibits progressive wing shape divergence and no divergence in wing size during nine generations. Differential survivorship was associated with shape differences, supporting the hypothesis that the shape variability has an adaptive meaning in relation to temperature.

Shape variation arises from interactions between genes controlling different traits during development and pattern formation. Therefore, in order to determine the genetic basis of shape and its evolutionary significance a dual approach is necessary, employing quantitative developmental genetics and population genetics.

Some connections between quantitative and developmental genetics have recently been provided as regards wing pattern formation. GARCIA-BELLIDO, RIPOLL and MORATA (1973) showed that the wing blade appears to be subdivided in earlier development into two distinct portions originating from primordial cells in the imaginal disc, whose descendants (polyclones) never cross a certain demarcation line. This line represents the boundary of the two wing compartments (posterior and anteior) which show different and specific cell dynamics during development (GARCIA-BELLIDO 1977).

The compartments appear to be units for the genetic control of development and responsible for size and shape of organs and appendages (LAWRENCE and MORATA 1976). Very recent studies on quantitative inheritance in Drosophila support this idea: CAVICCHI, PEZZOLI and GIORGI (1981) and CAVICCHI *et al.* (1981) studying variations in body dimensions in lines selected for short wing, or in lines exposed to sudden temperature variations, found that metric traits (vein lengths and their distances) belonging to the same wing compartment were well coordinated in their phenotypic expression. THOMPSON, HELLACK and KENNEDY (1982) and THOMPSON and WOODRUFF (1982), dealing with polygenes affecting the expression of vein mutants, found an interdependence between modifiers affecting veins within the same compartment.

The developmental genetic reasons for size and shape variations may be investigated by studying cell size and number variation. The contribution of cell size and number in determining wing surface varies in relation to the origin of the studied populations. Inadequate nutrition and artificial selection for body size (ZARAPKIN 1934; ROBERTSON 1959b) appear to be associated with changes in cell number, whereas variations in body size produced by temperature seem solely related to variations in cell size (ALPATOV 1930; ROBERTSON 1959a). Moreover, cell size appears genetically determined as shown by the selection procedure (ROBERTSON 1959a). It has also been suggested that cell number may undergo genetic control by genes acting on the mitotic rate during larval growth of the wing disc whose expression might also be dependent on genes controlling the two wing compartments (GARCIA-BELLIDO, RIPOLL and MORATA 1976).

On the basis of these findings we have attempted to connect developmental

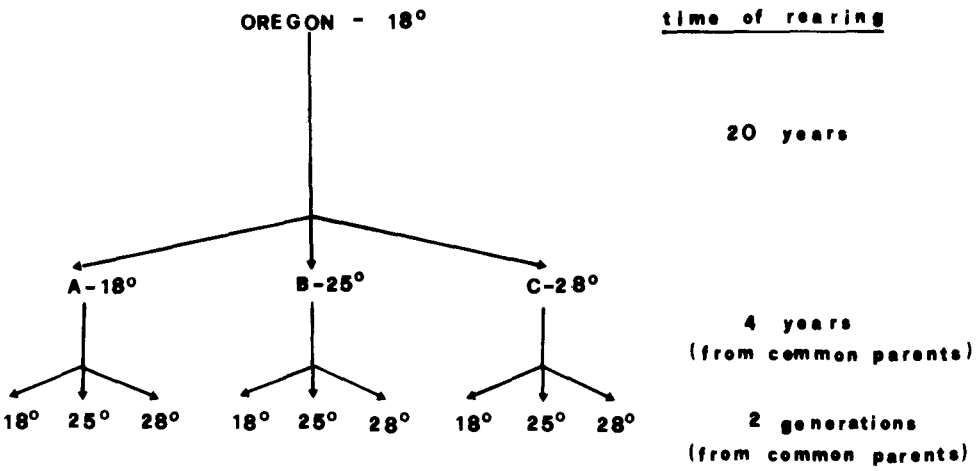

FIGURE 1.—Experimental design. Egg samples from 20 pairs of flies from the Oregon strain kept for 20 yr at 18° were placed at 18°, 25° and 28° to found the A, B and C populations, respectively. After 4 yr of mass rearing, from each population 18 pairs were chosen and allowed to lay eggs for 24 hr in their own environment and were then transferred to the other two temperatures for 1-day laying. Measurements were performed after an additional single-culture generation at each temperature.

genetics to population genetics by studying the genetic basis of size and shape variations induced by temperature in the Drosophila wing in relation to its developmental pattern. It is known that wing size is highly correlated with body size (ROBERTSON 1962; TANTAWY and RAKHA 1964; SOKOLOFF 1966; CAVICCHI, PEZZOLI and GIORGI 1981) and fitness components (TANTAWY and VETUKHIV 1960; TANTAWY and RAKHA 1964; PIERAGOSTINI, SANGIORGI and CAVICCHI 1979). Therefore, wing size may be confidently used as an index of body size in a given population.

MATERIALS AND METHODS

The starting population consisted of an Oregon laboratory strain reared for almost 20 yr at a constant temperature of 18°. We preferred a laboratory rather than a wild strain because we consider that 20 yr of continuous rearing at this temperature represent a sufficieint time to make it well adapted to its environment. In addition we were encouraged by the fact that the strain displayed a heritability of 0.3 for both thorax and wing length when estimated by both Lerner's method and parent-offspring regression.

Eggs laid from 20 pairs of flies were used to found new mass populations (Figure 1): the first (A) was maintained at the same 18° temperature; the second (B) and the third (C) were kept for 4 yr (about 140 generations) at 25° and 28°, respectively. The last temperature represents the upper extreme thermic environment for the survival of our strain; few progeny were obtained from each generation until the 1st yr of rearing when a gradual improvement was seen. After 4 yr, 18 pairs were chosen from each population (A, B and C) and allowed to lay for 1 day at each temperature (18°, 25°, 28°).

The flies belonging to the A population transferred to 28° and those from the C one transferred to 18° displayed a very low fitness, whereas those from the B population showed a good survivorship at all temperatures.

To minimize physiological effects determined by previous rearing at different temperatures, one subsequent generation was reared in each environment: two pairs within each progeny were

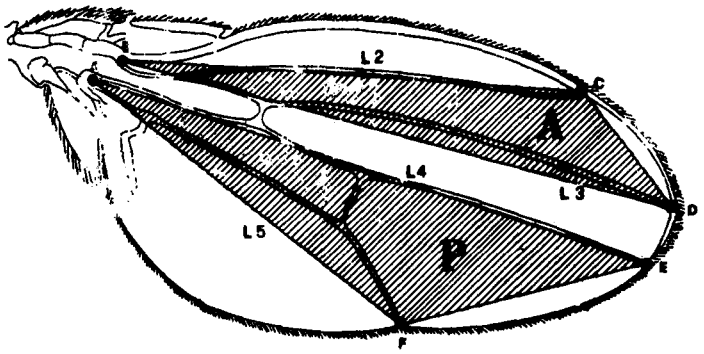

FIGURE 2.—Graphic representation of the Drosophila wing. The region chosen to represent the posterior (P) compartment is delimited by L4, L5 veins, measured as straight lines (AE = L4 and AF = L5) and their distance (EF = L4–L5 distance). The region delimited by L2, L3 veins (BC = L2 and BD = L3) and their distance (CD = L2–L3 distance) was considered to represent the anterior (A) compartment.

randomly sampled and reared again in single culture, changing vials every day for 3 days. The progeny number obtained in the fittest vials never exceeded 40, so that, considering the quantity of standard medium in a culture (no less than 15 ml), crowding was avoided. The progeny obtained from only one of the two pairs and, hence, from a total of nine families (replicate pairs) were taken for measurements. The right wing of seven females from each family (when available) was pulled out and mounted on a slide. The length of L2, L3, L4 and L5 veins and the distance, at the margin of the wing, between L2, L3 and L4, L5 veins (Figure 2) were measured under a microscope at magnification ×50, with an ocular micrometer of 100 divisions; all measurements were then converted into millimeters × 10^2. Because the boundary between the two wing compartments lies just beside the L4 vein, different effects induced by temperature on the two groups of veins were taken as wing shape variation resulting from changes in the developmental pattern of the wing.

Size and shape differences between populations and transfers were analyzed by univariate and multivariate analysis. The univariate analysis of size was performed by a joint analysis of variance between populations and transfers. Of the multivariate statistics we chose Mahalanobis' distance (D^2) which has been considered the most appropriate method for size and shape analysis (ATCHLEY 1980; KUNKEL and CHERRY 1980). Total D^2 was partitioned into size and shape components following the same theoretical background proposed by SPIELMAN (1973) and TEMPLETON (1977). Our method is essentially that used by Spielman except that we transformed our raw data into values on uncorrelated coordinates by the pivotal condensation method (see RAO 1970) computer programmed by MURTY and ARUNACHALAM (1967) instead of principal component analysis. As far as correlated traits are concerned, some components corresponding to very low eigenvalues can be discarded by principal component analysis.These represent minor axes of variation within a group but not necessarily minor axes of variation between groups. The test for significance of D^2, size^2 and shape^2 was based on F-test of the Hotelling T^2.

Variations in cell size and number were evaluated considering two wing surfaces that approximately correspond to the wing compartments (posterior and anterior). For the posterior compartment the area of the triangle whose sides are represented by the L4 and L5 veins and the distance between them at the margin of the wing was estimated, and for the anterior compartment the area delimited by L2, L3 veins and the distance between them was considered (Figure 2). Areas were computed by Erone's formula

$$S = \sqrt{p(p-a)(p-b)(p-c)}$$

where p is half the perimeter and a, b and c are the lengths of the sides of the triangles.

Cell area was estimated under a microscope at a total magnification ×275 by counting the number of bristles (cells) present on a dorsal surface of 96.8 × 10^{-4} mm^2 limited by a reticle

placed in the eyepiece. The average cell area was estimated by dividing the area of the reticle by the number of cells counted. For the posterior compartment the reticle was placed close to the crossvein and at the same distance from the end of the wing in the anterior compartment. Cell density differs in different wing regions and does not appear to be compartment dependent; nevertheless, the cells are regularly arranged on the wing surface, and counts in different regions are quite well correlated (ROBERTSON 1959a; DELCOUR and LINTS 1966). In this experiment disruption of the correlation will, therefore, be considered evidence of a different response of the two compartments to temperature.

Cell number was obtained by dividing wing size by cell area; because areas and number of cells were not found normally distributed, the measurements were converted to natural logarithms (ROBERTSON 1959a). In this form, wing area is the sum of cell area and number.

Genetic differences among populations were detected by crossing the original A population with the B and C ones. The crosses were performed two generations after transferring the populations to a common environment at 25°. Parents and F_1's were raised simultaneously with F_2's. All of the reciprocal crosses were made. Seven female right wings from each of ten families per reciprocal cross were measured.

RESULTS

Vein lengths and their distances: Mean values of vein lengths and their distances in the B and C populations kept for 4 yr at 25° and 28°, respectively, and in the original population (A) two generations after their transfers at the three temperatures are shown in Figure 3. The populations (A, B, C) appear quite differentiated within each environment (18°, 25°, 28°), showing a persistent temperature-dependent cline in wing size most evident at 18°: flies reared at warm temperatures show smaller wings in all environments considered. Different traits show quite different responses both as effects of transfer and of prolonged rearing at different temperatures; the distance between L2 and L3 veins (anterior compartment) shows the smallest variation.

The analysis of variance (ANOVA) given in Table 1 provides estimates for the effect of transfers on both populations and genotypes within populations as well as the effect of prolonged rearing at different temperatures. Highly significant differences between temperatures and between population mean squares are observed, whereas the amount of variance between families is almost the same, or less than family × temperature interaction. Population × temperature interaction is not significant when compared with the between families for L3, L4 veins and for the distances between L4–L5 and L2–L3 veins, whereas significant interaction family × temperature is observed. Because the families are the replicate pairs of flies sampled from each population at each temperature, the between families variance represents variability due to genotypes within populations. Accordingly, the family × temperature interaction represents a genotype-environment interaction, where genotype means "average genotype" because flies within any family may differ genetically due to segregation and recombination. Intraclass correlations quoted in the same table summarize the results. Intraclass correlation represents a measure of the resemblance between individuals within each class; if the components of the variances "between" and "within" are summed, the intraclass correlation is the ratio between the class component and the sum of components.

No difference between veins belonging to different compartments can be observed; in particular, L3 and L4 veins and likewise L2 and L5 show quite

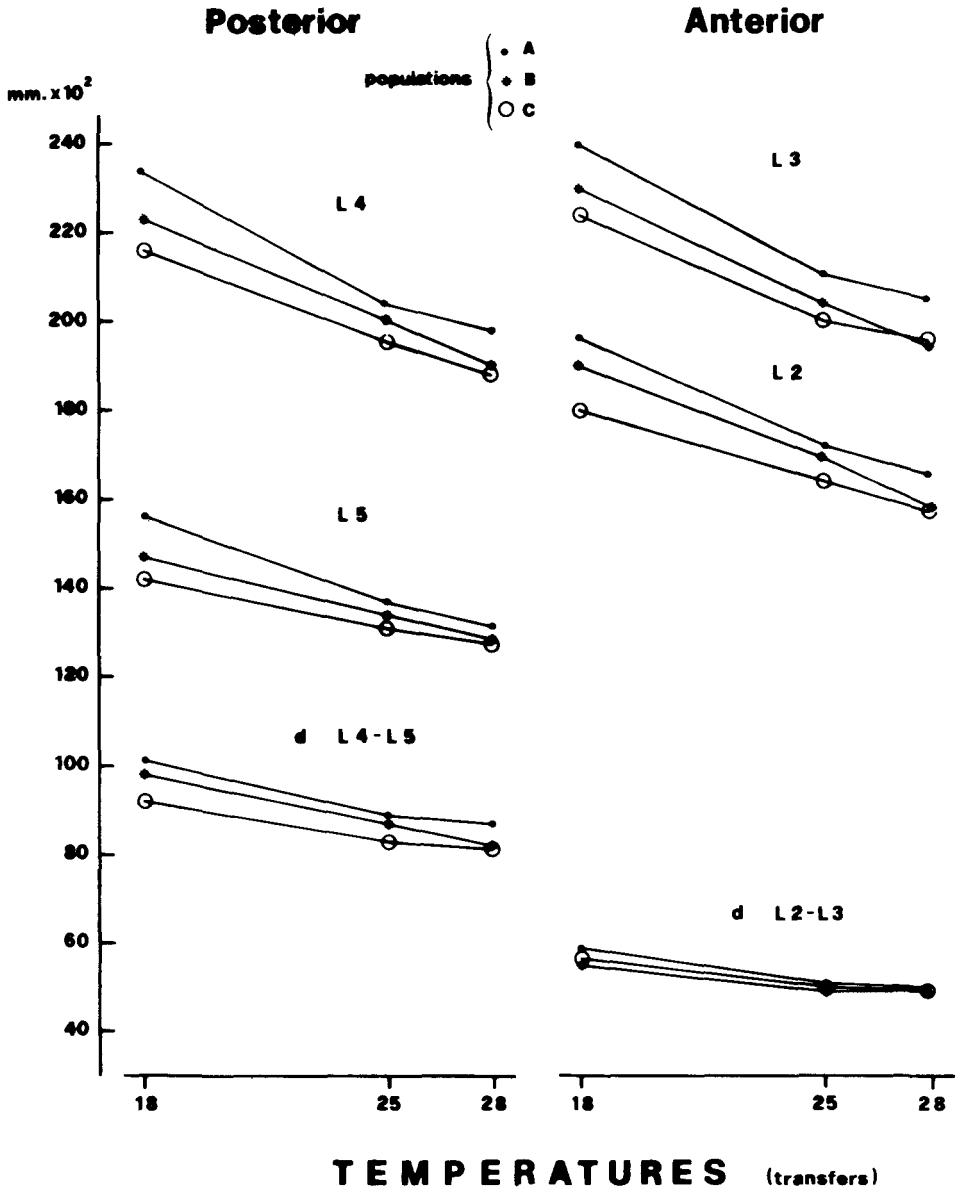

FIGURE 3.—Mean values of the different wing traits measured within compartments in the A, B and C populations transferred to three temperatures (18°, 25° and 28°).

similar behavior. This is not surprising, since both L3 and L4 veins seem to be affected by polygenes associated with chromosome *III* (THOMPSON 1975; THOMPSON and WOODRUFF 1982), whereas those for lengthening the L2 vein are associated with chromosome *II*; the L5 vein seems affected by polygenes and modifiers located on both chromosomes *II* and *III* (THOMPSON and WOODRUFF 1982). On the other hand (Figure 3), we can see that the distances

TABLE 1

ANOVA to test the effect of temperature on populations and genotypes within populations

Source	d.f.	Posterior			Anterior		
		L4 length	L5 length	L4–L5 distance	L2 length	L3 length	L2–L3 distance
Between temperatures (T)	2	42505.59** (0.901)	14347.99** (0.733)	7598.80** (0.589)	33486.42** (0.789)	46368.33** (0.812)	2514.15** (0.557)
Between populations (P)	2	5400.61** (0.529)	2161.31* (0.356)	1556.79** (0.281)	4328.95* (0.454)	5899.33** (0.541)	138.29* (0.059)
P × T	4	172.61 (0.058)	347.91** (0.279)	87.89 (0.039)	309.29* (0.138)	116.11 (0.032)	19.68 (0.012)
Between families (F)	23	81.96 (0.002)	42.54 (0.0)	38.75 (0.003)	87.64 (0.063)	63.66 (0.0)	12.44 (0.0)
F × T	46	80.86** (0.374)	45.12** (0.396)	37.51** (0.126)	57.53** (0.261)	90.12** (0.392)	54.74** (0.729)
Within families	393	17.49	9.09	20.06	18.35	18.44	3.19

Temperatures are the three environments of transfer; families are genotypes within populations. Results are expressed as mean squares in $mm^2 \times 10^4$. Numbers in parentheses are intraclass correlations (see text).

* Significant at 5% level.

** Significant at 1% level.

between veins in the posterior and anterior compartments show a quite different behavior, suggesting that the way in which the veins are placed in the two compartments changes as an effect of temperature.

Both size and shape variations induced by temperature have been studied by multivariate analysis. Tables 2 and 3 summarize the average size and shape distance between temperatures within populations (Table 2) and between populations within temperatures (Table 3). The analysis was performed on both the wing traits taken as a whole (posterior and anterior) and on those belonging to either the anterior or posterior compartment considered separately. The sums of the statistics obtained on each compartment (posterior plus anterior) are reported too.

Obviously, the distance (D^2) obtained as a sum is overestimated and larger than that obtained in the joint analysis (posterior and anterior), in which the correlation between traits of different compartments is taken into account. In addition, when a different number of characters is dealt with, the significance level is not the same in different analyses (see Table 2 and 3 footnotes). Significance of the shape components within compartments reveals only allometric variations in the characters belonging to the same compartment. Therefore, in order to compare the behavior of the two compartments, size^2 and shape^2 percent on total D^2 are also given in the tables both for the sum (posterior plus anterior) and the joint analysis (posterior and anterior).

If the characters belonging to different compartments are subjected to different controls for their expression, the amount of shape variation in the joint analysis must be greater than that obtained in the sum of the within-compartment analyses (posterior plus anterior).

If we first consider the effect of transfers (between temperatures, Table 2) on the original population (A) we note that the size component largely accounts for the phenotypic changes observed. The percent contribution of size and shape is almost the same in posterior plus anterior and posterior and anterior, suggesting that the two compartments react quite similarly to transfers. In the B and C populations the amount of the shape contribution increases; moreover, in the population exposed to more severe environmental selection (C), the contribution of shape is 11% greater in posterior and anterior when compared with posterior plus anterior, indicating that the two compartments react differently to transfers.

In the between-populations within-temperatures analysis (Table 3), total D^2 appears markedly reduced but highly significant. It is noteworthy that the contribution of shape is proportionally increased, being almost the same or larger than that of size in the posterior and anterior analysis. The percent differences between shape contributions in posterior plus anterior and posterior and anterior are very large (19–31%), indicating a great deal of compartment × population interaction. The prolonged rearing at different temperatures, therefore, seems to have produced different genetic effects on the two compartments.

In general, when real values are considered, the largest discrimination values are observed at 18° which represents the original environment; the selected

TABLE 2

Average size², shape² and total (D^2) distance between temperatures within populations for the posterior (Post) and anterior (Ant) compartments

Compartment	Populations								
	A			B			C		
	Size²	Shape²	D^2	Size²	Shape²	D^2	Size²	Shape²	D^2
Post	27.10**	0.07	27.17**	20.86**	1.15**	22.01**	12.84**	2.30**	15.15**
Ant	27.91**	0.42*	28.33**	24.26**	2.79**	27.05**	20.42**	0.98**	21.41**
Post + Ant[a]	55.01 (99)	0.49 (1)	55.50	45.12 (92)	3.94 (8)	49.06	33.26 (91)	3.28 (9)	36.56
Post & Ant[b]	30.90** (97)	0.86** (3)	31.76**	47.19** (90)	5.27** (10)	52.46**	19.91** (80)	4.97** (20)	24.89**

Numbers in parentheses are size² and shape² percent on total D^2. The within-compartment analysis (Post or Ant) is significant when D^2 (or size² or shape²) is greater than 0.33 at 5% level and greater than 0.48 at 1% level, whereas for the joint analysis the statistics have to be greater than 0.56 or 0.76 to reach significance at 5 and 1% levels, respectively.

[a] These numbers are the sum of the statistics obtained in the separate analysis of the two compartments.

[b] These numbers are the statistics obtained in the joint analysis of the two compartments.

* Significant at 5% level.

** Significant at 1% level.

TABLE 3

Average size², shape² and total (D^2) *distance between populations within temperatures for the posterior (Post) and anterion (Ant) compartments*

Compartment	Temperatures								
	18°			25°			28°		
	Size²	Shape²	D^2	Size²	Shape²	D^2	Size²	Shape²	D^2
Post	6.32**	0.66**	6.98**	1.60**	0.14	1.74**	1.29**	0.84**	2.14**
Ant	3.74**	2.77**	6.51**	2.03**	0.85**	2.88**	1.58**	0.59**	2.17**
Post + Ant[a]	10.06 (75)	3.43 (25)	13.49	3.63 (79)	0.99 (21)	4.62	2.87 (67)	1.43 (33)	4.31
Post & Ant[b]	4.96** (51)	4.75** (49)	9.71**	3.11** (60)	2.08** (40)	5.19**	0.85** (36)	1.51** (64)	2.36**

Numbers in parentheses are size² and shape² percent on total D^2. The within-compartment analysis (Post or Ant) is significant when D^2 (or size² or shape²) is greater than 0.33 at 5% level and greater than 0.48 at 1% level, whereas for the joint analysis the statistics have to be greater than 0.56 or 0.76 to reach significance at 5 and 1% levels, respectively.

[a] These numbers are the sum of the statistics obtained in the separate analysis of the two compartments.

[b] These numbers are the statistics obtained in the joint analysis of the two compartments.

* Significant at 5% level.

** Significant at 1% level.

TABLE 4

Differences of F_1 and F_2 reciprocal crosses for the different traits in the posterior and anterior compartments

Cross[a]	Posterior			Anterior		
	L4 length	L5 length	L4–L5 distance	L2 length	L3 length	L2–L3 distance
			Difference of F_1 reciprocals			
A × B – B × A	3.50***	1.87**	1.55**	2.40***	2.94***	0.86*
A × C – C × A	5.11***	2.25***	1.31***	3.33***	5.74***	0.62**
			Difference of F_2 reciprocals			
A × B – B × A	2.94***	1.59***	1.90***	2.21***	3.34***	1.06**
A × C – C × A	3.75***	0.31	0.89*	0.27	−0.60	1.07**

The crosses were performed at 25°. All comparisons are in mm × 10^2.

[a] Female parent given first.

*, ** and *** Significant at 5, 1 and 0.5% levels, respectively.

populations (B and C), therefore, exhibit a reduced response to transfers as a probable consequence of either reduced genetic variability or developmental canalization. Shape estimates are significant in most of the within-compartment analyses (posterior or anterior) in agreement with the different genetic determinations of veins belonging to the same compartment. However, the shape values are higher in the joint analysis (posterior and anterior) than in posterior plus anterior, suggesting that veins belonging to the same compartment also have a common genetic and/or developmental control.

The genetic basis of the between-population differences observed was detected by crossing the original population A with B and C. The crosses were performed at 25° two generations after their transfer. We chose this temperature because the C population kept at the warmest temperature (28°) displayed a very low viability when transferred to the original environment (18°), as did the original A population when transferred to 28°, suggesting that they were well adapted to their own environments.

Wide differences between reciprocal crosses are observed (Table 4), especially between the A and C populations. Sex-linked genes do not seem to be involved because a difference between reciprocal crosses would be expected only in the heterogametic sex in F_1 and in the homogametic sex in F_2 (MATHER and JINKS 1971). The differences persist in the F_2 generation of A × B and B × A crosses, whereas there is a marked decrease when A and C populations are considered. In view of this, the differences may indicate a maternal effect rather than cytoplasmic inheritance. Similar maternal effects on body size have been described in natural populations of *D. pseudoobscura* (PROUT 1959; ANDERSON 1968) and *D. subobscura* (MCFARQHUAR and ROBERTSON 1963).

The comparisons, F_1 − midparent, F_1 − F_2 and F_2 − midparent (Table 5), are, therefore, reported for each cross. Considerable heterosis is observed in the F_1 generation, especially in A × C and C × A crosses. The comparisons, F_1 − F_2, show a decrease in F_2 means which is relatively larger in the reciprocal

TABLE 5

F_1 − midparent (MP), F_1 − F and F − midparent differences for the different traits in the posterior and anterior compartments

Cross[a]	Posterior			Anterior		
	L4 length	L5 length	L4–L5 distance	L2 length	L3 length	L2–L3 distance
			F_1 − MP			
A × B	5.47***	3.64***	2.02***	5.29***	5.44***	1.76***
B × A	1.97***	1.71***	0.47	2.89***	2.50***	0.90**
A × C	10.35***	6.24***	4.38***	9.22***	10.83***	2.21***
C × A	5.24***	3.99***	3.07***	5.89***	5.09***	1.59***
			F_1 − F_2			
A × B	3.43***	2.48***	0.43	3.12***	2.92***	−0.57
B × A	2.87***	2.21***	0.78*	2.93***	3.32***	−0.37
A × C	3.50***	2.30***	0.73*	2.94***	4.30***	−0.51
C × A	2.14**	0.36	0.31	−0.12	−2.04**	−0.06
			F_2 − MP			
A × B	2.04**	1.16**	1.59***	2.17***	2.52***	2.33***
B × A	−0.90	−0.43	−0.31	−0.04	−0.82	1.27***
A × C	6.85***	3.94***	3.65***	6.28***	6.53***	2.72***
C × A	3.10***	3.63***	2.76***	6.01***	7.13***	1.64***

The crosses were performed at 25°. All comparisons are in mm × 10^2.
[a] Female parent given first.
*, ** and *** Significant at 5, 1 and 0.5% levels, respectively.

crosses between the A and B populations than in those between the A and C ones. The C × A cross shows a reduction of L4 vein, an increase in L3 vein and constancy of the other traits. The distances between veins show slight positive variations in the posterior compartment but negative changes in the anterior one. This determines an increase in heterosis in the F_2 generation for the distance L2–L3 in all of the crosses and a slight decrease for the L4–L5 distance. In addition, when the comparisons of F_2 − midparent are considered, a maintenance of heterosis in the F_2 generation is observed in all of the crosses with the exception of the B × A one; the heterosis observed in the A × B cross may depend on the persistence of maternal influences. The greatest heterosis is observed in the crosses between the most distant populations.

In Table 6 the within-families variances of parental, F_1 and F_2 generations are given. The mean squares of reciprocal crosses appear fairly homogeneous so that they are pooled. The variances contain both genetic and environmental components; however, the differences among parental populations and hybrid generations should be largely genetic. F_1 and F_2 variances are compared with those of parents and F_2/F_1 ratios are also given.

TABLE 6

Comparison of within-families variances for the different traits in the posterior and anterior compartments in parents and hybrids from the populations kept at different temperatures

Population	Cross[a]	d.f.	Posterior			Anterior		
			L4 length	L5 length	L4–L5 distance	L2 length	L3 length	L2–L3 distance
			Mean squares				Mean squares	
A and B	Parents (P)	80	8.113	5.458	2.944	5.764	6.254	2.759
	F_1	80	7.737	3.813	1.717	6.561	6.316	1.656
	F_2	113	9.678	3.544	4.434	6.809	8.675	2.718
	Ratio			*F*-test			*F*-test	
	P/F_1		1.05	1.43*	1.71***	<1	<1	1.67**
	F_2/F_1		1.25	<1	2.58***	1.04	1.37	1.64**
	F_2/P		1.19	<1	1.51**	1.18	1.39	<1
			Mean squares				Mean squares	
A and C	Parents (P)	79	11.717	5.629	4.272	7.195	9.703	3.587
	F_1	80	9.441	3.373	2.269	6.990	8.523	2.821
	F_2	117	21.439	7.972	6.367	11.147	18.597	2.846
	Ratio			*F*-test			*F*-test	
	P/F_1		1.24	1.67**	1.88***	1.02	1.14	1.27
	F_2/F_1		2.27***	2.36***	2.81***	1.55**	2.18***	1.01
	F_2/P		1.83***	1.42*	1.49**	1.55**	1.92***	<1

The crosses were performed at 25°. Mean squares are $mm^2 \times 10^4$.

[a] Reciprocals are pooled.

*, ** and *** Significant at 5, 1 and 0.5% levels, respectively.

The crosses between the A and B populations show smaller F_1 variances than those of parents for L5 vein and L4–L5 distance in the posterior compartment and for L2–L3 distance in the anterior one. Only the F_2 variance of L4–L5 distance is larger than those of parents and F_1's, whereas that of L2–L3 distance exceeds only the F_1 variance.

In the crosses between the two extreme populations (A and C), the F_2 variances are highly significant when compared with either F_1 or parental variances with the exception of the L2–L3 distance (anterior compartment). Only the F_1 variances of L5 vein and of L4–L5 distance (posterior compartment) are significantly smaller than those of parents.

On the whole, although the F_2 − midparent comparisons (Table 5) are usually positive, the results suggest that the three populations have different coadapted gene arrays that are disrupted by cross.

Wing area, cell area and number: Mean values of wing area, cell area and number of the two wing compartments relative to the B and C populations kept for 4 yr at 25° and 28°, respectively, and to the original one (A) two generations after their transfers at the three temperatures are shown in Figure 4. The two compartments show quite different responses both as an effect of prolonged rearing at different temperatures and as an effect of transfer. The size of the posterior compartment appears more affected by prolonged rearing at the three temperatures than the anterior one, and both of them show quite similar behavior to transfers. The divergence among populations is more evident at the 18° temperature at which the B and, even more, the C population show a relatively small recovery of the original size. This may depend on either a reduction of genetic variability or developmental canalization as a consequence of temperature-directed selection on body size. Temperature, therefore, induces steady variation in wing surface, especially on the posterior portion of the wing.

In the anterior compartment cell area and number decrease in all populations with increasing environmental temperature, but cell number only decreases slightly. Cell area displays only slight differences among populations. In the posterior compartment, cell area shows a greater response to both prolonged rearing at different temperatures and transfers. Cell number behaves differently in the three populations: in the original one (A), when environmental temperature increases, cell number slightly decreases ($P < 0.10$); in the B and, notably, in the C population ($P < 0.005$), it is lower at colder temperatures. Both cell area and number appear to be involved in wing size determination.

The ANOVA analysis reported in Table 7 provides the statistics for the results just given. The comments given for Table 1 also apply to this table. In the posterior compartment highly significant population × temperature interaction is observed for all wing parameters, larger than the mean differences between populations and temperatures for wing area and cell number. It is only for cell area that between-temperature and between-population differences account for more variance than the interaction, population × temperature. Different genotypes within populations show both additive (between families) and interaction (family × temperature) components for all three wing

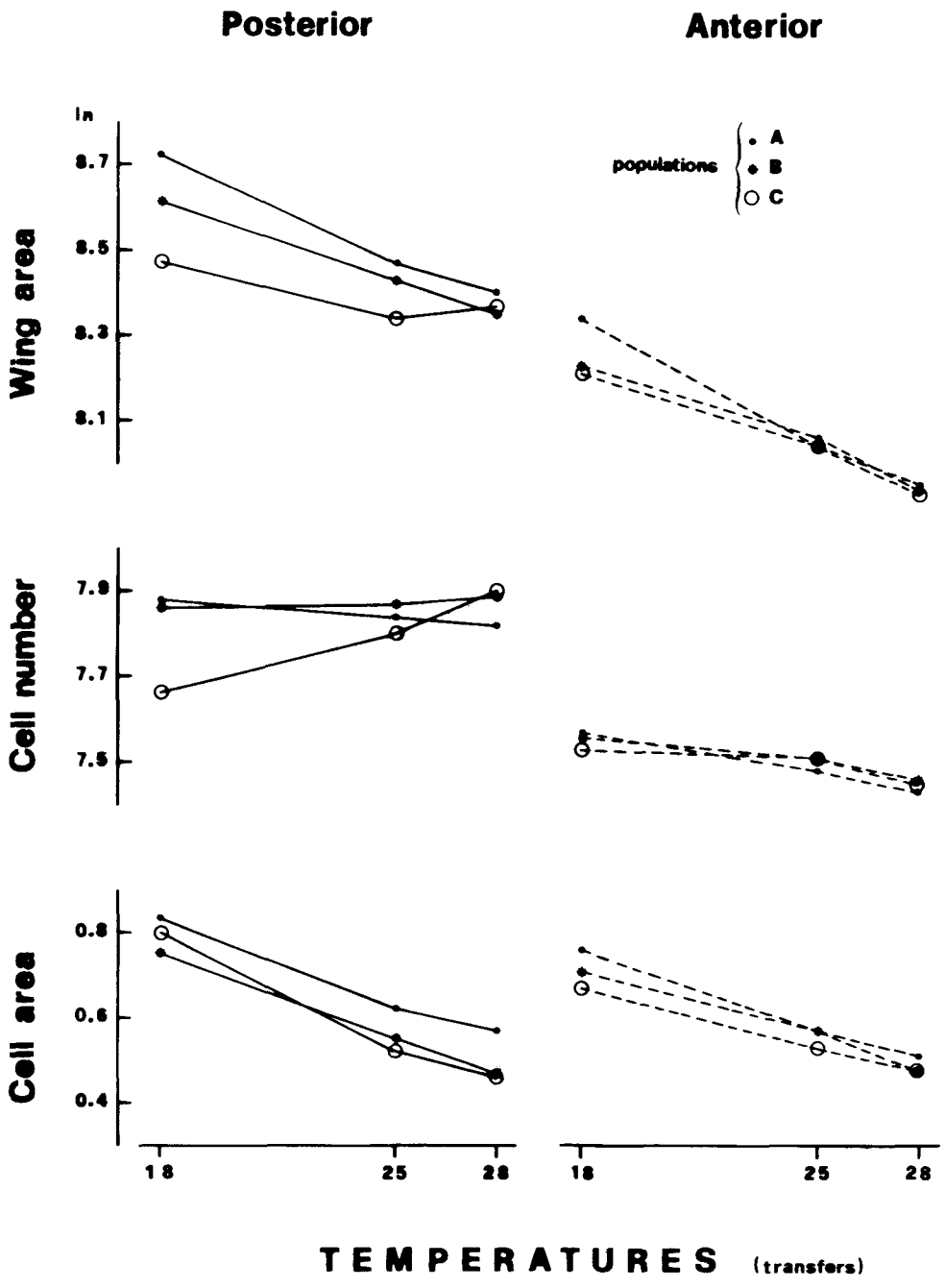

FIGURE 4.—Mean values of wing area, cell number and cell area in the two compartments in the A, B and C populations transferred to three temperatures (18°, 25° and 28°).

parameters. Conversely, in the anterior compartment a slight population × temperature interaction is observed for wing area. Cell area shows only a significant between-populations variance; family × temperature interaction is

TABLE 7

ANOVA to test the effect of temperature on populations and genotypes within populations

Source	d.f.	Posterior			Anterior		
		Wing area	Cell no.	Cell area	Wing area	Cell no.	Cell area
Between temperatures (T)	2	4.444 (0.374)	0.200 (0.0)	3.529** (0.787)	4.515** (0.484)	0.523** (0.147)	2.080** (0.740)
Between populations (P)	2	0.255 (0.0)	0.259 (0.0)	0.482* (0.452)	0.072 (0.0)	0.023 (0.0)	0.126* (0.152)
P × T	4	1.206** (0.632)	0.305** (0.292)	0.045** (0.183)	0.092* (0.040)	0.019 (0.0)	0.012 (0.0)
Between families (F)	23	0.063** (0.181)	0.043** (0.099)	0.009** (0.112)	0.028 (0.007)	0.034 (0.011)	0.010 (0.026)
F × T	46	0.021** (0.202)	0.020** (0.105)	0.003* (0.034)	0.024** (0.491)	0.029** (0.109)	0.007** (0.132)
Within families	393	0.008	0.009	0.002	0.014	0.016	0.003

Temperatures are the three environments of transfer; families are genotypes within populations. Results are expressed as $\ln^2$. Numbers in parentheses are intraclass correlations (see text).

* Significant at 5% level.

** Significant at 1% level.

significant for all considered parameters. The wing parameters considered, therefore, appear to be subjected to different controls in the two compartments, also showing a certain degree of interdependence within each compartment.

How cell area and number contribute to wing surface within the transferred populations has also been studied by regression analysis. Table 8 gives the linear regression coefficients between cell number, cell area and wing area within compartments for all transfers in the three populations. The regression between cell area and number has been omitted because, using a logarithmic scale, it appears as the complement to unity, with changed sign, of the regression coefficients between cell number and wing area. Indeed, if we consider the regression coefficient of the A population kept at 18° between cell number and wing area which is equal to 0.493 ± 0.052, than the coefficient between cell number and area is -0.507 ± 0.052.

Considering first the behavior of the original A population, we can see that the relationship between cell number and wing area is significantly positive and increases when flies are transferred to warmer temperatures in both compartments. Conversely, the relationship between cell number and area decreases, displaying a significant and inverse regression coefficient at 18° (-0.507 ± 0.052 in the posterior compartment and -0.374 ± 0.056 in the anterior one) and a nonsignificant relationship at 28°, being 0.086 ± 0.088 in the posterior compartment and -0.052 ± 0.072 in the anterior one.

Cell area and wing area show an inverse and significant relationship at 18° in the posterior compartment; but not in the anterior one; at the warmest temperature, the relationship is positive and highly significant in both compartments. In the B and C populations cell number increases its contribution to wing size particularly in the posterior compartment, whereas the regression

TABLE 8

Linear regression coefficients between cell number, cell area and wing area in the posterior and anterior compartments for the A, B and C populations transferred to three temperatures (18°, 25° and 28°)

Temperature of transfer	Posterior				Anterior			
	Cell no. – Wing area		Cell area – Wing area		Cell no. – Wing area		Cell area – Wing area	
	b	s_b	b	s_b	b	s_b	b	s_b
				A Population				
18°	0.493	± 0.052***	−0.327	± 0.136*	0.726	± 0.056***	−0.214	± 0.253
25°	0.729	± 0.081***	0.276	± 0.218	0.919	± 0.058***	0.508	± 0.361
28°	1.086	± 0.088***	1.235	± 0.241**	0.948	± 0.072***	0.785	± 0.296**
				B Population				
18°	0.652	± 0.076***	0.247	± 0.244	0.729	± 0.072***	0.220	± 0.209
25°	0.704	± 0.062***	0.111	± 0.192	0.766	± 0.067***	0.304	± 0.203
28°	0.745	± 0.076***	0.302	± 0.211	0.860	± 0.048***	−0.012	± 0.353
				C Population				
18°	0.780	± 0.055***	−0.078	± 0.276	0.608	± 0.062***	−0.130	± 0.181
25°	0.928	± 0.052***	0.530	± 0.345	0.792	± 0.054***	−0.024	± 0.270
28°	0.878	± 0.036***	−0.462	± 0.436	0.901	± 0.046***	0.176	± 0.383

Regression coefficients are ln.
*, ** and *** Significant at 5, 1 and 0.5% levels, respectively.

coefficients between cell area and wing area are never significant. In general, transfers and prolonged rearing at warm temperatures produce both an increase in cell number – wing area and, therefore, a proportional decrease in cell number – cell area relationships. Cell size and number, therefore, appear to be relevant but independent parameters for the determination of wing surface and seem affected by natural selection in different ways. This is further supported by the crosses between the A population and the B and C ones.

Table 9 gives the differences of F_1 and F_2 reciprocals. Some significant differences are observed in both F_1 and F_2 generations, particularly for cell number. The sign of differences for cell number is opposite to that of cell area in most comparisons, confirming a balance of the two parameters for wing surface determination.

Because of the variability in the differences of reciprocals for the different traits, the F_1 to midparent, F_1 to F_2 and F_2 to midparent comparisons are given for each cross in Table 10. Heterosis is observed on wing area in both compartments, larger in A × C and C × A crosses and in the anterior compartment, even though the area of the anterior compartment shows a slight between-populations divergence in the environment in which the crosses were performed (25°). In the anterior compartment, heterosis for wing area seems dependent on the heterosis of cell number, whereas in the posterior compartment, it is dependent on heterosis of cell area. This discrepancy partially disappears in F_2's in which the persistence of heterosis for both compartment

TABLE 9

Differences of F_1 and F_2 reciprocal crosses for the different traits in the posterior and anterior compartments

Cross[a]	Posterior			Anterior		
	Wing area	Cell no.	Cell area	Wing area	Cell no.	Cell area
			Difference of F_1 reciprocals			
A × B–B × A	0.022	−0.011	0.032*	0.036	0.021	0.015
A × C–C × A	0.00	−0.027**	0.027	−0.024	−0.036**	0.012
			Difference of F_2 reciprocals			
A × B–B × A	0.038**	0.033**	0.005	0.020	−0.007	0.027
A × C–C × A	0.010	−0.018**	0.028	0.073***	0.046***	0.027

The crosses were performed at 25°. All comparisons are in ln.

[a] Female parent given first.

*, ** and *** Significant at 5, 1 and 0.5% levels, respectively.

surfaces is mostly dependent on cell number. Cell number generally increases in F_2's (F_1 to F_2 comparisons) so that an increase in heterosis is observed. The effect on wing area is particularly evident in the anterior compartment and less in the posterior one because of the decrease in cell area.

The behavior of the three wing parameters in F_1 and F_2 crosses is quite different in the posterior compartment, where the selection was more effective. It may be argued that the three wing parameters have a distinct genetic determination and that wing surface is the product of a balance of genes that control cell size and number. This balance is not revealed by the anterior compartment, which only exhibits a high correlation between wing area and cell number, possibly because of the low response to selection.

In Table 11 the within-families variances of the parental, F_1 and F_2 generations are given (pooling reciprocals). F_1 and F_2 variances are compared with those of parents and F_2/F_1 ratios are also given. Crosses between the A and B populations show F_1 variances significantly smaller than those of parents for wing area and cell number in the posterior compartment and for cell number only in the anterior one. Only the F_2 variance of cell area in the posterior compartment exceeds that of the parents. In the crosses between the A and C populations, F_1 variances of wing area and cell number are lower than those of parents in both compartments; the F_2's are significantly more variable than the F_1's in only the posterior compartment. Cell size shows an F_2 variance larger than that of parents in both compartments and larger than F_1 variance in the posterior compartment.

This segregational pattern seems to be further evidence that cell area is the wing parameter most affected by temperature and that the two compartments are independent targets for selection. The results also confirm the wing area and cell number are closely related parameters.

TABLE 10

F_1 − midparent (MP), F_1 − F_2 and F_2 − midparent differences for the different traits in the posterior and anterior compartments

Cross[a]	Posterior			Anterior		
	Wing area	Cell no.	Cell area	Wing area	Cell no.	Cell area
			F_1 − MP			
A × B	0.046***	−0.013	0.059***	0.117***	0.088***	0.029
B × A	0.024	−0.003	0.027	0.081***	0.067***	0.014
A × C	0.089***	0.014	0.075***	0.110***	0.067***	0.043
C × A	0.089***	0.041***	0.048**	0.134***	0.103***	0.031
			F_1 − F_2			
A × B	0.008	−0.023**	0.031*	0.002	0.003	−0.001
B × A	0.024	0.020**	0.004	−0.015	−0.025**	0.010
A × C	0.020	−0.007	0.027	−0.042*	−0.061***	0.019
C × A	0.030***	0.002	0.028	0.055*	0.021	0.034
			F_2 − MP			
A × B	0.038**	0.009	0.029	0.115***	0.085***	0.030
B × A	0.00	−0.024**	0.024	0.096***	0.092***	0.004
A × C	0.068***	0.021**	0.047**	0.152***	0.128***	0.024
C × A	0.059***	0.039***	0.020	0.079***	0.082***	−0.003

The crosses were performed at 25°. All comparisons are in ln.

[a] Female parent given first.

*, ** and *** Significant at 5, 1 and 0.5% levels, respectively.

DISCUSSION

Our work started with the aim of investigating the genetic basis of the developmental mechanisms responsible for body size and shape changes in laboratory populations of *D. melanogaster* when temperature is the only environmental factor involved.

We confirm that environmental temperature induces steady variations in body size: increasing temperature decreases body size; the induced variations appear genetically determined and, therefore, temperature-dependent selection seems to have operated on our populations. Wing size and shape of the populations kept at 25° and 28° appear affected by genetic, physiological and developmental influences. When wing vein lengths and their distances are considered, heterosis is observed in F_1's, greater in the crosses between A and C populations than between the A and B ones. A decrease in heterosis is observed in F_2's, but mean values never go below the midparent with the exception of the B × A cross. The within-family variances increase in F_2's, particularly in the crosses between the original population (A) and that kept in the warmer environment (C).

In general the amount of heterosis and segregation displayed by crosses is proportional to the severity of environmental selection. Even though F_2 break-

TABLE 11

Comparison of within-families variances for the different traits in the posterior and anterior compartments in parents and hybrids from the populations kept at different temperatures

Population	Cross[a]	d.f.	Posterior			Anterior		
			Wing area	Cell no.	Cell area	Wing area	Cell no.	Cell area
			Mean squares			Mean squares		
A and B	Parents (P)	80	7.49	9.12	1.21	22.86	29.37	3.13
	F_1	80	3.05	5.59	2.04	18.69	9.70	2.30
	F_2	113	3.77	5.27	2.15	9.84	11.34	2.08
	Ratio			*F*-test			*F*-test	
	P/F_1		2.46***	1.63**	<1	1.22	3.03***	1.36
	F_2/F_1		1.23	<1	1.05	<1	1.17	<1
	F_2/P		<1	<1	1.78***	<1	<1	<1
			Mean squares			Mean squares		
A and C	Parents (P)	79	7.38	8.60	1.63	18.15	20.06	1.97
	F_1	80	3.26	4.91	1.95	8.30	13.03	4.92
	F_2	117	10.15	10.57	2.85	11.30	15.73	4.46
	Ratio			*F*-test			*F*-test	
	P/F_1		2.26***	1.75**	<1	2.19***	1.54*	<1
	F_2/F_1		3.11***	2.15***	1.46*	1.36	1.21	<1
	F_2/P		1.38	1.23	1.75**	<1	<1	2.26***

The crosses were performed at 25°. Mean squares are $\ln^2 \times 10^4$.

[a] Reciprocals are pooled.

*, ** and *** Significant at 5, 1 and 0.5% levels, respectively.

down is not shown, different coadapted gene arrays probably exist in the three populations.

Many maternal effects are involved in the expression of vein lengths both in F_1 and F_2 generations and to a greater extent in the F_1 crosses between the A and C populations, suggesting that the amount of interaction between the genes controlling wing length and maternal environment is related to the phenotypic differentiation which occurred.

The performance of the four veins in this experiment as regards the expression of both phenotypic and genetic variability agrees with their genetic determination (THOMPSON 1975; THOMPSON and WOODRUFF 1982); this seems further evidence that genes controlling body size play a direct role in adaptation to temperature. On the other hand, their combined behavior appears strictly dependent on the developmental unit (compartment) in which they exist during late development. This is revealed by the shape analysis we have performed that shows both a high correlation between genetic and wing shape divergence and a dependence of shape variation on different response of the two compartments to selection. These results suggest that a category of genes is responsible for the expression of groups of veins and their reciprocal position and indicate the existence of a probable prepattern in wing vein formation.

The existence of a prepattern for vein determination is also assumed in some other studies of clonal (GARCIA-BELLIDO and MERRIAM 1969; GARCIA-BELLIDO, RIPOLL and MORATA 1973; GARCIA-BELLIDO 1977) and polygenic (THOMPSON, HELLACK and KENNEDY 1982; THOMPSON and WOODRUFF 1982; S. CAVICCHI, D. GUERRA and D. LA PORTA, unpublished data) analyses of vein pattern formation in which it is argued that some genes control the development of a single compartment and others single elements within a compartment. These findings confirm the view expressed by LAWERENCE and MORATA (1976) and in our previous works (CAVICCHI, PEZZOLI and GIORGI 1981; CAVICCHI *et al.* (1981) that compartments represent units of genetic control of size and shape.

Our results show that, during development, temperature-dependent selection operates in different ways on the genes that control the cell dynamics of the two wing compartments. Cell area is the wing parameter mainly affected by temperature in both compartments, also showing a marked response when the three populations are transferred to three temperatures. In fact, cell area shows a larger additive variance than that exhibited by the other two wing parameters both in the transfers and in the crosses. Although cell area appears to be a very important parameter in determining wing size variations among populations, it only has a slight effect on wing size differences between flies within populations. This is because within groups the variability between flies estimated on the basis of cell area is never correlated with the variability between flies estimated on wing area. Moreover, when the results obtained by transferring the populations to different temperatures and by crossing them are considered, the genetic differentiation that occurred for wing area, particularly in the posterior compartment, may have been produced by selection on genes other than those controlling the cell area.

On the other hand, wing area appears closely related to cell number. Cell

number has been shown to be under genetic control (GARCIA-BELLIDO, RIPOLL and MORATA 1976). Moreover, as shown in the crosses between the A and C populations for the posterior compartment, cell number seems to be controlled by genes selected by temperature, even though the number appears to compensate for cell size variations. This compensation is carried out both at the level of single organisms and the population as a whole. The first level is active in the original A population kept at 18° in which a significant inverse relationship between cell area and number is observed. This may be regarded as a physiological buffer for maintaining the stability of wing and, in general, body size in a stable environment. The second level is active when an important environmental factor, such as temperature, produces selective forces on genes that are responsible for body size. This level is evident in the C population in which cell area and number no longer show a relationship but exhibit an opposite trend of response to transfers.

We are in doubt as to whether the segregation for cell number observed in A × C crosses is a consequence of selective forces acting on specific genes or the result of the close relationship existing with wing area. Considering the buffering properties exhibited by this parameter, we are more inclined toward the second hypothesis. Accordingly, the genes that control wing area may have a pleiotropic effect on genes controlling cell number and this may represent an important genetic buffer for the maintenance of body size stability.

Therefore, also on the basis of the results obtained when the wing veins were considered, we may infer that selection for environmental temperature is active both on genes that control the spatial organization of the wing compartments and on genes controlling cell size. As a consequence, the genes for spatial organization have to be active during development for a longer time than those controlling vein patterns and cell area, at least until the end of the cell proliferation in the wing disc, *i.e.*, 24 hr after pupa formation. Moreover, temperature-dependent selection differs in the two compartments and this constitutes the origin of the wing shape variations observed. The slower development of the anterior compartment, as revealed by clonal analysis (GARCIA-BELLIDO and MERRIAM 1971), could account for its greater buffering properties.

The different behaviors of the two compartments allow us to argue that they represent individual units of selection. Hence, the two compartments being two different units of development, in our case the unit of selection is represented by a unit of development.

The developmental events connected with temperature seem to be important factors in population differentiation. In agreement with previous studies cited in this paper, we think that size variation has adaptive meaning even though it is known that at all temperatures larger flies have higher fitness and they are expected to be favored by selection. Temperature has a large effect on developmental time and the duration of the larval period that is positively correlated with body size (ROBERTSON 1963); this might represent a developmental mechanism that imposes a limit on size at different temperatures.

In our previous study (CAVICCHI, GIORGI and MOCHI 1978) shape variation was found to be associated with reproductive fitness in relation to temperature; in this work we show that the shape variation covers half the total morphological distance between populations kept at different temperatures and originate from genetic changes of the developmental program.

This program has been genetically fixed and may be modified by natural selection. Therefore, shape variation has an adaptive meaning; however, the complexity of genetic and developmental events involved in the divergence between populations inhabiting contrasting environments for temperature makes it difficult to establish a closer connection between the observed variation and fitness.

Developmental biology tries to establish the steps of pattern formation and its genetic control; population biology deals with the evolutionary significance of pattern variation. We think, therefore, that an approach combining population and developmental genetics may be a useful tool to investigate evolution.

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