STUDIES IN QUANTITATIVE INHERITANCE. XII. CELL SIZE AND NUMBER IN RELATION TO GENETIC AND ENVIRONMENTAL VARIATION OF BODY SIZE IN DROSOPHILA

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INDIVIDUALS may differ in body size for many distinct reasons, both environmental and genetic, and it is of some interest to know how far different causes of variation and also different kinds of genetic behavior are associated with characteristic differences in development. Mere numerical record of genetic variation of body size can identify different types of genetic behavior but lumps together different effects which must be recognized and studied if a fuller understanding is to be achieved. Previous publications in this series have been concerned with various aspects of the genetic variation of body size in *Drosophila melanogaster* as revealed by selection, inbreeding, chromosome interchange between lines and other tests. It is now necessary to examine such situations from a rather different viewpoint and see whether the combination of genetic and physiological evidence can take the analysis to a deeper level.

Ideally the size of an animal or an organ can be described in terms of the size and number of its cells and it would be valuable to know how the cell size and number relations change in different genetic and environmental situations. Such information should, at the same time, throw light on the regulation of body size and of genetic differences in this capacity.

It so happens that an insect like Drosophila is quite well suited for such a study. DOBZHANSKY (1929) first pointed out that changes in cell size and number might be estimated from the cell density in the wing membrane which consists of a double layer of cells, each bearing a tiny bristle. The cell outlines can be seen shortly after eclosion but later become obliterated. The number of bristles which occur in a given area of upper or lower surface of the wing provides a measure of the surface area of a cell or cell size. Although gradients in bristle density occur, the cells are regularly arranged and counts in different regions of the wing are quite highly correlated. It is well known that wing and body size vary together when nutrition is altered and many observations (ROBERTSON and REEVE 1952; REEVE and ROBERTSON 1953) have demonstrated a high genetic correlation as well and so differences in wing area generally afford a reliable indication of comparable differences in body size. Hence records of wing area and cell density provide the basic data for this study in which the variation of size and cellular constitution of a single organ, which is highly correlated with body size, is used as an indication of genetic or environmental differences which influence body size generally.

With respect to earlier work, DOBZHANSKY (1929) demonstrated a striking correlation between wing cell size and estimates of total chromosome volume—disregarding the Y—among the progeny of a cross between diploid and triploid flies which produces a variety of numerically unbalanced chromosome combinations. ALPATOV (1930) studied the changes caused by rearing flies at different temperatures and levels of crowding and reported a general tendency for crowding to be associated with changes in both cell size and number while temperature affected cell size. ZARAPKIN (1934) compared cell size in large and small selected strains of *Drosophila funebris* and concluded that changes in cell number were entirely responsible for the observed differences in wing size. More recently ROBERTSON and REEVE (1952) reported that differences in wing area between a large and small strain of *Drosophila melanogaster*—also selected for wing size—could be attributed to changes in cell size. However, in the small strain wing size was disproportionately reduced—relative to thorax length—by sex-linked genes and this may account for the apparent discrepancy with ZARAPKIN's data, especially since DOBZHANSKY (1929) noted that the sex-linked miniature which reduces wing size does so *via* cell size. BREHME (1941), in a study of the development of different Minute mutants which grow more slowly than normal, found the wing cells reduced in size and suggested that reduction of body size could be attributed to change in cell size. BARIGOZZI (1951) reported differences in cell size when the Y chromosome from different stocks was placed against a standard genetic background. These various observations underlined the need for a more systematic study which lead to the collection of rather extensive data, which, for reasons of space, cannot be accommodated in a single paper. Hence the present paper deals with the effects of environmental variation, estimates of genetic variation in wild populations, and the results of selection for large and small cell size. The next paper in this series will be concerned with the changes in cell size and number which are associated with selection for differences in body size, crosses between lines and other special genetic situations.

MATERIAL AND METHODS

General: In view of the correlation between counts in different regions of the wing and the labor involved in scoring many individuals, cell size has been estimated from a single region, midway between veins III and IV and a little distal to the major cross vein. The area examined represents approximately one percent of the total wing area at 25°C. Wings are mounted on slides to allow projection of an enlarged image on a ground glass screen. To facilitate counting and provide a check on the homogeneity of the bristle distribution the screen was provided with a ruled grid, divided into a number of equal areas. Wing area is determined from a magnified outline with the aid of a planimeter. The measure of cell size refers to the wing. How far the cell size and number relations in the wing hold also for the rest of the exoskeleton which has a similar embryological origin and growth during the larval period, will be considered later; naturally there is no reason to expect parallel behavior in different kinds of tissue like gut or gonad.

General details of culture on the maize meal molasses medium, measurement of live flies and so on have been described in the first number of this series (ROBERTSON and REEVE 1952). Comparison of the average dimensions of different series is based generally on records for 40–50 females drawn equally from four or five replicated cultures.

The effect of inadequate nutrition on the relations between cell size and number in individuals of different genotype have been studied by culturing larvae on a chemically defined, aseptic medium developed by SANG (1956). Suboptimal levels of specific, essential nutrients reduce adult size, which can be influenced by many different kinds of nutritional imbalance. It is thus possible to discover how individuals of the same genotype respond to different treatments which produce an equivalent reduction in size and also how far genetically diverse individuals differ in response to the same treatment.

Statistical analysis: The analysis of the data presents a few problems. Since the area of the wing may be regarded as the product of cell size and number, a log transformation is required to convert these variables to an additive scale. Accordingly all records of wing area and cell area were converted to natural logarithms before analysis. The estimate of "cell size" is simply the area in which bristles are counted divided by their number. In the tables which follow, mean wing area and cell area or cell size are expressed in terms of log squared hundredths of a millimeter. The region of the wing scored was chosen because it is fairly central and the bristles are regularly distributed. SCHATZ (1951) working with various wing mutants described striking changes in the pattern of bristle density. However, many tests indicate that major heterogeneity of bristle density is absent in the material studied here and that average cell area in the region studied is quite adequate for comparison with changes in wing size. Since the counting grid was divided into six equal areas, it was possible to check the uniformity of the bristle density. The variation of a unit area may be regarded as made up of a component (c) common to all such units of the total area studied and an error component (i) to give $x=c+i$ as the variance of a unit; the approach is similar to that used by REEVE and ROBERTSON (1954) in the study of the number of bristles on successive abdominal sternites. The variance of the total area of n units is given by $V\,\Sigma\,(x) = n^2\,\sigma_c^2 + n\,\sigma_i^2$ while the sum of the variances of the units areas is represented by $\Sigma\,(V\,x) = n\,\sigma_c^2 + n\,\sigma_i^2$. From these relations we can deduce

$$\sigma_c^2 = \frac{V\,(\Sigma\,x) - \Sigma\,(V\,x)}{n(n\text{-}1)}\,;\ \sigma_i^2 = \frac{n\,\Sigma(V\,x) - V\,(\Sigma\,x)}{n(n\text{-}1)}$$

If the wing cells and their bristles are entirely homogeneous, σ_i^2 will represent the random variation arising from the application of a fixed outline to a discontinuous distribution; departure from a uniform distribution will inflate σ_i^2. σ_c^2 represents the best estimate of the variation of cell size after the error variation has been allowed for. When cell size is derived from the total bristle count, the correlation between wing and cell size must be corrected to allow for the inclusion of this error variance.

In practice it turns out that, although minor heterogeneity is present, there is

considerable consistency among a variety of genotypes in the estimates of σ_i^2. Evidence is available from a series of inbred lines of diverse origin, of crosses between them and also from various wild stocks and selected strains. Table 1 shows the average error variance of a total count of six units ($\sigma_i^2/6$), weighted by the degrees of freedom in each sample and from this we conclude that the average value of 0.000,782, derived from the pooled samples is a suitable estimate of the error variance of total area. When the accumulation of data had demonstrated the consistency of σ_i^2 this was turned to advantage by using the average value and the variance of the total count to calculate σ_c^2 indirectly, thereby lightening the effort of computation. In the data presented below estimates of the variance of cell size refer to σ_c^2 and regressions of wing on cell size allow for this. Errors of counting and positioning of the grid make a negligible contribution to the variance of total count; this was checked by repeat counts on a number of wings.

It is worth noting in Table 1 that the estimates of σ_i^2 from inbred line crosses and wild stocks are about the same, i.e., there is no evidence of any change in the degree of heterogeneity of bristle pattern as a result of inbreeding.

Log cell number is estimated from the difference between log wing area and log cell area. For graphical representation the regression of log wing area on log cell area provides a convenient guide to changes in cell size or number which accompany a given change in wing size. Thus if the cell number is constant we expect a regression slope of unity. Values intermediate between zero and unity suggest an inverse relation between the two variables while values greater than one imply that both are changing in the same sense. With these considerations in mind we are now in a position to consider what happens to cell size and number in different situations.

EXPERIMENTAL RESULTS

Environmental influences

Temperature: It is well known that larvae grown at lower temperatures produce bigger flies (ALPATOV 1930) and also that the relative size of different parts of the body vary according to temperature (IMAI 1934). Tests on wild stocks have shown that between 30°C and 18°C wing area changes two to two and a half times as much as the square of thorax length. This effect of temperature is the most notable exception to the regularity with which wing and thorax dimensions show similar proportional change. Since ALPATOV's (1930) tests, carried out on a

TABLE 1

Error variance of cell counts per total area in different genotypes

Genotype	Degrees of freedom	Error variance
Inbred lines	356	0.000,773
Crosses between lines	255	0.000,769
Wild populations	814	0.000,789
Weighted average	...	0.000,782

single strain, suggested that variation of wing size due to temperature is a function of cell size, it is convenient to begin by seeing how far this is generally true. For this purpose, pairs of lines, long inbred by brother-sister mating, together with their F_1 crosses were reared at three temperatures: 18°, 25°, and 30°C. The crosses were made between the lines N_2 and C_6, N_2 and E_4, R_2 and C_9, C_9 and C_{10}; the symbols N, R, E and C refer to the origin of the lines from the Nettlebed, Renfrew, Crianlarich and Edinburgh wild stocks. Five cultures of eggs were generally set up for each line and cross and 6–8 females were scored from each culture. Figure 1 a-d shows the mean values of wing area and cell size; the following features are to be noted:

(i) Taking the average of the crosses as representative of the behavior of non-inbred wild flies, it appears that a change of temperature from 30° to 18°C increases wing area by some 45 percent. A glance at the different graphs in Figure 1 shows that this change can be attributed almost entirely to a change in cell size and that cell number remains comparatively constant. The line with a slope of one is drawn through the average wing area and cell size of the crossbred flies reared at the three temperatures and it is clear that the points for the crossbred flies fall closely about this line in every case.

(ii) Although the inbred lines follow the same general rule they show less consistency in their response to temperature, especially when cultured at 30°C. Wing area is smaller than anticipated from the relations observed at 18° and 25° C, especially in lines C_6, C_{10} and R_2. Since 30° C is near the upper limit of growth and survival it is to be expected that inbred lines will be more adversely affected than the crosses and the discrepancy in wing size is presumably a reflection of this. The disproportionate reduction of wing area at the higher temperature is reflected mainly in a lower cell number. We can estimate log cell number from the difference between average log wing and cell area and such estimates for the various series at 30°C, expressed as deviations from estimated cell number at 18°C, are set out in Table 2. The inbreds show a consistently negative deviation with an average of -0.092 compared with -0.012 for the crosses.

(iii) Inspection of the graphs shows that the relations between cell size and number for a given wing size are not necessarily the same; the special case of flies cultured at 30°C are excluded from this consideration. The point is well made by comparing wing area and cell size of lines N_2 and E_4 at 25°C in Figure 1b. Although the wing area in the lines is about the same, the cell sizes work out at 0.703 and 0.583 log units—a highly significant difference. A similar situation is seen in Figure 1c with respect to the dimensions of R_2 and C_9 at both 18°C and 25°C. This raises the general problem of genetic variation in the cell size and number relations, independent of wing size—a topic to be considered later.

(iv) Although all genotypes, apart from the special changes at 30°C, tend to follow the same pattern of response to temperature change nevertheless the extent of the shift up and down the slope of unity is not constant, i.e., the difference in wing area between the contrasted genotypes is not the same for a given temperature difference. Thus in Figure 1a, the F_1 of the cross between N_2 and C_6 signifi-

TABLE 2

Difference between estimated log cell number at 30°C and 18°C

Parents	Deviations	
	Inbred lines	Crosses
C_6	−0.081	−0.027
N_2	−0.010	
E_4	−0.166	−0.018
N_2	−0.022	
R_2	−0.055	−0.013
C_9	−0.070	
C_{10}	−0.231	−0.025
C_6	−0.101	
Average	−0.092	−0.012

cantly exceeds the larger line N_2 at 18°C, but at 25°C it is slightly smaller. In Figure 1b N_2 is bigger than E_4 at 18°C but is smaller at 30°C. In Figure 1c the F_1 between R_2 and C_9 is much larger than either parent at 18°C whereas at 25°C all genotypes have about the same size. Finally in Figure 1d, at 25°C the cross exceeds both parent lines which are about the same size, while at 18°C C_{10} and the F_1 are very similar and considerably exceed C_6. As a result of this genotype environment interaction the size of the F_1 in relation to the size of the parents may differ according to the temperature. Hence dominance deviations estimated from the comparison of the sizes of parents F_1, F_2 and backcrosses would be quite different according to the temperature during development.

(v) In recent years there has been discussion of two aspects of the response to environmental variation of individuals which differ genetically in such a way that we may reasonably regard one genotype as conferring greater fitness than another, as when we compare inbred lines and crosses. On the one hand, there is plenty of evidence that the latter achieve greater stability of phenotype in that certain characters like size and fertility are less variable. But there is also the possibility that the fitter types have greater capacity to respond adaptively to different conditions so that, in certain respects they will be more variable (SCHMALHAUSEN 1949; THODAY 1955). Such effects are familiar in plants but apparently rather difficult to demonstrate with assurance in animals; a good example is described by SMITH and SMITH (1954).

In the present context the evidence rests on whether the crosses show greater or less response to a given temperature difference than their inbred parents. For this purpose we can compare performance at 25°C and 18°C; tests at 30°C are excluded for the reasons given above. The tendency for wing size to be disproportionately reduced in inbred lines at 30°C could be regarded as evidence of the greater stability of crosses.

Treating the eight sets of data from inbred lines and four from crosses, as two groups for comparison, the crosses do indeed show a greater difference in wing area and cell size between 18° and 25°C than the inbreds. Table 3 shows the average difference in wing and cell size at the two temperatures. For wing area, the average difference works out at 0.218 and 0.249 units for inbred and crosses respectively, while for cell size the corresponding values are 0.193 and 0.268, with a difference of 0.075, which is significant at the 0.02 level of probability.

Although the difference for wing size is not statistically significant since it goes in the same direction as cell size, the effect is probably real. Thus these comparisons of performance at 25°C and 30°C illustrate the greater ability of cross-

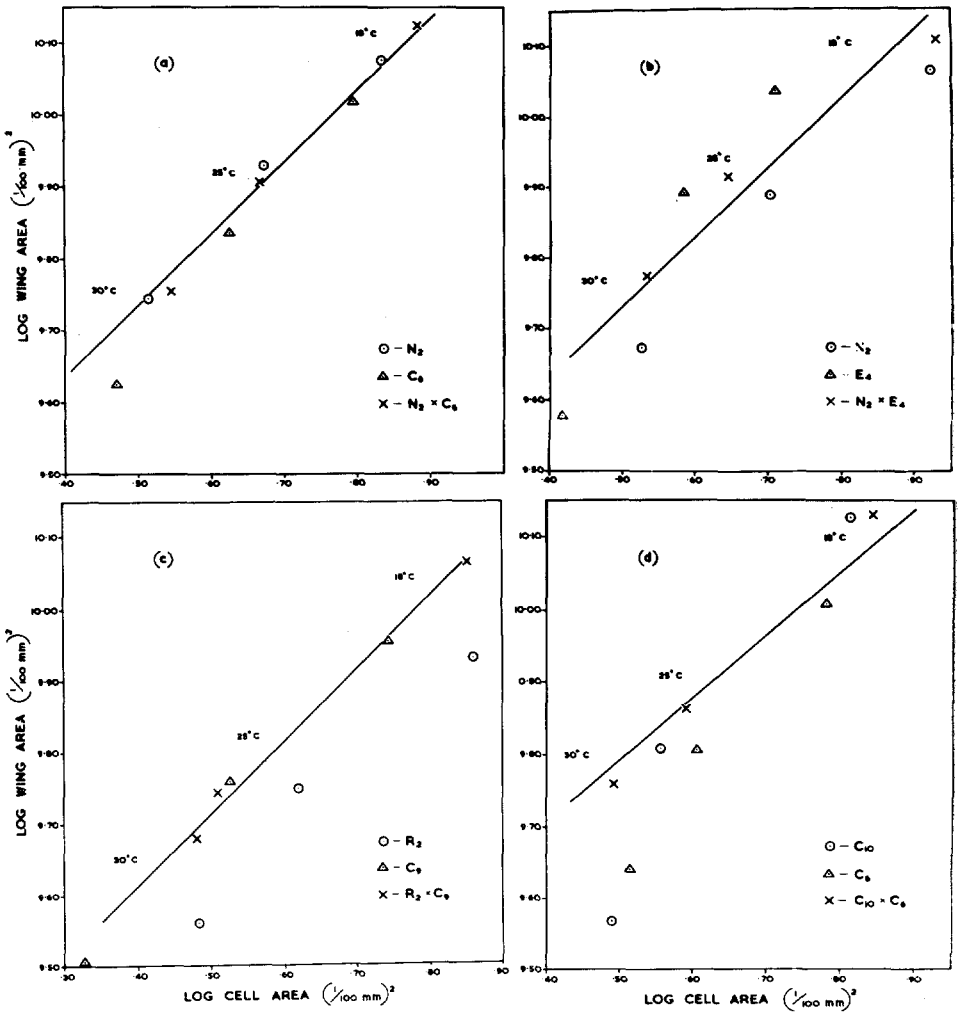

FIGURE 1.—Cell and wing areas in inbred lines and crosses at different temperatures.

TABLE 3

Average difference in wing and cell area between inbred and crossbred flies reared at 18°C and 25°C in log units

	Wing area	Cell area
Inbred lines	0.218 ± 0.018	0.193 ± 0.016
Crosses	0.249 ± 0.025	0.268 ± 0.022
Difference	0.031	0.075
P	>0.05	<0.02 > 0.01

breds to withstand more extreme conditions, while performance at 25°C and 18°C points to their greater responsiveness to this particular environmental change.

(vi) The inbred line N_2 was used in the cross to C_6 and also E_4. The tests were separated by an interval of five weeks and there is no reason to suppose that N_2 changed genetically in that period. However, at corresponding temperatures, the wing area in the second test is less than in the first; this is shown in Figure 2. At 30°C and 25°C the differences between the estimates of both cell and wing area in the two experiments are about the same, although at 18°C wing area is relatively a little bigger in the later test. Environmental differences between the tests may be due either to temperature or nutrition. It is improbable that temperature differences are the cause. It is obvious from Figure 2 that most of the observed difference in wing size must be attributed to cell number, so there is an interesting contrast between the effects of temperature, which influence cell size and of nutrition, which here influences cell number. This suggests that the next logical step is to examine the effects of variable nutrition on cell size and number; such experiments are dealt with next.

The effects of variable nutrition: Nutritional effects have been studied by rearing larvae on chemically defined aseptic media which contain all the essential nutrients in excess except for certain specific deficiencies; the most favorable diet is provided by medium C of SANG (1956). Suboptimal levels of any essential nutrient reduce body size. This procedure is preferable to the reduction of body size by crowding on live media since it enables us to compare different genotypes under similar conditions of nutritional imbalance, while the homogeneous, aseptic medium reduces within culture variance to a low level. The compounds which have been reduced in concentration comprise casein, ribonucleic acid and choline. Three unrelated wild populations, namely Pacific, Gabarros and Kaduna have been reared on media deficient in one or other respect; in the Kaduna population only casein and choline deficiency has been studied.

The data are summarized in Figure 3a, b, c in terms of the average cell number and cell area recorded for the different treatments. Since we are not concerned with differences between strains in nutritional requirements, this aspect of the data will not be considered here. The first point to note is the general tendency

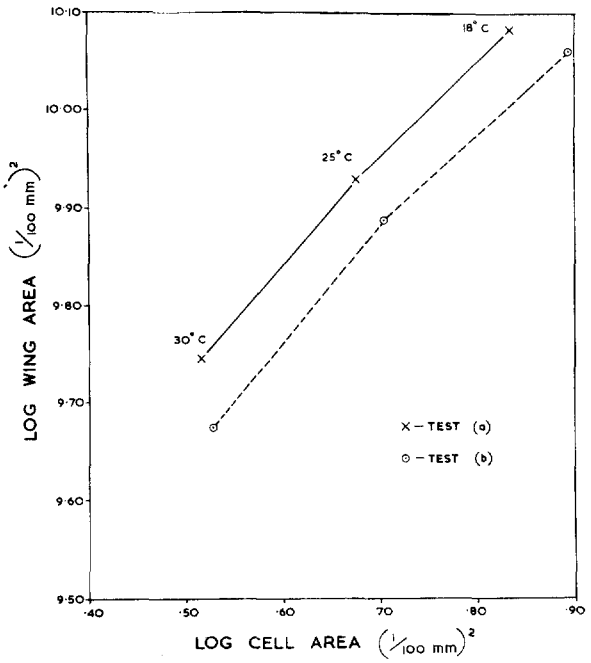

FIGURE 2.—Dimensions of line N_2 in different tests.

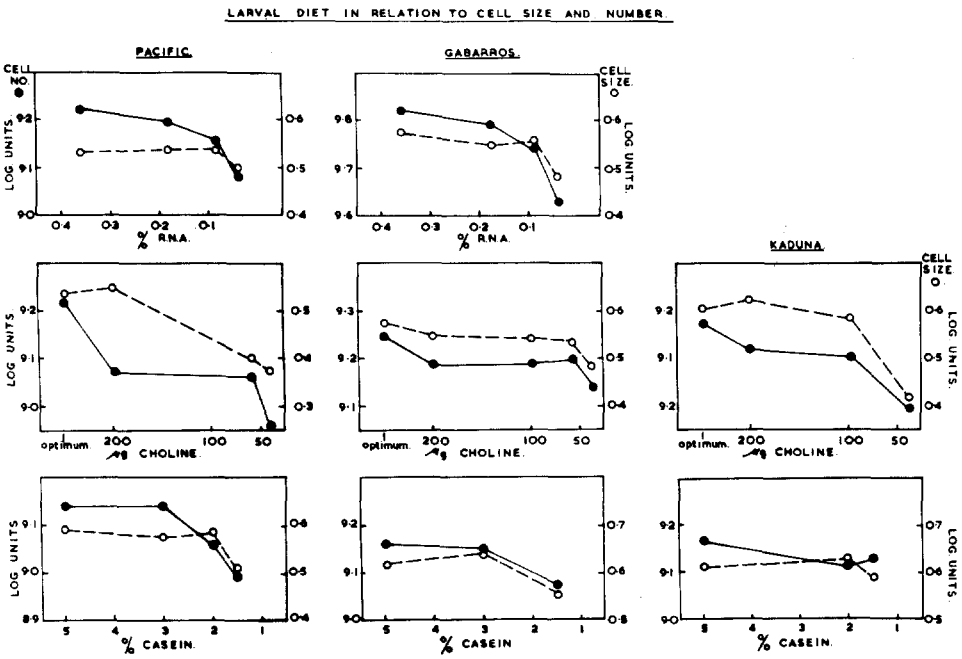

FIGURE 3.—Cell size and number in wild strains reared on chemically defined aseptic media which differ in concentration of particular essential nutrients.

for nutritional imbalance to affect cell number more readily than cell area. This is especially evident in the Pacific and Kaduna populations in which a considerable decline in wing size may leave cell size unchanged. The Gabarros strain apparently differs from the other two in that cell size is more readily affected. When conditions become too adverse, both cell size and cell number are reduced. A particularly interesting situation is presented by the effect of choline deficiency in the Pacific population. The initial decline in wing size due to culturing on the synthetic medium supplied with 200 μg of choline instead of the usual live yeast or "optimum" medium involves a decline in cell number alone. But the difference in wing area between flies reared on media with 200 and 60 μg choline is apparently almost entirely due to a reduction of cell area whereas further reduction of choline concentration is accompanied by decline in both cell size and cell number.

The most important feature of these results is the general tendency for unbalanced diets which lengthen the period of development and reduce body size to be associated with a decline in cell number rather than cell size. Other experiments, with inbred lines and crosses, not dealt with here, support this general conclusion. It will be recalled that the differences of wing size between the two tests with line N_2 were attributed to differences of cell number. So this behavior appears to be quite typical unless starvation is too acute.

Genetic variation

Comparison of wild populations: It is convenient to consider first the dimensions of different wild populations. Table 4 summarizes the data for seven populations derived from widely separated localities and kept in the laboratory for varying periods of time, generally several years, but only a few weeks in the case of Pacific. The populations are tabulated in order of diminishing size. There are well defined differences in body size between the populations; the extremes differ by some 11 percent in terms of squared thorax length. Average wing and thorax size are highly correlated ($r=0.72$). The end column of Table 4 lists the difference between log wing area and log squared thorax length, which provides a measure of relative wing size. Five of the strains are similar in this respect, while one,

TABLE 4

Average wing area, cell area, cell number and squared thorax length in wild populations; in log units

Populations	Thorax	Wing area	Cell area	Cell number	Wing-thorax difference
Renfrew	9.420	9.837	0.537	9.300	0.417
Gabarros	9.381	9.907	0.599	9.308	0.526
Sao Paulo	9.380	9.848	0.552	9.296	0.468
Pacific	9.353	9.806	0.559	9.247	0.453
Crianlarich	9.348	9.799	0.549	9.250	0.451
Ischia	9.329	9.783	0.580	9.203	0.454
Edinburgh	9.312	9.765	0.577	9.188	0.453
Standard error	0.008	0.006	0.006	0.006	....

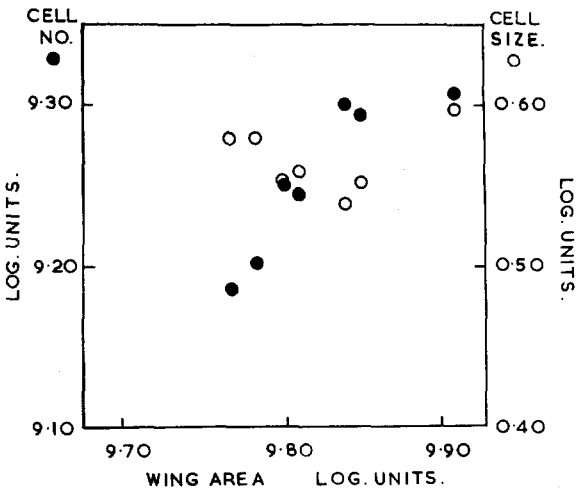

FIGURE 4.—Cell size and number in wild populations.

Renfrew, has relatively small wings and another, Gabarros, relatively large wings, and these differences are associated with larger and smaller cell area. This may indicate that major variation in relative wing size is associated with change in cell area rather than number. The data are represented graphically in Figure 4 by plotting average log cell area and log cell number against log wing area. This brings out clearly the importance of cell number; the regression of wing area on cell number works out at 0.90 ± 0.19. One population, Gabarros, deviates more than the others from the regression line and this is obviously associated with its larger relative wing size. Average cell area is comparatively constant. There is some evidence of an inverse relation between average cell area and cell number but this is quite minor compared with the major variation of cell number. The significance of this relative constancy of cell area in populations which differ in body size will be discussed later.

The variation of cell size. General: The genetic contribution to total phenotypic variance can be roughly estimated by comparing the variance among individuals of a wild population with the variance of appropriate genetically uniform individuals. General experience suggests that crosses between inbred lines are suitable for such comparisons since their general reaction to environmental variation is much the same as that of wild individuals. The variance of any character for such genetically uniform flies can be taken as a measure of environmental effects. Records of wing and cell size from several different wild populations and also from crosses between various inbred lines are available from experiments carried out at different times. Since the estimates of variance and covariance within each of the two categories present a considerable hemogeneity such within-culture estimates have been pooled to provide a reliable picture of the average variance under optimal conditions. The estimates are set out in Table 5 in which the components have been multiplied by 10^6 and rounded to the nearest ten to make com-

TABLE 5

Genetic and environmental components of variance and covariance (log units: $\times 10^6$)

Genotype	Direct estimates						Indirect estimates				
	d.f.	σ_S^2	σ_C^2	cov_{SC}	b_{SC}	r	σ_N^2	cov_{SN}	b_{SN}	cov_{NC}	b_{NC}
Variable	271	2190	1640	330	0.20	0.18	3160	1850	0.59	—1300	—0.80
Uniform	89	710	510	0.00	0.00	0.00	1220	710	0.58	— 510	—1.00
"Genetic" effects	...	1480	1130	330	0.30	0.26	1940	1140	0.59	— 790	—0.71
Percentage of total	...	67	69	100	...	...	61	62	...	....	...

S, *C* and *N* refer to wing area, cell area and cell number.

parison easier. Estimates of genetic effects are simply the difference between corresponding components for wild and genetically uniform flies, i.e. genetic and environmental effects are assumed to combine additively on a log scale, which, although unlikely to be strictly true, is a good enough approximation when environmental conditions are favorable.

As expected from previous work, there is substantial genetic variation of wing size, accounting for a little under 70 percent of the total variance. Genetic variation of cell size works out at about the same level. A regular feature of wild populations is the low, but consistently positive regression of wing area on cell area which works out at 0.20. This correlation is evidently entirely genetic in origin since the covariance terms in the crosses is zero i.e. the nutritional variation within cultures leaves general cell size unchanged, as might be expected from the evidence presented earlier in the section dealing with nutrition. The genetic regression of wing on cell area rises to 0.3. This correlation could arise from an association between wing cell size and either general body size or relative wing size, or both could contribute to the variance. However, general experience of the high level of genetic correlation between wing and thorax size in wild populations and also the finding that log squared thorax length and log cell area are also correlated to a similar degree in the wild populations examined (r=0.21 for 130 degrees of freedom) suggest that the observed correlation between wing and cell area reflects variation of general body size. Thus although most of the variation of wing and hence body size is associated with variation of cell number, nevertheless an appreciable fraction is associated with variation of cell size.

It may be further inferred that there is a high negative correlation between cell size and number as the following considerations show. Since the area of the wing can be regarded as the product of average cell size (C) and number, (N), or the sum of the log values, the variance of wing size (S) can be expressed as

$$\sigma_S^2 = \sigma_C^2 + \sigma_N^2 + 2\ cov\ CN$$

from which by substitution and rearrangement it follows that

$$\sigma_N^2 = \sigma_S^2 + \sigma_C^2 - 2\ cov\ SC$$
$$cov\ SN = \sigma_S^2 - cov\ SC$$
$$cov\ CN = cov\ SC - \sigma_C^2$$

Such indirect estimates of the variance of cell number together with the covari-

ance between wing size and cell number and also cell size and number are shown on the right hand side of Table 5. They draw attention to the high negative correlation between cell size and cell number evident in both environmental ($r = -1.0$) and also genetic effects ($r = -0.54$). Such indirect estimates must naturally be treated with a certain reserve, since error variance in the estimates of average wing cell size will contribute to apparent negative covariance between cell size and number. It is most improbable that the observed negative covariance is merely due to this fact as the later results show. The negative covariance of cell size and number suggests that genetically different individuals of the same wing size may differ in cell size and number. Such variation tends to obscure underlying relationship associated with change of wing size and special tests are needed to clarify the situation.

The effects of selection: Since there is plenty of genetic variation influencing cell size in wild populations, further information about its properties was gotten by selection for large and small cell size in three different wild populations. Selection was carried out for 2, 3, and 9 generations in the Pacific, Pobla de Lillet and Gabarros populations respectively. The procedure was as follows: from each of four cultures, 15 pairs of newly emerged flies were drawn at random, the left wing cut off carefully, mounted and the extreme four pairs with the highest or lowest cell density were selected from each culture and combined with similarly selected individuals to form the parents of successive generations. Thus each generation 16 pairs of flies were chosen from a total of 60 pairs scored. The removal of a wing had no obvious adverse effect on the flies, which mated with alacrity when the selected parents were put together. Throughout these tests there was no evidence of any change in survival, as measured by the proportion of eggs cultured which became adults. Samples from the unselected population were set up sufficiently often to compare the effects of selection in either direction. The results with the different populations are described in turn.

(a) *The Pobla de Lillet population.* In this test selection ran for three generations. Table 6 shows the average cell size in the successive generations, sexes averaged, and also the differences between the selected strains.

Cell size in different generations cannot be compared directly since controls were not raised and it is known that temperature fluctuated about 3°C during this

TABLE 6

The effects of selecting for large and small cell size in the Pobla de Lillet population

Mean cell size of the selected strains: sexes averaged, log units			
Generation	Large	Small	Difference
1	0.550	0.509	0.041
2	0.550	0.489	0.061
3	0.559	0.489	0.070
Cumulated selection differential			0.284
Estimated heritability			0.247
Standard deviation in unselected population			0.050

selection experiment. This does not affect the comparisons between the selected strains reared together. Evidently cell size responds quickly to selection and after three generations the strains differed by some seven percent which corresponds to about one and a half times the standard deviation of cell size in the unselected population. Heritability can be estimated from the ratio of final difference between the selected strains to the cumulated selection differential i.e., the sum of the average selection differentials per generation, estimated as the deviation of the selected parents from their culture means. With this criterion, heritability works out at about 25 percent.

It might be thought that the selection response is due to a change in the pattern of cell density rather than a change in average wing cell size. To check this counts were carried out on 24 flies of each strain in three additional regions of the wing where the density differs somewhat. These regions were located in the distal region of the marginal cell (II), between veins II and III about three quarters of the way along the veins (III) and roughly in the middle of the third posterior cell (IV); the usual region for counting is labelled 1. The ratio of cell density in the small to that in the large cell strains worked out as follows for the different regions:

I	II	III	IV
1.076	1.062	1.070	1.053

Thus, although minor shifts in pattern may have occurred, these are certainly trivial compared with the average change in cell size throughout the wing.

From the last generation of selection and also the unselected population eggs were collected and set up in five cultures; virgin flies were also crossed reciprocally. Since the direction of the cross made no difference the data have been combined; ten females were scored from each culture; the relevant comparisons are shown in Table 7.

Since the difference between the selected strains in this test and at the end of selection is virtually identical—0.076 versus 0.070 units—the generation of relaxed selection was apparently without effect. Comparing the selected strains with controls, the large moved further away from the initial level than the small cell strain i.e. 0.045 compared with 0.031 units. Wing size has declined in both selected strains, especially the small cell strain. The decline in wing size of the large cell strain is unexpected in view of the positive genetic correlation found

TABLE 7

Comparison of selected strains and crosses with the unselected population: females only

Genotype	Deviation from unselected (log units)		
	Cell area	Cell number	Wing area
Large cell strain	0.045**	—0.074	—0.029*
Small cell strain	—0.031**	—0.014	—0.045*
Cross	—0.006	—0.013	—0.019

* Indicates significance of the 0.05 level of probability.
** Indicates significance of the 0.01 level of probability.

among individuals of the unselected stock. The F_1 flies do not differ appreciably from the average values found in the unselected stock, suggesting that the re-establishment of more normal cell size and number relations and wing size are interrelated.

To summarize, this experiment shows that (a) there is an inverse relation between cell size and number, with respect to selection for large cell size since striking increase in cell area is accompanied by decline in cell number. (b) It is easier to change the cell size and number relations in favor of larger cells and fewer of them than *vice versa*. (c) The selection for small cell size which leads to a decline in wing size may be relevant to the positive correlation between wing and cell size found among individuals of the unselected population (Table 5).

(b) *The Pacific population:* Two generations of selection for large and cell size were carried out in this population; the results are summarized in Table 8. The response was quite striking, amounting to a difference of some seven percent between the selected strains—equivalent to about one and a half times the standard deviation. Again selection was more effective upward than downward leading to a deviation from the control of 0.043 log units as compared with 0.028 and this is reflected in the estimates of heritability which work out at 0.58 and 0.47 respectively; these values are higher than in the last experiment. Apparently selection has affected only the cell size and number relations apparently independently of wing size, since this has remained constant.

(c) *The Gabarros population:* The selection response is summarized in Figure 5, a-c. The top graphs (5a) which show the deviation from the controls in successive generations, indicates that selection has produced a striking effect in either direction. By generation 9 the cell area in one strain is about 20 percent greater than in the other. The nature of the response is rather different in the two directions; in the large cell strain there was a fairly steady response for five generations, after which response apparently ceased. In the other strain, the initial response was slower and then speeded up and was apparently still continuing when the experiment was discontinued at generation 9. If records of cell size were the only data available, i.e., if cell size were being treated as a "character", the nature of the selection response might invite comparison with one or other of the characteristic types of response found when other characters are selected for. However, it is easy to show that inferences based on such comparisons would be of little value, while heritability estimates derived from the apparent response

TABLE 8

Response to selection for cell size in the Pacific population

Selected strain	Deviation from unselected				
	Cell area	Cell no.	Wing area	Selection differential	Heritability
Large cell	0.043**	—0.048	—0.005	0.074	0.58
Small cell	—0.028**	0.029	0.001	0.064	0.44
Standard deviation of cell size = 0.045					

** Indicates significance of the 0.01 level of probability.

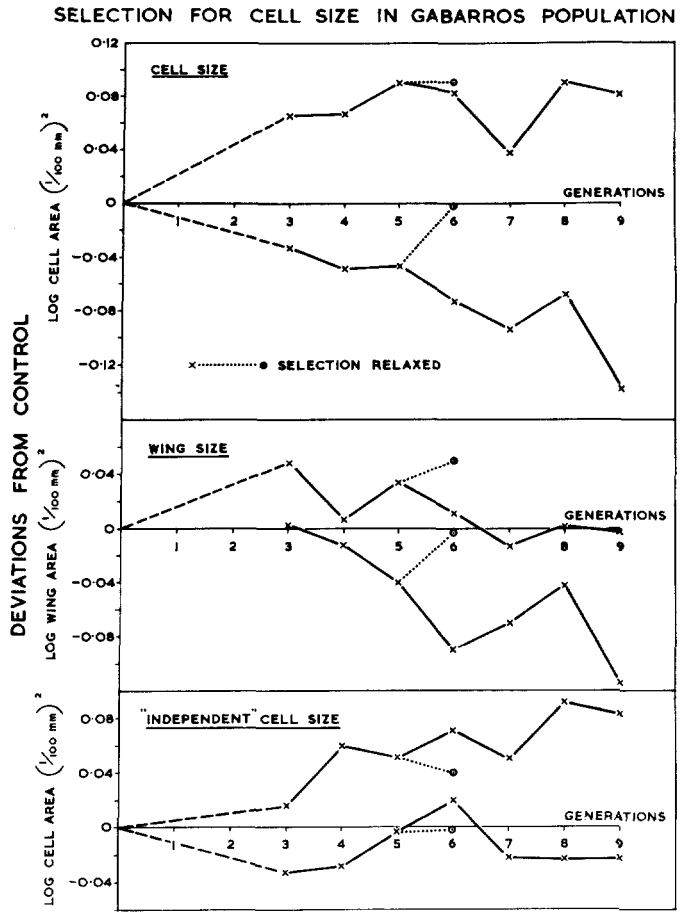

FIGURE 5.—In the bottom graph the points are calculated by subtracting the deviation from control for wing area from the corresponding deviation for cell area. This gives an indication of shift in the inverse relations between cell size and number—hence the term "independent" cell size.

would be very misleading. Figure 5b shows what happened to wing size during selection. In the large cell strain, wing size increased during the first three generations and then declined irregularly to fluctuate about the control level. Thus the initial response to selection apparently involved effects correlated with body size rather than merely changes in the cell size and number relations within the wing, but in view of the data presented later, the correlation may be due to linkage.

In the small cell strain the picture is reversed. The first three generations of selection leave wing size unchanged, but from generation 4 onwards there is a drastic decline in wing size, so that by the end of the experiment, the latter has fallen about ten percent below the original level. The later response to selection

is due to genetic changes which affect general cell size, rather than changes in the cell size and number relations within the wing.

Since average wing and cell size may be expressed as deviations from the control level, the changes in cell area which are apparently independent of the changes in wing area and which involve inverse changes in cell number can be estimated by subtracting the deviation for wing from that for cell size; this is illustrated in Figure 5c. With this procedure, there is a marked asymmetry of response; in the large cell strain the cell area is increased about eight percent while in the other strain the decline is only about two percent. This recalls the similar trend in the Pobla de Lillet selection, suggesting that it is a general rule for wild populations, that it is easier to shift the cell size and number relations, within the wing, in favor of larger cells and fewer of them. The substantial reduction of wing size effected by selection for cell size alone is reassuring evidence that the measure of cell size is a perfectly adequate indicator of average cell size in the wing.

At generations 3, 4, 7 and 9, thorax length was also recorded to see whether the changes in wing size reflect changes in general body size. The comparisons are set out in Table 9, in terms of deviations from the average values for the unselected population. The dimensions of thorax are expressed as the log of the square of the length, to make the deviations more comparable with wing area; only females were studied. Since the data are in natural logs and the deviations are not too great, the values in Table 9 can be converted into approximate percentage differences by multiplying by 100.

Allowing for the likelihood that equivalent changes in body size are not expressed by precisely equivalent changes in the log of wing area and the square of thorax length, and also for minor fluctuations of temperature which, as noted earlier, affect wing more than they affect thorax size, the trend of the deviations in both selected strains leaves little doubt that changes in wing area which accompany changes in cell area reflect changes in general body size. At generation 5 eggs were set up from the selected and unselected strains in five replicates at 18°C, 25°C and 30°C; ten females per culture were scored. The effects of temperature will be considered later; at present we are concerned only with the dimensions of the strains reared at 25°C after a single generation of relaxed selection as shown

TABLE 9

Wing and thorax size in the Gabarros selection experiment

	Deviations from controls; in log units			
	Large cell strain		Small cell strain	
Generation	Wing	Thorax	Wing	Thorax
3	0.049	0.044	0.001	0.017
4	0.006	0.089	—0.012	0.070
5	0.033	—0.013	—0.071	—0.019
6	0.001	0.004	—0.041	—0.037
7	—0.002	—0.020	—0.115	—0.086

in Figure 5. Since the flies in this test were not raised along with the flies of generation 6, the two groups can be compared by reference to their deviations from the control level.

In the large cell strain, cell size was apparently unchanged after a generation without selection, but wing area increased. In the small cell strain wing and cell size reverted to the control level. There is complete correlation between the wing and cell size in the behavior of the small cell strain, whereas in the large cell strain the increase in wing size involves a shift in the cell size and number relations.

Since the genetic variation of cell size is made up of qualitatively different effects which contribute very unequally to the response in either direction, estimates of heritability derived from the ratio of response to cumulated selection differential might conceivably have some empirical value if merely variation in wing cell size alone were considered, but they would have scant biological significance. Perhaps a slightly more meaningful picture can be arrived at by confining such estimates to periods during which there is either little or no change in wing size or to periods when there is a high correlation between wing and cell size.

In the former situation, variation in the inverse relations between cell size and number predominates, whereas in the latter the physiological nature of the variation differs. Thus by reference to Figure 5, one might estimate heritability for the independent effects in generations 7 through 9 in the large cell strain, and for the first three generations in the other strain. Also if one assumes that selection for cell is essentially selection for wing size after this period, and is further prepared to relate the selection differential for cell to the response in wing size, a heritability estimate for the latter can be computed. Such estimates are admittedly very rough approximations; but they are summarized in Table 10.

At the end of the selection experiment the strain selected for small cell size was crossed to the unselected population, and the F_1 reared along with the two parent strains. The results shown in Table 11 are expressed as deviations from the average values recorded for the small cell strain. The last column refers to relative wing size i.e. log wing area minus log squared thorax length.

As noted earlier, the small cell strain is appreciably smaller than the unselected population; squared thorax length is some eight percent smaller. When crossed to the unselected stock, average thorax size of the F_1 coincides almost exactly with that of the unselected stock, so the difference in general body size created by selection for small cell sizes behaves as entirely recessive. Wing and cell area also increase in the F_1 beyond the level of the small cell strain but at first sight there

TABLE 10

Heritability estimates for wing and cell size in the Gabarros for selection

Strain	Dimensions	Generation interval	Response log units	Selection differential	Heritability
Large cell	Cell size	6–8	0.028	0.129	0.22
Small cell	Cell size	1–3	0.024	0.155	0.15
Small cell	Wing size	4–9	0.115	0.296	0.39

TABLE 11

The effects of crossing the small cell Gabarros strain to the unselected population: in log units

	Deviations from the small cell strain				
	Squared thorax length	Cell area	Cell no.	Wing area	Wing—thorax
Unselected	0.077	0.167	—0.044	0.123	0.046
Cross	0.078	0.116	—0.045	0.070	—0.008
Difference	0.001	0.051	0.011	0.053	0.054

is a discrepancy in that wing size, unlike thorax size, is not increased up to the control level. This can be explained by referring to the differences in relative wing size listed in the end column of Table 11. The cross and the selected strain have the same relative wing size which is less than that of the unselected stock. Furthermore, this discrepancy between the unselected flies and the F_1 can be associated with the relatively larger cell area of the former, since cell number works out at the same value for the F_1 and the unselected stock. Thus, in this cross relatively larger wing size, associated with correspondingly larger wing cells behaves as recessive. Apart from this difference in wing-thorax ratio, there has been a complete restoration of body size and cell number in the wing.

Thus selection for small cell size has involved a syndrome of effects which include general body size as well as the cell size and number relations in the wing. The recessive and other behavior recalls the parallel indications from the Pobla de Lillet strain selected for small cell size in which reduction in cell size affected wing size rather than merely the inverse relation between cell size and number. The evidence suggests, therefore, that selection for small cell size has uncovered rather important properties of the genetic variation of body size generally. This topic will be considered in more detail later.

As noted earlier, the various strains and also the cross between the selected strains were reared at 18°C, 25°C and 30°C after five generations of selection. The idea behind this test was as follows. Wing and thorax are highly correlated genetically and reflect variation in total mass. But the genetic correlation is incomplete and the wing-thorax ratio may differ. For example, disproportionate reduction of wing size has been reported in one line in which small wing size was selected for (ROBERTSON and REEVE 1952). In the survey of wild populations dealt with in Table 4 one stock—Gabarros—has relatively bigger wings than the others which are much alike, while we have just considered the case of the difference in wing-thorax ratio between the small cell strain and the control Gabarros stock. In all these instances the differences in relative wing size has been correlated with changes in cell size, just as the changes in wing-thorax ratio affected by rearing larvae at different temperatures are associated with changes of cell size. Nevertheless, the most notable property of the wing-thorax ratio is its comparative constancy within and between populations, under given conditions, as might be expected since there is likely to be an optimum relationship with respect to flying ability. Stability of the relationship between temperature during growth

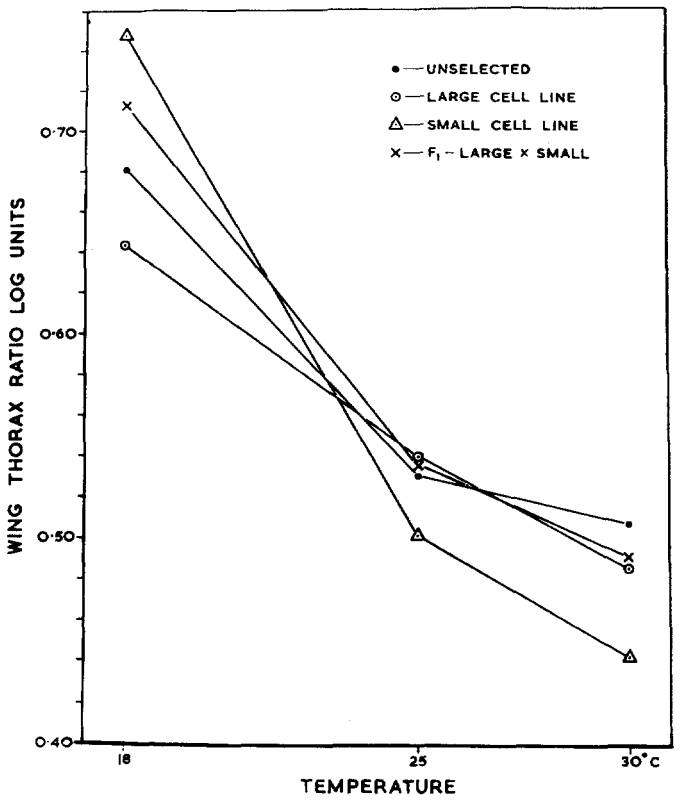

FIGURE 6.—Wing-thorax ratio and temperature.

and the wing-thorax ratio, presents an aspect of genetically controlled regulation of organ size and genotypes probably differ in this respect. Change in the cell size and number relations in the organ concerned, seems particularly likely to interfere with the processes concerned in maintaining the typical relationship.

This appears to be the case. Figure 6 shows the curves relating the wing-thorax ratio (log wing area means log squared thorax length) and temperature for selected lines and their F_1 as well as the unselected stock. The unselected and large cell strains are rather similar in response, whereas the small cell strain is clearly different, especially at 18°C at which temperature the wing-thorax ratio is considerably greater than in the unselected stock, although at 25°C it is lower indicating that the difference in ratio, noted at the end of the selection experiment had been established quite early. The cross is like the unselected and large strains at 25°C and 30°C, although at 18°C the ratio is shifted in favor of that characteristic of the small cell line. It appears therefore, that a shift in favor of large cells and fewer of them is compatible with the normal growth of the wing in relation to body size at different temperatures, whereas the reciprocal type of change is not.

DISCUSSION

As noted in the introductory statements only part of the available data have been dealt with in this paper, which will be followed by an account of what happens to cell size and number when body size is altered by different kinds of genetic change. Hence a complete discussion must await these additional results. However, the present data point to a number of inferences which can now be considered.

Since only part of the body has been studied, generalizations about changes in cell size and number in other parts of the body have to take account of the manner of growth peculiar to this organ. On embryological grounds it is reasonable to assume that adult size is determined by the time the larva pupates. But WADDINGTON (1940) and others have shown that considerable growth, including cell division, accompanies the process of differentiation of the wing and other exoskeletal parts in the pupal period. Although the particular kinds of change in cell size and number in the wing which accompany a given change of body size may also occur in other parts of the body derived from imaginal discs, there is no *à priori* reason why this should be so and only further work can settle this interesting point. This consideration, however, does not affect the value of changes of cell size and number in the wing as indicators of different kinds of genetic and environmental effect.

With respect to environmental variation which affects wing and body size there is a substantial increase in body size as the temperature during growth is lowered from 30°C to 18°C; between these limits wing area increases about 45 percent. As IMAI (1934) noted, body proportions do not remain unchanged at different temperatures; in particular wing area changes 2–2½ times the rate at which the square of thorax length changes. Such a well marked reaction probably has adaptive significance which might be sought in the relations between body mass, rate of wing beat, wing area and prevailing temperature. Comparison of the performance of a number of inbred lines and crosses showed that these considerable changes in wing area are apparently effected almost entirely by change of cell area. Clear-cut gene-environment interaction occurred in the degree of response to a given change of temperature. Although all genotypes followed the same general pattern of change via cell size, they differed in how much wing size was increased or decreased by the same change of temperature. As a result the size relations between say a pair of inbred lines and their F_1 may be drastically altered by changing the temperature a few degrees; and it was noted that such behavior would confer limited significance on formal estimates of dominance deviations derived from parent, F_1, F_2 and backcross data. There is some indication that crosses between inbred lines show a greater response to a decline of temperature from 25°C to 18°C, i.e., they increased in size proportionately more than their parents. At 30°C wing size was smaller than expected in some of the inbred lines, due to a reduction of cell number. Thus a shift from 25°C to 30°C reveals the greater resistance of crosses to extreme conditions while the change from 25°C to 18°C draws attention to their greater responsiveness to environ-

mental change; no doubt both features are ultimately attributable to their greater capacity for homeostatic adjustment during growth.

In the comparisons of the Gabarros strains selected for large and small cell size, thorax length was also recorded and it was possible to compare the wing-thorax relationship or relative wing size at different temperatures. The strain selected for large cells and the unselected strain were alike while the small strain differed, suggesting that the alteration of the cell size and number relations in favor of smaller cells and more of them may provide a more effective way of altering the processes which control the characteristic relationship between wing and general body size at different temperatures than the reciprocal type of change.

Variation in the chemical composition and quantity of food almost certainly accounts, apart from temperature fluctuation, for the greater part of the environmental variation to which *D. melanogaster* is customarily exposed. The interrelations between genotype, nutritional requirements and pattern of development may be explored by finding the effects on cell size and number of different, defined suboptimal diets. It is known (SANG 1956) that the reduction of specific nutrients below a certain level reduces body size and lengthens the period of larval development. Since the minimal requirements of such nutrients are not absolute, but are bound to vary according to the composition of the rest of the medium (BEGG and ROBERTSON 1950), an almost indefinitely large variety of alternative suboptimal diets could be tested. Casein deficiency almost certainly involves deficiency of at most a few essential amino acids. Although slow growth to small size can generally take place in the absence of RNA, indicating a limited ability to synthesize the constituent purines and pyrimidines (SANG 1957) additional RNA is generally necessary for normal growth. Choline is an essential nutrient; since its chief function is probably the provision of methyl groups, various processes of metabolism are probably affected by suboptimal levels. Thus, although shortages of any of these nutrients may lead to a similar reduction in body size, it is likely that different metabolic processes constitute limiting factors.

In spite of this fact, the general response to such different treatments on the part of individuals of the three populations tested is much the same, although the different populations are not identical in their response to the same treatments. There is a strong indication that adverse nutrition which reduces body size and hence wing size, does so by reducing the number of cells while cell area tends to be affected only with more extreme treatments. The same conclusion was drawn from the zero covariance between wing area and cell area with respect to within-culture variance of the crosses between inbred lines, while the nutritional differences between the two tests with the line N_2 were also associated with change of cell number; other unpublished observations have provided additional evidence of this behavior. Hence, provided nutritional variance is not too great, cell area appears comparatively stable.

Clear evidence of the regulation of body size when the larval diet is inadequate has been reported by SANG (1958). For example, a suboptimal supply of protein in the larval diet leads to a lengthening of the larval period, without reduction

of body size below the level attained on an adequate diet. But with further reduction of the protein level a point is reached where body size declines. Genotypes differ with respect to their ability to regulate body size on suboptimal diets (ROBERTSON, in press). It is likely that the tendency for cell size to remain constant in spite of nutritional variation is related to this capacity for regulation.

The contrast between the effects of temperature, which, apart from the special case of the inbred lines at 30°C, are associated with changing cell size and constant cell number and the effects of adverse nutrition, shows how different kinds of environmental change, which may lead to an equivalent difference of body size, may produce these effects in quite different ways. It follows that genetic changes which influence temperature sensitive processes or the ability to utilize particular diets may betray such difference in the cell size and number relations.

The genetic effects which have been studied under favorable conditions of nutrition at a constant temperature may be considered next. Estimates of the total genetic variance of cell size and number in wild populations from comparisons of the variance of genetically uniform crosses between inbred lines suggests that most of the individual variation is genetic in origin. It may be inferred that the variation of wing size is predominantly associated with variation of cell number, although there is an appreciable genetic correlation between wing and cell size as well. In addition there is a substantial contribution from effects which involve an inverse relation between cell size and number i.e. genetically different individuals of the same wing size may differ in cell size and number. Selection on three different populations showed that it was possible to shift the cell size and number, within limits, without affecting wing size.

It might at first seem rather odd that cell size in the wing should vary so much. It must be remembered that the cells are completely flattened so that cell "size" refers rather to surface area than to volume and hence different degrees of stretching would appear as variation of cell area. However, since there is a distinct association between the length of the little bristles carried by the cells and their area, it is rather improbable that such mechanical deformation is very important. The possibility of varying levels of polyteny cannot be ignored. Many insect tissues have highly polytene cells, and although it is generally accepted that imaginal disc nuclei are diploid (KURNICK and HERSKOWITZ 1952), it is conceivable that polyteny, as in the wing scales of Ephestia (HENKE and POHLY 1952) might arise during the pupal period of growth. DOBZHANSKY (1929) showed a high correlation between cell size and estimates of chromosome volume, in a series of genotypes with atypical chromosome number. Work with other animals, notably that of FANKHAUSER (1945) with amphibia, has shown that polyploid cells are larger but this does not affect final organ size due to regulation, which results in an inverse relation between cell size and number when diploid and polyploid types are compared. Apart from these general observations little can be said at present about the basis for the apparent differences in cell size, which will have to be studied biochemically.

The genetically determined processes which stabilize body size in a population

about a characteristic mean are such as to reconcile genetic variation with comparative phenotypic uniformity. The variation of the cell size and number relations probably demonstrates genetic differences in growth which are regulated to ensure stability of final size. It is to be expected that populations will differ in the degree of latitude in this respect as the selection experiments suggested. Thus in the Pacific population there was a striking shift in the cell size and number relations without any change of thorax or wing size. In the Pobla de Lillet population, selection for large cells lead to an inverse change in cell number, and only a slight decline in wing size, which may have been due to linkage or sampling, while in the Gabarros test, selection for large cells also indicated considerable scope for changing the cell size and number relations without affecting wing size. With selection for small cell size however, the picture is rather different, since in both the Pobla de Lillet and Gabarros tests, continued selection lead to a decline in wing size. It appears therefore that the scope for shifting the allocation of materials for growth in favor of smaller cells and more of them is very much less than the reciprocal change. It appears, therefore, that selection for small cell size provides a direct way of selecting gene combinations which destroy the conditions which favor the stability of body size. Strains selected for small cell size are likely to prove useful tools for the genetic study of stabilizing selection (SCHMALHAUSEN 1949) and of the regulation of body size generally; further work along these lines is in progress.

This inference is supported by the purely genetic evidence. Both in the Pobla de Lillet and, especially, in the Gabarros test, the smaller wing size which results from the selection of small cell size behaves in a recessive manner. In the latter case, the average thorax size of the unselected stock and of the F_1 of the cross between it and the small cell strain almost coincide. Previous experience of backcrossing strains selected for small body size to the unselected population (ROBERTSON and REEVE 1954) would lead us to expect a positive departure from intermediacy in the F_1, but this entirely recessive behavior is unprecedented. This suggests that selection for small body size on the one hand, and selection for small cell size on the other, involve different kinds of developmental change which are correlated with differences in genetic behavior. If the selection for small cell size has led to gene arrays which undermine the stability of body size, it is not surprising that such effects should behave as recessive, or perhaps, to use a more exact term, hypostatic to the more normal gene arrays contributed by the unselected parents. Such behavior recalls the property of genetic differences which characterize lines long selected for small body size that have apparently reached fixation (ROBERTSON 1954, 1955). On the earlier evidence it was suggested that continued selection for small body size, eventually breaks up and discards epistatic combinations which favor more normal size in favor of hypostatic effects, thereby accelerating the progress to fixation. It is worth noting that the immediate return to normal size when selection was relaxed in the Gabarros small cell strain is not inconsistant with interaction between a number of genes, whose joint effect is dissipated by segregation. These considerations pose a query as to

the status of the genetic correlation between wing and cell size in the wild populations. This point will be taken up in the next paper of this series in the light of further evidence.

Thus the record of cell size and number in relation to organ and body size brings various dynamic aspects of growth within reach of experimental modification and genetic study. The inference that differences in genetic behavior may be associated with characteristic differences in development points to a more sophisticated analysis of genetic variation which will lead to a synthesis between the concepts of population and developmental genetics—two disciplines which are maintained in a state of unprofitable isolation.

SUMMARY

1. The effects of different kinds of genetic and environmental variation on wing size in *Drosophila melanogaster* have been studied in terms of the changes in cell size and number in the wing. Each wing cell carries a small bristle and so average bristle density in the wing membrane provides the estimate of cell size. Since wing and body size are highly correlated, wing area can be taken generally as a measure of body size. Hence records of wing and cell area, converted to a logarithmic scale, provide the basic data.

2. The higher the temperature during larval growth the smaller the wing and body size and such variation is expressed almost entirely by changes in cell area alone. This was demonstrated by rearing a number of inbred lines and crosses at 18°C, 25°C and 30°C. In addition, the following points were noted:

(a) In inbred lines cultured at 30°C there is often a disproportionate reduction of wing size due to fewer cells; the crosses are unaffected by the suboptimal temperature in this way.

(b) There is a tendency for crosses to show a greater range of cell and wing size between 25°C and 18°C than their inbred parents.

(c) Genotype differ in the relative effect on wing size of culturing larvae at two different temperatures and so the relation between F_1 and parent size may vary considerably according to the temperature during growth.

3. The effects of inadequate nutrition in reducing body size have been studied by rearing larvae of different wild stocks on chemically defined aseptic media, deficient in alternative essential nutrients. Provided the diet is not too unfavorable reduction of wing size is accompanied by change in cell number while cell size remains comparatively constant. But with more extreme conditions cell size is reduced as well.

4. Average thorax length, wing and cell area and cell number were determined on seven unrelated wild populations which differed in average size. Differences in cell number account almost entirely for the difference in wing size and between the populations.

5. The relative contribution of genetic segregation and within-culture environmental variance to the total phenotypic variance of wing size, cell size and cell number in several wild populations, was estimated by comparing the variance

components with those derived from a number of genetically uniform crosses between inbred lines. Almost 70 percent of the variance of wing, cell size and cell number is genetic in origin. In wild populations there is almost always a correlation between wing and cell area which accounts for a phenotypic regression of wing on cell area of about 0.2 in log terms. This is entirely genetic since the environmental covariance is zero and the genetic regression works out at 0.3. Although most of the genetic variance of wing and presumably body size is associated with variation in cell number, an appreciable fraction is associated with variation in cell size.

6. Genetically different individuals of the same wing size may differ in cell size and number. Variation in such inverse relations between cell size and number makes an important contribution to the total genetic variation of cell size, and is regarded as tangible evidence of genetic differences in development which are compatible with stability of final wing and body size.

7. Genetic variation in the cell size and number relations, apparently independently of wing size, has been clearly demonstrated by selection for large and small cell size in three different wild populations. Heritabilities ranged from about 0.2 to 0.5. It proved easier to shift the relations in favor of large cells and fewer of them than *vice versa*. It proved impossible to make a permanent increase in body size by selecting for larger cells whereas selection for small cell size lead to a decline.

8. The genetic effects responsible for small body size in a strain, selected for small cells, behaved as completely recessive or hypostatic in a backcross to the unselected stock. Such behavior contrasts sharply with the relatively more additive behavior in similar crosses when the small parent is the product of selection for small body size. Hence different criteria of selection may apparently lead to the same results—in terms of body size—but, in reality, may affect different developmental processes and involve different kinds of genetic behavior. It is suggested that selection for small cell size provides a direct way of breaking up epistatic combinations which normally ensure the stability of body size in the population.

9. The inference that differences in genetic behavior may be associated with characteristic differences in development points to more informative methods of analysing genetic variation and eventual synthesis of concepts derived from the fields of both population and developmental genetics.

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LITERATURE CITED

ALPATOV, W. W., 1930 Phenotypical variation in body and cell size of *Drosophila melanogaster*. Biol. Bull. **58**: 85–103.

BARIGOZZI, C., 1951 The influence of the Y chromosome on quantitative characters of *Drosophila melanogaster*. Heredity **5**: 415–432.

BEGG, M., and F. W. ROBERTSON, 1950 The nutritional requirements of *Drosophila melanogaster*. J. Exptl. Biol. **26**: 380–387.

BREHME, K. S., 1941 Development of the minute phenotype in *Drosophila melanogaster*. A comparative study of the growth of three minute mutants. J. Exptl. Zool. **88**(1): 135–160.

DOBZHANSKY, TH., 1929 The influence of the quantity and quality of the chromosomal material on the size of the cells in *Drosophila melanogaster*. Arch. Entwicklungsmech. Organ. **115**: 363–379.

FANKHAUSER, G., 1945 Maintenance of normal structure in heteroploid salamander larvae, through compensation of changes in cell size by adjustment in cell number and cell shape. J. Exptl. Zool. **100**: 445–455.

HENKE, K., and H. J. POHLEY, 1952 Differentielle Zellteilungen und Polyploidie bei der Schuppenbildung der Mehlmotte *Ephestia kuhniella*. Zeit. ges. Naturw. 7b, **2**: 67–79.

IMAI, T., 1934 The influence of temperature on variation and inheritance of bodily dimensions in *Drosophila melanogaster*. Arch. Entwicklungsmech. Organ. **128**: 634–660.

KURNICK, N. B., and I. H. HERSKOWITZ, 1952 The estimation of polyteny in Drosophila salivary gland nuclei based on determination of desoxyribonucleic acid content. Jour. Cellular Comp. Physiol. **39**: 281–299.

REEVE, E. C. R., and F. W. ROBERTSON, 1953 Studies in quantitative inheritance. II. Analysis of a strain of *Drosophila melanogaster* selected for long wings. J. Genet. **51**: 276–316.

1954 Studies in quantitative inheritance. VI. Sternite chaeta number: a metameric quantitative character. Z. Ind. Abst. Vererb. **86**: 269–288.

ROBERTSON, F. W., 1954 Studies in quantitative inheritance. V. Chromosome analyses of crosses between selected and unselected lines of different body size in *Drosophila melanogaster*. J. Genet. **52**: 494–515.

1955 Selection response and the properties of genetic variation. Cold Spring Harbor Symposia Quant. Biol. **20**: 166–177.

ROBERTSON, F. W., and E. C. R. REEVE, 1952 Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in *Drosophila melanogaster*. J. Genet. **50**: 416–448.

1954 Studies in quantitative inheritance. VII. Crosses between strains of different body size in *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **86**: 424–438.

ROBERTSON, F. W., 1959 Gene-environment interaction in relation to the nutrition and growth of Drosophila. pp. 89–98. *Biological Contributions*. Edited by M. R. WHEELER. Univ. Texas Publ. **5914**.

SANG, J. H., 1956 The quantitative nutritional requirements of *Drosophila melanogaster*. J. Exptl. Biol. **33**: 45–72.

1957 Utilization of dietary purines and pyrimidines by *Drosophila melanogaster*. Proc. Roy. Soc. Edinburgh. B. **66**: 339–359.

1958 Circumstances affecting the nutritional requirements of *Drosophila melanogaster*. Ann. N.Y. Acad. Sci. **7**: 352–365.

SCHATZ, E., 1951 Über die Formbildung der Flügel bei Hitxmodifikationen und Mutationen von *Drosophila melanogaster*. Biol. Bull. **70**: 305–353.

SCHMALHAUSEN, I. I., 1949 *Factors of Evolution*. The Blakiston Co. Philadelphia.

SMITH, J. M., and S. M. SMITH, 1954 Genetics and cytology of *Drosophila subobscura*. VIII. Heterozygosity, viability and rate of development. J. Genet. **52**: 152–164.

THODAY, J. M., 1955 Balance, heterozygosity and developmental stability. Cold Spring Harbor Symposia Quant. Biol. **20**: 318–326.

WADDINGTON, C. H., 1940 The genetic control of wing development in Drosophila. J. Genet. **41**: 75–139.

ZARAPKIN, S. R., 1934 Analyse der genotypisch und durch Aussenfaktoren Bedingten Grössenunterscheide bei *Drosophila funebris* II. Verhältnis zwischen Körpergrösse und Zellenzahl Z. Ind. Abst. Vererb. **68**: 172–184.