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GENETICAL CONTROL OF STABILITY IN DEVELOPMENT

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I. CANALISATION AND STABILISATION

CELLS and tissues may follow a variety of paths during the normal course of development and differentiation, even though they are the same or closely similar in genotype. These paths are, however, far from haphazard: as Waddington (1942) has emphasised, they are limited in number and sharply distinct from one another. Intermediates are absent, so that development may be said to be canalised.

The choice of path from among the possibilities open to the cell may be determined immediately by a particular nuclear relation. Thus in dicœcious animals the decision between male and female development commonly depends on segregation of the X-Y difference, on whether the zygote is carrying XX or XY chromosomes. This genetical difference is sometimes large, involving whole chromosomes or even more than one chromosome; but in other cases it seems to be much more simple, consisting perhaps in little more than a single gene, if we may judge by the lack of cytological differentiation in mosquitoes (Gilchrist and Haldane, 1947), by the ease of transference of the switching function in fishes (Winge and Ditlevsen, 1947; Gordon, 1947), and by the ability of the YY type to survive and function in axolotls (Humphrey, 1942) and asparagus (Rick and Hanna, 1943). In a few species (see White, 1945), the course of sexual development is switched by external causes. Environmental switches of this kind are rare, most probably because they have been replaced by the more certain and more precise switching that genes can give (Waddington loc. cit.).

These are cases where the differences are seen in the development of complete individuals. The same canalisation is, however, observable in the differentiation of tissues within a single soma, and here there is no ground for regarding the switch into one channel or another as being due to any difference, even the smallest, in genotype. Rather the critical difference is to be sought in the cytoplasm which is changing serially under the control of a constant nuclear genotype, the earliest difference being in the cytoplasm of the egg cell, where it was laid down by the relation of the cell to maternal tissue, or to

such external forces as gravity (Mather 1948*a* and *b*). But whatever the critical difference, whether genetic or environmental as we see it in sex determination, or cytoplasmic as we see it in differentiation, the principle is the same. One of a limited number of courses will be followed in development, depending on a difference often so simple as to seem almost trivial. Indeed the difference may be demonstrably trivial under other circumstances, for the changeover of sex determination in fishes from one gene to another shows us that a genetic element whose segregation is critical in one strain may be without influence on sexual development in another strain of the same species. Nor do external forces have such an effect at later stages in development as they appear to have in determining the cytoplasmic arrangement of the egg in some species, though they can hardly have ceased to operate.

Thus, differences which are critical at one time or under one set of circumstances are ineffective on other occasions. This obviously suggests that the genotype is adjusted to confer on them their power of switching development, to build on an initial small difference in the sense that, given the initial difference, the genotype will determine a divergence. Experiment has confirmed that such is the case. Alteration of the genotype can lead to a digression in the course of the channel into which development is switched. Genotypes can be produced in which, for example, the X-Y difference no longer determines operative males versus females, or in which the normal course of tissue differentiation breaks down. The course of development, with its various alternatives no matter how they are switched, is inherent in the genotype : the outcome of development is successful, and the intermediates between the various channels are absent, because natural selection has adjusted the genotype to give just this result.

This regulation of canalisation cannot stop at building on the initial and critical differences : once the initial difference between the development of male and female or between tissues of the same soma has been laid down, later disturbances of kinds normally encountered appear unable to divert the development from its destined course. It can hardly be doubted, however, that disturbances must occur. The environment can never be without its action, which may well differ in different parts of a soma as well as vary from one individual to another. Furthermore, there must be chance upsets in the cells themselves, failures, for example, of the cytoplasm to partition equally in both bulk and content at cell division where it should be equal, or to achieve a precise inequality where such inequality is normal. Such disturbances must tend to be accumulative, yet they appear to have no serious effect on the course of development. It would thus appear that the genotype which can be adjusted to offer a series of alternative channels can also be adjusted to keep

these channels within narrow bounds ; that it can be adjusted not only to build up, where advantageous, the effects of initial differences within the cell, but also to suppress them where they would be harmful.

The manifestation of mutant genes affords some evidence of this stabilising power of the genotype (Waddington l.c.). The expression of, for example, the gene " eyeless " in *Drosophila* is much more variable both between individuals and between the two sides of a single individual than is that of its normal allelomorph. The genotype appears to have been adjusted by natural selection to give a relatively uniform development of eye size in the normal fly, but the stability vanishes once the course of development is changed by the introduction of the mutant gene. The old balance is no longer effective and a new adjustment of the genotype would be necessary to achieve a corresponding stability in the new circumstances of development. Such lower variation between normal individuals will in large part be due to reduction in expression of the differences which must generally exist between their genotypes ; but the lesser differences between the two eyes of the same normal fly must be ascribed to a better regulation of the individual's own development, for there is no ground for regarding the genotype as other than effectively uniform within a fly.

The study of asymmetry affords therefore a means of investigating the genotypic stabilisation of developmental processes. Eye size is not, however, a convenient character to study metrically, because irregularity in shape can make the area difficult to measure directly and the high number of facets makes their counting troublesome. The number of sternopleural chaetæ in *Drosophila melanogaster* affords a much more convenient character. These chaetæ, lying between the fore and mid legs, are readily counted ; they normally number between 8 and 12 on each side ; they commonly show a measure of asymmetry ; they are known to be subject to heritable variation ; and their number is not so greatly upset by external conditions, such as crowding in the culture bottle, as are features like overall bodily dimensions. The present study has therefore been made on this character.

2. INBREEDING EXPERIMENTS

(a) Asymmetry as a reflection of instability

If the developmental paths are intrinsically the same on the two sides of the individual (implying, of course, not only that the genotype is the same, but that the seriation of cytoplasmic changes in differentiation is the same, and that there is no consistent difference in the action of external agencies) the expression of the character will be symmetrical, barring local upsets. Any asymmetry which may appear will be an expression of such local disturbances, whether arising from differences of environment or upsets of cell development ; but since

such disturbances (if they are fairly and wholly to be regarded as chance disturbances and not as, at least up to a point, regular features of development) should have equal chances of affecting each side, the average expression of the character should be symmetrical, even though any given individual may differ bilaterally. In other words, if we plot the frequency distribution of the chance difference between the sides, excess on one being arbitrarily taken as positive and on the other as negative, it should be symmetrical about a mean of zero within the limits of sampling variation. Where all the individuals contributing to the distribution are of the same genotype, the spread of the distribution, measured by its variance, will depend on the frequency and magnitude of the local upsets, and also on the capacity of that genotype to stabilise development in spite of upset. Thus given comparable circumstances the capacity of two genotypes for coping with upset may be compared through the variances of their distributions of differences between the two sides. Where the frequency distribution is obtained from individuals whose genotypes are not uniform, the variance may also reflect genetical differences, but it may still be taken as measuring the stabilising power of some average genotype in the sense that difference between the variances given by two populations raised under similar conditions would indicate a disparity in the ability of their overall genotypes to cope with disturbances in development.

Even where the average expressions of the character on the two sides were not exactly equal, so that there was a slight bias in favour of one of them or in other words a slight consistent differentiation between the sides in development, the variance of the distribution of differences between the sides would still, when corrected for the departure of the mean from zero, be a measure of stabilising power of the genotype. But if the averages of the sides were very different, as is the case with the expression of polydactyly in poultry (Fisher, 1935), the variance might well become a complex and unreliable measure of stabilising power, because developmental processes on the two sides must then be, for one reason or another, intrinsically different and so might react differently to upsets of similar cause.

Our first step must therefore be to check on bias between the sides. Given an absence or near absence of bias, we can take the variance of the bilateral difference as a measure of stabilising capacity, provided too that we are satisfied that different genotypes are compared over similar ranges of circumstances.

With these considerations in mind the experiments were begun using two long inbred stocks, Oregon (O) and Samarkand (S). Cultures of the two stocks, their reciprocal F_1s, and F_2s from these F_1s were raised on a number of occasions, a single pair being used as the parents of each culture and up to four cultures of a kind being produced on any one occasion. Though genetical variation is known

to exist in the O stock, and probably exists in S as well, its amount is so small that the stocks may be regarded as homogenic without serious error. The F_1s may therefore be taken as homogenic apart from differences between the reciprocals in the sex chromosomes of their males. The F_2s cannot of course be homogenic, but again apart from the sex chromosome differences of the two reciprocals, the various cultures will be genotypically comparable.

The numbers of sternopleural chaetæ were counted on each side of 20 males and 20 females in each of 25 cultures of the parent stocks (1000 flies from each stock), in each of 25 cultures from the two reciprocal F_1s (1000 flies from each reciprocal) and in each of 20 cultures from the two reciprocal F_2s (800 flies from each reciprocal). The number of chaeta on the right side (R) was subtracted from that on the left side (L) for each fly, a positive value thus indicating excess on the left and a negative value excess on the right.

The mean L—R difference should be zero if no bias of development is present. The average of L—R is given for the two series of parents, F_1s and F_2s in table 1, together with the average gross numbers of chaetæ, *i.e.* the average of L+R.

TABLE 1

Average values of L—R and L+R in parents, F_1s and F_2s

Generation	Bias (L—R)		Gross numbers of chaetæ (L+R)	
	Females	Males	Females	Males
Parents O	0·05	0·12	18·78	18·67
Parents S	0·08	0·16	19·76	19·62
F_1s O×S	0·05	0·16	19·44	18·93
F_1s S×O	0·03	0·08	19·35	18·90
F_2s O×S	−0·01	0·03	18·95	18·84
F_2s S×O	−0·11	0·02	19·24	18·89

The excess of L over R is significant in O, S and the two F_1s, but not in the F_2s where indeed R exceeds L on the average in females. It thus appears that there may be a bias in flies of a particular kind, but that this varies with the genotype and is in any case small. Taking flies of later generations (described below) also into account, the average bias was found to be 0·0303 of a chaeta over 8800 females, and 0·0926 over 8800 males, or 0·0615 over 17,600 flies of both sexes. Even in males this is only about 1 per cent. of the average number of chaetæ on a single side or 0·5 per cent. of the average number for the two sides taken together.

It is of interest to notice that the sex difference is consistent, not only in the generations of table 1, but also in the later generations.

The mean value of L—R in males exceeds that in females by 0·0623 over the whole 17,600 flies. For some reason males tend to a greater extent than females to produce slightly more chaetæ on L than on R. The higher bias of males is not the consequence of a higher gross number of chaetæ, for this is actually smaller than in females (table 1).

The average value of L—R also varies significantly between cultures of parents, F_1s and F_2s, and the variation is greater between cultures raised on different occasions than between those raised at the same time. Here again, however, the contribution of differences between cultures is small, being less than 2 per cent. of the variation within a single culture, even when measured between cultures raised on different occasions. Variation between cultures raised on the same occasion is barely significant ($P = 0{\cdot}049$). There is no good evidence of differences between the two parents, or between the reciprocals in F_1 and F_2, in the bias they show. Nor is there evidence of interaction in effect of generations, sex and culture.

The bilateral bias thus varies with sex, genotype and culture, but is always small. It can hardly be taken as indicating differences between the inherent developmental paths of the two sides sufficiently large to invalidate our use of the frequency distribution of L—R as a basis for comparing the stabilising capacities of the genotypes. The variances of the distributions of L—R differences given by the various cultures have, however, been corrected for the bias in nearly all later calculations in this Section (those of 2(*d*) being the exception), *i.e.* the variance of the distribution of L—R in each culture has been taken round the mean of L—R and not round zero.

(*b*) *Oregon, Samarkand and their F_1s and F_2s*

The variances of L—R are set out for the parents, F_1s and F_2s in table 2, males and females being given separately. Since there were 25 cultures of each parent and F_1, with 20 flies of each sex counted, each of these sex variances is based on $25 \times 19 = 475$ degrees of freedom (N). The pooled variance (V) of each parent and each reciprocal F_1 will thus be based on $N = 950$ degrees of freedom, and V for both reciprocal F_1s taken together on $N = 1900$. With 20 cultures of each F_2 V for the separate sexes will be based on $N = 380$, the pooled V for each of the F_2s on $N = 760$, and V for both F_2s together on $N = 1520$.

In the cases of parents and F_1s, estimates of V are available from 25 cultures, and from 20 in the F_2s. These 25 or 20 estimates may be tested for agreement with others by the method described by Stevens in the appendix to a paper by Fabergé (1936). In effect, this method compares the sum of squares of the 25 (or 20) estimates of V calculated round the overall mean estimate ($\bar{V}$), with the expected variance of V, found as $2\bar{V}^2$, to give a χ^2 for $N = 24$ (or 19). In no case was there any indication of heterogeneity of the estimates

of V from the individual cultures. Indeed summing the χ^2s over sexes and generations we find a value of 212·45 for N = 268 (=8×24+4×19). The variation between cultures is thus sub-normal if anything. The differences in conditions from one culture bottle to another have no detectable effect on the variation in asymmetry, even though they have a small effect on the bias. This finding is important in two ways. It tells us that conditions are sufficiently alike from one bottle to another to permit simple comparisons of the properties of genotypes grown in different cultures; and it suggests that the environmental component in asymmetry is not large, so that such asymmetry as is seen very likely springs in a large measure, not from local differences in the environment, but from chance cellular upsets in development.

Given that simple comparisons between genotypes raised in different cultures are legitimate, we may consider the implications of the figures in table 2. The most striking feature is provided by

TABLE 2

Variances of L—R (V) in parents, F_1s and F_2s

V in	Parents		F_1		F_2	
	O	S	O×S	S×O	O×S	S×O
Females . . .	2·0976	2·3854	1·8305	1·7751	1·4891	1·7108
Males . . .	2·0916	2·3116	1·8151	1·9247	1·7967	1·7405
Sexes pooled . .	2·0946	2·3485	1·8228	1·8499	1·6429	1·7257
	2·2216		1·8363		1·6843	

the differences between the generations. The Vs of the parent lines O and S can be compared by the method of Fisher (1950, p. 227). Their difference is not quite significant, having P just below 10 per cent. The pooled value of 1·8363 for the two F_1s taken together falls short of V for the lower parent (O) with a probability of between 3 and 4 per cent. The pooled value for both F_2s falls short of the F_1 V with a probability of between 7 and 8 per cent. Though extremely suggestive none of these comparisons is unambiguously significant in itself; but if we compare parents, F_1s and F_2s in a single test we find $\chi^2 = 36{\cdot}23$ for N = 2, even where the two parents are pooled. There can thus be no doubt that the different generations differ in their Vs, and there is reasonable presumptive evidence that O and S also differ in this respect. Evidently different genotypes give different degrees of stability in development. The further implications of these comparisons will be discussed in a later section.

It will be seen from table 2 that males and females are alike in V in the two parents, O being lower than S. The sexes do not appear to agree so well in the F_1 of S×O and the F_2 of O×S, though agreement in F_1(O×S) and F_2(S×O) is as good as in the parents. This suggests that the X chromosome of O carries one or more dominant genes for lower variance, or greater stability to put it in developmental terms. The action of such a gene or genes would result (i) in the males of F_1(S×O) having a higher V than either the males of the reciprocal F_1 or the females of both reciprocals, these females and the O×S males being alike in V ; and (ii) in the females of F_2(O×S) having a lower V than the females of F_2(S×O) or both sets of F_2 males, these F_2(S×O) females and both sets of males being alike in V.

Qualitatively these expectations are fully realised by the data, but when statistical tests are applied it is found that though higher than both sets of F_1 females and F_1(O×S) males, the F_1(S×O) males do not exceed them significantly in V ($\chi^2_{[1]} = 0{\cdot}73$) ; and equally that although lower than both sets of F_2 males and the F_2(S×O) females, the F_2(O×S) females fall short of them in V to an extent which is barely significant even when it is taken into account that the deviation is in the predicted direction ($\chi^2_{[1]} = 3{\cdot}40$). Thus, although suggesting, perhaps strongly, that the X chromosome of O carries a dominant gene or genes increasing the stability of development, the data cannot be taken as conclusively demonstrating this point.

Be the position in regard to sex linkage as it may, there can be no doubt that the variances of parents, F_1 and F_2 differ, so that the parent lines O and S must differ in genes affecting the stability of development. These genes should be segregating in the F_2s, but none of the tests so far described could reveal such segregation, for the F_2s have had to be considered as wholes, and, though not generally uniform like parents and F_1s, all the cultures of F_2 must have comparable ranges at genotypes. Later generations from the crosses, however, make possible tests of segregation, as we shall now see.

(c) *Later generations from the crosses*

Breeding was continued from the F_2s in two experiments made at different times. These experiments followed the same essential pattern : generations up to F_{10} were raised, all matings being of single pairs throughout. Each experiment traced back to four F_1 cultures, two of each reciprocal cross, made simultaneously. Two F_2s were raised from each F_1, *i.e.* four F_2s from each reciprocal, or eight in all. Two F_3s were next raised from each F_2 culture, making sixteen in all. In the first experiment this doubling process was continued for one further generation to give thirty-two F_4s, a pair from each F_3. Only one of each twin pair of F_4 cultures was, however, used to give parents for F_5, two F_5 cultures being set up from each of the sixteen F_4s so taken. There were thus thirty-two F_5s and the doubling

process had stopped. Sixteen F_5 cultures, one from each twin pair, were similarly used to give parents for F_6, two F_6s being made from each of these F_5s, and the process was repeated to F_{10}. The flies were taken as parents of the next generation without conscious selection : the first two females and the first two males to be counted were used. Nor was any conscious selection exercised in taking one of a pair of cultures to give parents of the next generation. As already stated, all matings were of a single pair, and the members of a pair were always full sibs. The matings being biparental, the generations were not F_3, F_4, etc., in the strict mendelian sense (see Mather, 1949), but this notation will be retained for convenience.

The second experiment was like the first in structure, but the doubling process stopped a generation earlier so that there were only sixteen F_4s, two from each of eight of the F_3s. In both experiments the reciprocal crosses contributed equally to the cultures of the various generations, at least in the early stages. When, however, failure occurred in both of a pair of cultures raised from the same parent culture of the previous generation, the lineage was allowed to lapse. Two lineages had lapsed by F_{10} in the eight descendants from each reciprocal cross in the first experiment, and one of the four from each reciprocal in the second experiment. More frequently, however, one culture of a pair failed, the two cultures for the next generation being then raised from the survivor. Twenty flies of each sex were counted from each culture and only cultures yielding this minimum were used. No weighting has been employed in the analysis to accommodate lost cultures or lineages. The numbers of cultures available in each generation are shown in table 3.

TABLE 3

Numbers of cultures in each generation of the two experiments. The descendants of the reciprocal crosses are shown separately

Generation	1	2
F_3	8+8	8+8
F_4	13+13	8+8
F_5	12+14	8+7
F_6	13+14	8+7
F_7	13+12	6+8
F_8	14+14	6+7
F_9	13+12	6+5
F_{10}	7+12	5+4

The various cultures of F_2 will contain comparable ranges of genotypes and so will not be expected to differ for genetical reasons in the estimates they give of V. This will no longer be true in F_3, since the genetical differences of the parents taken from F_2 will be reflected in the genotypes present in the F_3 progenies. We must

therefore expect the variation in V between cultures to be greater than random if bilateral stability is under genic control. The members of each twin pair of F_3 cultures will be genetically no more alike on the average than members of different pairs; but in F_4 and later generations this will no longer be true. Genetically produced differences in V should on the average be less between cultures of the same pair than between different pairs, and while under the breeding system used differences between pairs (*i.e.* between lineages) should increase with the generations, those within pairs should decrease. Finally, there should be a genetical correlation of offspring cultures with parent cultures from F_3/F_4 onwards if genic segregation is occurring. These experiments provide therefore a variety of tests of our conclusion that O and S differ in genes affecting developmental stability. The tests may not be intrinsically sensitive, as the genetical variation will be confounded with the high sampling variation of V, but the observations available should suffice to give some sign of genic segregation if it is taking place.

The values of V are given separately for the sexes in each generation in table 4. Data from the two experiments have been combined because their results proved to be homogeneous over the complete range of generations. It will be seen that V is lower in females than in males in every generation, and while the difference is significant in no single generation, it can hardly be doubted over the data as a whole. This difference is not an intrinsic property of the sexes as we can see from the parent stocks, but it is of course just as might be expected if sex-linked genes of the kind we have already come to suspect were segregating. It appeared that the sex-linked genes (assuming them to exist) favouring a lower variance were dominant, so that the males, which show the effect of sex-linked recessives more commonly than do females, should have a higher average V. The magnitude of the sex differences in F_3—F_{10} is compatible with the evidence from F_1 and F_2. The later generations thus add to the evidence which F_1 and F_2 provided for the existence of sex-linked differences between O and S. There is no evidence that the sex difference varies between cultures, experiments, crosses or generations ($\chi^2_{[269]} = 278{\cdot}000$, $P = 0{\cdot}35$), such differences as might be expected being presumably so small as to be swamped in the joint tests.

The sex-linked genes might also express themselves in another way. The cross O×S would bring in two dominants for low V from the mother to one recessive for high V from the father, while S×O would bring in two recessives to one dominant. There should thus be a higher proportion of recessive genotypes and hence a higher average V in the later generations of S×O than of O×S. This is indeed the case over all but two of the generations (table 4) and the results taken together strongly suggest that the offspring of S×O have somewhat higher V's than do those of O×S, so adding further

to the evidence for sex-linked effects. The difference in average V between the reciprocals is slightly smaller than that between the sexes, so that the two comparisons agree sufficiently well. An item for the difference between reciprocals has been taken out of all the analyses, so that the comparison between cultures should not be distorted by the reciprocal difference.

The pooled or average values of V are also shown for each generation in table 4. Since these involve the sexes and reciprocals equally (or very nearly so) in each generation, they may be fairly used for comparison. The values of $\bar{V}$ from table 4, together with

TABLE 4

Variances of the sexes and of the reciprocals

Generation	Females	Males	O×S	S×O	Pooled
F_3 . .	1·92	2·16	1·91	2·17	2·0389
F_4 . .	1·83	2·05	1·86	2·03	1·9436
F_5 . .	1·96	2·28	2·01	2·22	2·1212
F_6 . .	1·86	2·10	1·99	1·97	1·9789
F_7 . .	1·98	1·99	1·80	2·17	1·9852
F_8 . .	1·98	1·99	1·87	2·10	1·9852
F_9 . .	1·72	1·99	1·77	1·95	1·8561
F_{10} . .	1·84	2·01	1·95	1·91	1·9285
Average .	1·89	2·07	1·89	2·06	1·9797

those for O, S, F_1 and F_2 from table 2, are plotted in fig. 1. It will be seen that $\bar{V}$ rises under inbreeding until F_5, by which generation it has progressed a long way back towards the mid-parent, which it would be expected to approach asymptotically. After F_5, however, $\bar{V}$ falls and seems to become more or less stable a little above the F_1 value. This behaviour suggests the intervention of selective forces favouring a greater stability, and hence lower $\bar{V}$, than is shown even by the O parent. The failure of cultures and even lineages recorded in table 3 would appear to offer the opportunity for selective changes in $\bar{V}$ and one can hardly doubt that competition within culture bottles must also have its effects in maintaining heterozygosity at a higher rate than expected in the absence of selection. It is therefore not unreasonable to ascribe this unusual behaviour under inbreeding to the action of the "natural" selection which is to be expected in experiments such as the present ones. Whether the continuation of the inbreeding lines beyond F_{10} for a number of generations more comparable to the hundreds which O and S have undergone, would have resulted eventually in values of $\bar{V}$ more comparable to those of the parents must remain a matter for surmise.

The pooled values of $\bar{V}$ in table 4 supply us also with the estimates of sampling variation needed for the analysis of the differences in V

between cultures. This analysis has been undertaken by Steven's method. V is used as the variate and subjected to an analysis of variance, items being taken out for the sex difference and its interactions, and for the differences between experiments and reciprocals and their interaction, which comparisons have already been considered. The sum of squares remaining is for differences between cultures, and after F_3 this can be partitioned further into items for differences

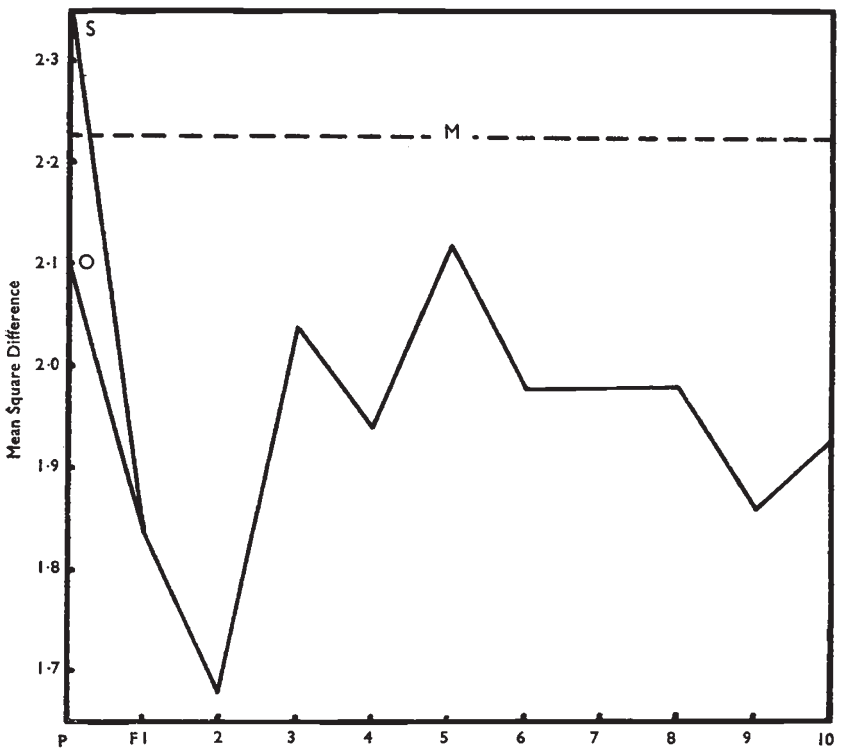

FIG. 1.—The mean square difference ($\bar{V}$) between the numbers of sternopleural chaetæ on the two sides of the flies in the parental lines, their crosses and the generations of the inbreeding experiments. The values shown are averaged over sexes, reciprocals and experiments. M is the mid-parent value, *i.e.* the average of the two parents, O and S.

between the twin pairs of cultures and for differences within these pairs. The sum of squares so obtained is divided by twice the square of $\bar{V}$ as estimated by pooling variation within cultures of the generation in question (*i.e.* $2\bar{V}^2$), and treated as χ^2s to give tests of significance of the culture differences. Since, however, the sum of squares rise with the numbers of degrees of freedom, the χ^2 values do not of themselves offer a simple means of assessing the size of the culture differences. Each χ^2 has therefore been divided by N, the number of degrees of freedom. When based on a reasonable number of degrees of freedom, the value so obtained will be close to 1 when the culture differences

are of the same size as the sampling variance. It will fall below 1 where culture differences are subnormal and rise above it where culture differences are greater than sampling variance would lead one to expect.

In the parents this measure of culture variation is about 1, and in F_1 and F_2 it falls below 1 (though not significantly) so that, as we have already observed, there is no evidence of culture differences exceeding the sampling variation expected on the basis of the variance within cultures, in the generations where comparison is between cultures of comparable genotypes. In F_3 and later generations on the other hand we should expect the measure of variation to exceed 1, as genic segregation should lead to the cultures being no longer comparable in genotype. Furthermore, the measure of variation for comparisons between twin pairs should tend to rise, though not very rapidly, as the genetic differences between pairs will tend to increase under the mating systems used, while variation within twin pairs should fall as inbreeding progresses.

The results of the analyses for all generations including parents, are given, in terms of our measure of variation, in table 5 and plotted in fig. 2.

TABLE 5

Variation between cultures, as measured when the sampling variation ($2\overline{V}^2$) *relevant to each generation is taken as unity. The variation in the sex difference* (i.e. *culture* × *sex interaction*) *is included for comparison. The number of degrees of freedom is shown in brackets*

Generation	Variation between cultures		Variation of sex difference
P	0·94 (48)		1·08 (48)
F_1	0·67 (48)		0·58 (48)
F_2	0·60 (38)		0.86 (38)
F_3	1·57 (28)		1·22 (28)
	Between pairs	Within pairs	
F_4	1·54 (20)	1·60 (18)	0·85 (38)
F_5	1·58 (18)	1·29 (19)	1·25 (37)
F_6	1·10 (18)	1·12 (20)	1·22 (38)
F_7	1·37 (18)	1·10 (17)	0·92 (35)
F_8	1·79 (17)	1·82 (20)	0·99 (37)
F_9	1·24 (16)	0·63 (16)	0·75 (32)
F_{10}	1·87 (13)	0·70 (11)	1·10 (24)

The variation in the sex difference of V between cultures is included in table 5 for comparison. This sex-culture interaction is not significant, so that its value oscillates round 1 in a random manner over the generations. It thus provides us with, so to speak, a random frame of reference with which to compare the values for variation between cultures. These do not exceed sampling variation until F_3, where the variation rises to 1·57, which is itself significant at the 5 per cent.

level. Thereafter, all the measures of variation between cultures are above unity, except for the item within twin pairs in F_9 and F_{10}. As we have seen, the variation within twin pairs should fall with the generations from a value in F_4 about equal to that between pairs (an expectation borne out by the data) to something approaching 1 in the late generations. Apart from a suprisingly, and no doubt

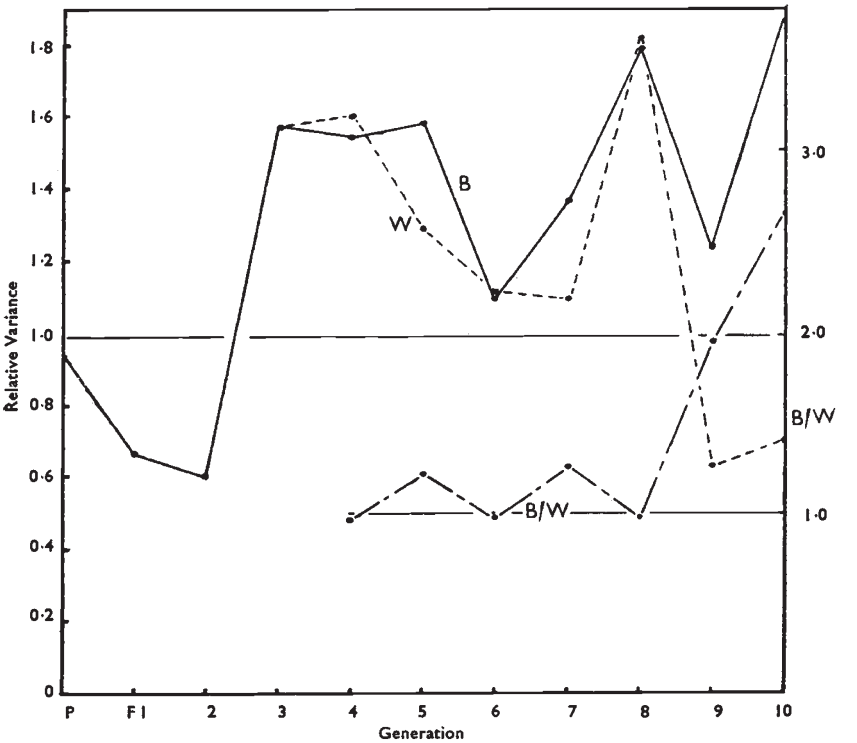

FIG. 2.—The relative variances between cultures ($2\bar{V}^2$ for the generation taken as unity) in the inbreeding experiments. After F_3 the relative variances are shown separately for comparisons between (B) and within (W) twin pairs of cultures. The ratio B/W is also plotted (scale on the right margin of the graph). Results are pooled over the two inbreeding experiments as these are homogeneous.

misleadingly, high value in F_8, the variation within pairs behaves as expected, the sub-normal values of F_9 and F_{10} being within the range of departure from 1 which the other data of the table, especially those from F_1 and F_2, show to be permissably ascribable to sampling variation.

The behaviour of the measure of variation between pairs is more erratic, but it certainly shows no steady fall and may indeed be regarded as compatible with the slow rise we should expect. Taken as a whole, the variation between cultures from F_3 on, is highly significant ($P<0{\cdot}0001$), so showing the expected contrast with the P, F_1 and F_2 generations. The mean value of the variation between

pairs in F_4—F_{10} (1·49) exceeds the mean within pairs (1·23), but the difference (though in the expected direction) is not fully significant. The ratio of the variation between, to that within pairs (B/W) should rise with the generations, as indeed it appears to do, even if a little erratically (fig. 2).

One further test of genic segregation remains to be applied, that of covariation between V in parent culture and offspring cultures. This can hardly be a sensitive test, for it must be obscured by the high sampling variation. Even so, however, taking the data of all generations together (*i.e.* pooling the covariances of F_4 on F_3, F_5 on F_4 and so on up to F_{10} on F_9), we find a positive regression of V in offspring culture on V in parent culture, the regression coefficient being 0·106. This is just significant at the 5 per cent. level when we take into account that it is positive as it must be if the correlation is genetic in origin. There is no evidence of any change in the value of the regression coefficient over the generations, but in view of the insensitivity of the test, we could not expect to detect such changes unless they were of a magnitude which could not readily be explained on genetical grounds.

Our many tests for the segregation of genes affecting the variance of the L—R difference have thus all been as successful as could be expected : our various expectations have been borne out reasonably well by the data. There can thus be little doubt of the existence of differences between the parent stocks, O and S, in respect of such genes, some of which furthermore appear to be sex-linked. The magnitude of the differences which segregation produces, seems however to be rather small. An inbreeding experiment, on the other hand, cannot be expected to tell us much about the magnitudes of differences which the extreme products of segregation can produce : response to selection should yield much more information. We shall therefore consider the effects of selection on V ; but before doing so we must examine a possible difficulty in the interpretation of the data presented so far, and one which is also relevant to the interpretation of results from selection experiments.

(d) The relation of asymmetry to chaeta number

So far we have assumed without discussion that V is a straightforward and adequate measure of stability. We know, however, that the number of sternopleural chaetæ (L+R) differs between the two lines O and S, and also varies among the families in the descendants of the cross. We must therefore ask whether change in L+R results in a direct developmental way in any corresponding change in V, and if so whether the relation so found would serve to explain the differences observed in V ; for if the changes in V merely reflected changes in the absolute number of chaetæ they could hardly be regarded at the same time as indicating genetic variation in the

control of stability as opposed to genetic variation in the magnitude of effect in development. We should expect that if such a relation exists, the higher the number of chaetæ, the more variable would be the manifestation, so that A = L+R and V would be positively correlated.

A number of considerations suggest that whatever the relation between A and V, it is not such as to make V interpretable wholly in terms of total chaeta number; that in fact the changes we see in V must be related at least in part to variation in the control of stability as opposed to magnitude of effect. In the first place, males

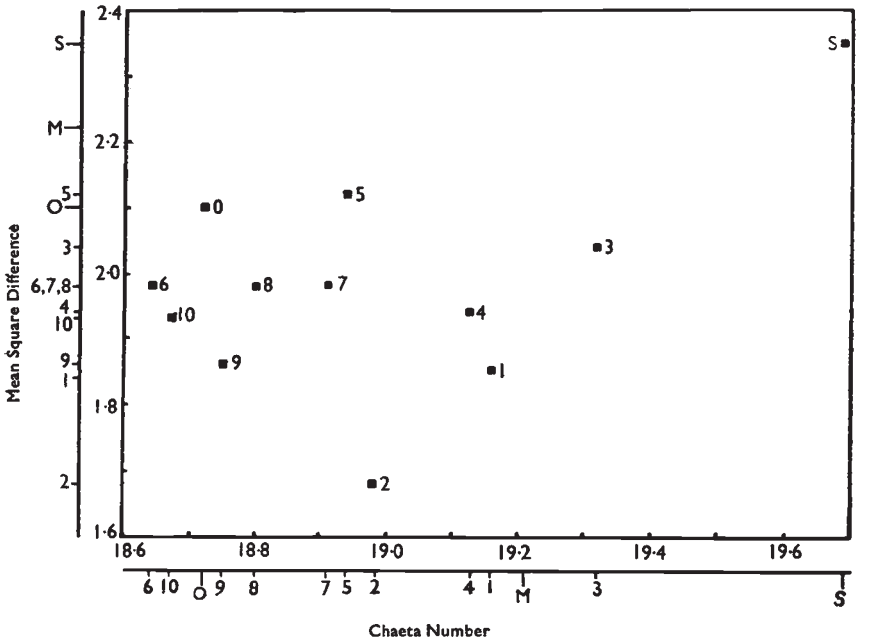

FIG. 3.—The relation of mean square difference (V) to gross chaeta number (A) for the different generations of the inbreeding experiments. The generation values of V are projected onto a single line on the left of the diagram and those of A onto a line at the bottom. M is the mid-parent value obtained by averaging the parents, O and S.

and females of O and S show similar values of V but differ in A; and at the same time A varies between cultures for environmental reasons, while V is homogeneous over cultures. The two inbreeding experiments were, as we have seen, homogeneous for the values they gave for V generation by generation, yet the second experiment regularly gave values of A about half a chaeta lower than did the first, possibly as a result of environmental differences. Furthermore, when we compare the mean values of A and V for the two stocks and for the various generations derived from their cross, we find little evidence of any simple relation between them (fig. 3).

The question has, however, been pursued further by finding the

regression of V on A within the stocks and their descendants and by making corresponding adjustments to the variation in V. All regressions were obtained by relating the value of V for a culture to the average value of A for that culture. If there is a correlation between A and V which is fixed by a developmental or scalar relation of the kind we are discussing, the regression so obtained should be equivalent to that resulting from comparisons within cultures. Characters under the control of at least partly separable genic (or environmental) systems could not, on the other hand, show such a fixed correlation. The regression found from comparisons within and between cultures would differ, and a genic correlation, even where linkage was involved, must vary as we move from the parents through the various generations of their crosses.

In estimating the regression of V on A, V has been calculated round a mean of zero for L—R. This departs from our procedure of earlier sections and, as we have already seen, is not completely justifiable, because the mean of L—R sometimes does depart a little from O. The departures of the means from O are so small, however, that the inaccuracy introduced thereby cannot be such as to invalidate our interpretation of the regression analyses.

TABLE 6

Regression of V on A

Parents	O	S	
	−0·04	0·75	
	O×S	S×O	Pooled
F_1	−0·02	0·07	0·02
F_2	0·42	0·00	0·19
	Expt. 1	Expt. 2	Pooled
F_3	0·41	0·32	0·35
F_4	0·22	0·34	0·27
F_5	0·28	0·28	0·28
F_6	0·32	−0·03	0·12
F_7	0·26	0·18	0·22
F_8	0·26	0·10	0·19
F_9	0·18	0·15	0·16
F_{10}	−0·11	0·21	0·06

Overall regression F_2-F_{10} = 0·20

The regressions of V on A are given in table 6, the values being shown separately for the two reciprocals in F_1 and F_2, and for the two experiments in F_3-F_{10}.

The most striking feature of the table is the variation in the regression of V on A. One parent, O, shows no evidence at all of

a relation, while the other, S, has a regression both highly significant and positive as would be expected. The difference between these two parental regressions is itself fully significant ($P = 0{\cdot}01\text{-}0{\cdot}001$). The regression in the case of S must be developmental, so that we are immediately faced with the situation that there are genetic differences in the developmental correlations themselves. Thus stability as measured by V can be related to absolute chaeta number, but it can also be divorced from it by genetic adjustment.

The reciprocal F_1s agree in showing little if any relation between V and A, being like the O parent in this respect. The reciprocal F_2s differ somewhat from one another, though not quite at a significant level ($P = 0{\cdot}07\text{-}0{\cdot}06$), one showing a relation of V and A, the other giving no evidence of any such relation. The later generations in both experiments show regressions intermediate between those of the parents. Taken by themselves, the regression in F_6 differs significantly at the 1 per cent. level between the two experiments, but this is the only such case and indeed if all the generations are considered together, the experiments agree perfectly well. No weight can therefore be given to the discrepancy in F_6, or, perhaps, to the difference between the reciprocal F_2s either. One trend is however clearer. There is a fall in the regression from F_3 to F_{10} which is significant at the 5 per cent. level at least. Such a fall in the relation between V and A would suggest that the correlation they show in these generations is at least in part due to linkages which become progressively resolved with the generations, and the absence of correlation in F_1 accords with this view. Certainly the relation between V and A revealed by these regressions is not the simple one that would be expected to invalidate the use of V as a measure of stability in development.

The effects of the regressions have, however, been investigated still further by adjusting the variation in V from culture to culture to allow for the relations observed. Using the culture means as the variants and taking all deviations from the generation mean, the sum of squares of V (*i.e.* $S(V^2)$) is corrected for the linear regression on A by subtracting $\frac{S^2(AV)}{S(A^2)}$ from it. One degree of freedom is used up in making the correction, which leaves a sum of squares measuring the variation in V unrelatable to the variation of A in that generation. The residual $S(V^2)$ can be divided by twice the square of the mean value of V in the generation ($2\bar{V}^2$) to give a χ^2 testing the heterogeneity of V over the cultures as described earlier. The value of $\bar{V}$ used in finding the divisor should itself be adjusted for relation to A, but this would involve calculating regressions inside the cultures and has not been done. The divisor may therefore be somewhat too high, so that the χ^2s may be too low, and the evidence for heterogeneity of V somewhat underestimated. The χ^2s have also been divided by

the corresponding numbers of degrees of freedom to give a measure of variation between cultures relative to $2\bar{V}^2$ taken as unity. The results of these tests are given in table 7. The two experiments have been pooled in the tests of F_3-F_{10}, and the two reciprocals in those of F_1-F_2, it having been shown by analyses of covariance that only F_4 and F_7 give differences between experiments verging on significance when the generations are taken individually, and that taking all generations together there is no indication of such differences.

TABLE 7

Differences in V between cultures after adjustment for regression on A

	χ^2	N	P	Relative variation between cultures
O . . .	21·79	23	0·55	0·95
S . . .	13·10	23	0·95	0·57
F_1 . . .	32·63	48	0·95	0·68
F_2 . . .	25·90	38	0·93	0·68
F_3 . . .	42·74	30	0·06	1·43
F_4 . . .	56·54	40	0·04	1·41
F_5 . . .	51·29	39	0·09	1·32
F_6 . . .	40·21	40	0·47	1·01
F_7 . . .	49·29	37	0·08	1·33
F_8 . . .	65·99	39	0·04	1·69
F_9 . . .	38·51	34	0·28	1·13
F_{10} . . .	37·82	26	0·06	1·46
F_3-F_{10} pooled .	382·39	285	0·001-0·0001	1·34

The picture given by this table is essentially the same as that obtained earlier when no allowance was made for any relation of V to A. There is no evidence of heterogeneity of the cultures in O, S, F_1 and F_2, where it is not expected, but good evidence in the generations from F_3 to F_{10}. Perhaps when taken individually, no one of these generations can be regarded as unambiguously revealing culture differences ; but all tend in the same direction, all but two have probabilities of less than 10 per cent., and when the χ^2s are pooled the significance is beyond dispute. The values shown in the last column for the relative variation between cultures are a little lower than those of table 5, in which relations between A and V are neglected. This is to be expected for, as we have seen, the divisor $2\bar{V}^2$ has not been adjusted for the regression. Even so, all generations from F_3 onwards show values greater than 1, while all earlier generations give values less than 1. Despite their underestimation, the differences between cultures show clearly in the generations where segregation would lead us to expect them, and are absent where segregation would not be effective.

Thus in large measure the variation in V cannot be referred to a

direct developmental relation with A : it can be altered independently of A, so that, in this sense at least, we have no reason to question the use of V as a measure of stability. We shall have further evidence of the separability of V and A from the results of selection, to which we must now turn.

3. SELECTION EXPERIMENTS

(a) The first experiment—Main lines

Both selection experiments commenced from crosses between the O and S stocks. The crosses were made reciprocally using single pairs of males and females. F_2s were raised from these F_1s, again using single pair matings. Selection began in F_2 (generation S-0), the two highest females being chosen out of twenty counted as mothers of the first high selected generation (S-1) and the two lowest females as mothers of the first low selected generation, and similarly with the males. The method of selecting parents was the same in later generations except of course that only high flies were taken in the high line and only low ones in the low line. Six F_2 cultures were used, four from $S \times O$ and two from $O \times S$.

The experiment was divided into two parts. In one, originating from one $S \times O$ and the $O \times S$ crosses, the high males and females from one F_2 culture were mated to give one high culture in S1, and similarly for the low line. This closed mating system was practised throughout, so that each line, high and low, comprised three separate sub-lines, each consisting of one culture per generation, with four parents, two of each sex. In the other part of the experiment, there were again three cultures in each line, high and low, in each generation with four parents per culture ; but the females taken as parents from culture A were mated to the males from B, the females from B to the males from C, and the females from C to the males from A. This system was varied after S-19 by reducing the number of cultures per line per generation reduced from three to two, but mating was still continued between cultures. This cyclical mating system will maintain a higher outbreeding and heterogeneity than the closed sub-line system, but the inbreeding of the inbred line will not of course itself be as great as if single pairs of full sibs had been used as parents.

After mating for a day or two in tubes, the parents were moved into the customary half-pint culture bottle and allowed to lay there for two days before removal. All counts were made on offspring from these bottles, except in a few cases where the first bottle having failed, flies were taken from a second culture in which the parents were placed after removal from the first bottle and in which they stayed for six or seven days.

A word is necessary about the rigour of selection. This was based

on the difference in sternopleural chaeta number between the left and right sides irrespective of sign. Sometimes, especially in the high lines, there was no doubt which flies to take as parents, two having higher differences than all others. Sometimes, however, one fly would have a greater difference than any other, to be followed by a group of several with the same next highest difference. One of this group would then be taken at random for use with the outstanding individual as joint parents. Where the highest difference was shown by a group of several flies, two were taken at random as parents. No cognisance was taken of the sign of the difference.

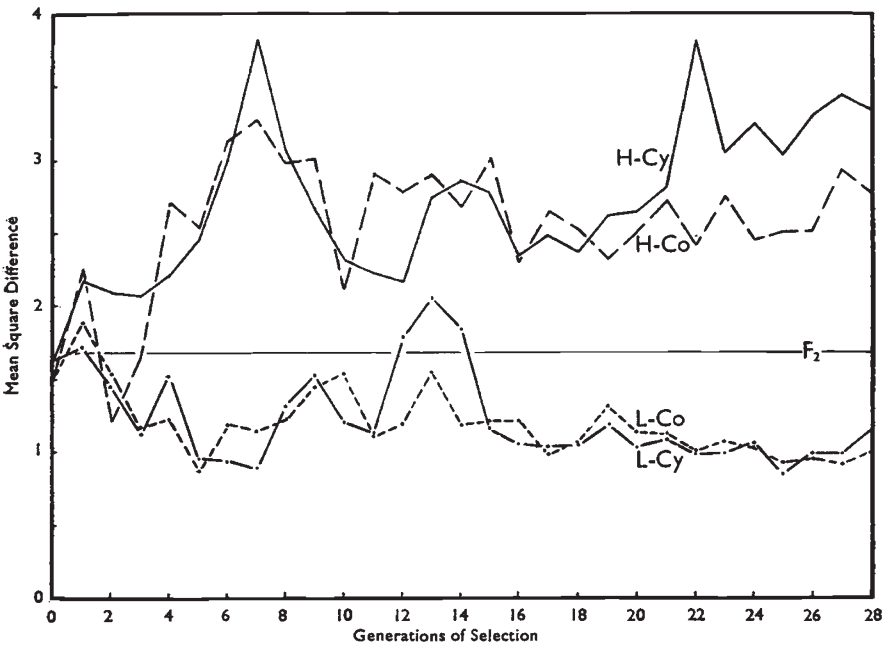

FIG. 4.—The changes in mean square difference between the sides (V) in the first selection experiment. F_2 is taken as generation S-0. H indicates lines selected for high difference and L those selected for low difference. Cy indicates the cyclical and Co the closed mating systems, as described in the text.

In the low line it was almost invariably the case that more than two flies showed the lowest possible difference, viz. equality on the two sides. Two were then taken at random for use as parents. As selection became effective in the low line, a rising proportion of flies showed no difference between the sides, so that the rigour of selection necessarily fell. No such consideration applies in the high line, of course.

The results of selection in both closed and cyclical lines are shown in fig. 4. The difference between the sides is measured by V taken about zero : no correction was made for the departure of the mean from O. Each point is the average of observations from three cultures,

except in the relatively few cases where one or, even more rarely, two of the three failed, and, of course, after S-19 in the cyclical lines, where only two cultures were raised.

Several features of the figure are obvious. There is clear evidence of response to both high and low selection in both closed and cyclical lines, so that there can be no doubt of the existence of heritable variation. The general behaviour of the lines also suggests polygenic variation, as will be discussed in more detail later. The response is less in the low than in the high lines, partly, doubtless, because of the falling rigour of selection in the low line, and partly also, one assumes, because there is in any case a limit to progress in a low line while there is, at least theoretically, no such limit on progress the other way. Even so the responses in the high line are surprisingly small; the root mean square difference advanced only from some 1·3 in F_2 to about 1·7 or 1·8 in the later generations. Progress was rapid at first but extremely slow later. Between S-9 and S-19, indeed, there is little evidence of change. After S-19, however, slow progress is clear. It is equal in the two low lines but is greater, perhaps not surprisingly, in the cyclical high line than in its closed counterpart. Circumstances unfortunately compelled the termination of the experiment at S-28, but if continuation had been possible, much larger differences might well have been produced in the cyclical high line.

The behaviour of all four lines is remarkable between S-6 and S-9. A peak of response is shown at S-7, or perhaps a little earlier in the low lines, only to be followed by a retrogression against selection shown by all four lines. The explanation is not obvious. The simultaneity of the four retrogressions suggests a common external influence, but since two lines retrogressed by falling and the others by rising, the change is not merely one of a simple stabilisation or increased fluctuation of the environment. Two possibilities suggest themselves. The high and low genotypes might respond differentially to a given environmental change. Though possible, this hardly seems likely, since the difference postulated would be one not merely of magnitude but of direction of response.

The other possibility depends on the correlated response that is often shown by fertility to selection for other characters. Fertility, as measured by the average number of flies hatching from the three culture bottles, fell sharply with the early responses to selection in all four lines (fig. 5). Now if, as was evidently the case in the selection lines of Mather and Harrison (1949), the chromosomes carrying the combination of genes of more extreme effect on the L—R difference also carried the combinations of genes causing greater reduction in fertility, any relaxation of selection would lead to retrogression of the chaeta character as the fertility character took charge (see Mather and Harrison). Should, therefore, the environment have become less stable in its effects on the chaeta difference between S-6 and S-9,

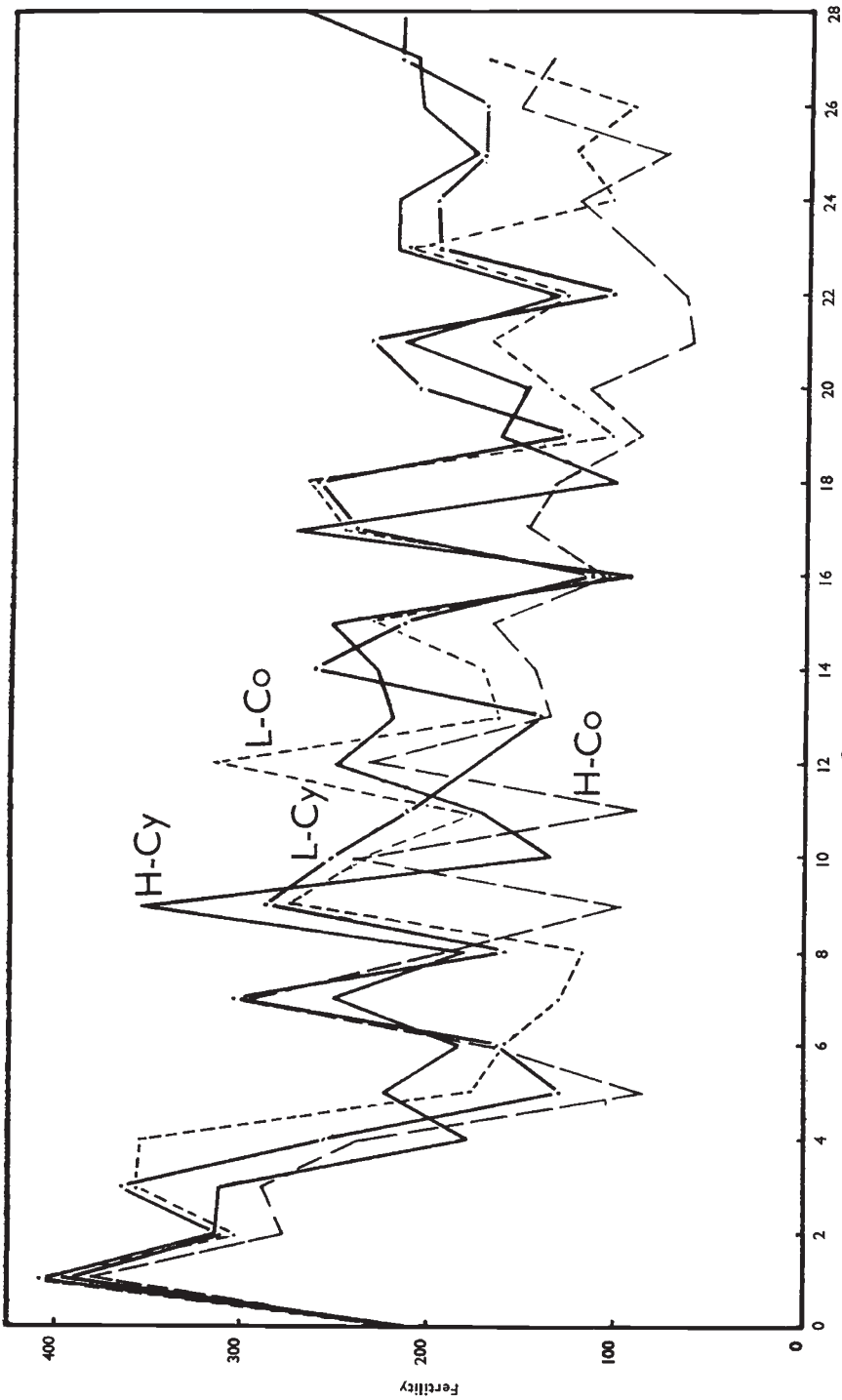

FIG. 5.—Fertility, as measured by average output per culture of all flies, in the four lines of the first selection experiment. H, L, Cy and Co as in fig. 4.

the non-heritable component of variation in L—R would have increased, with the result that the rigour of selection would fall and retrogression would set in. Deliberate relaxation of selection in later generations certainly did result in such retrogression of the lines, doubtless by this means (see Section 3 (*b*)), and there is a slight hint of fertility being higher in the four lines round S-8 and S-9 than round S-5 (fig. 5) ; but on the other hand, our earlier examination of the lines O and S and their F_1s and F_2s gave no hint of any marked effect of environmental differences on the variation of L—R between cultures. While this explanation of the simultaneous retrogressions seems the most reasonable, as being based on a known mechanism,

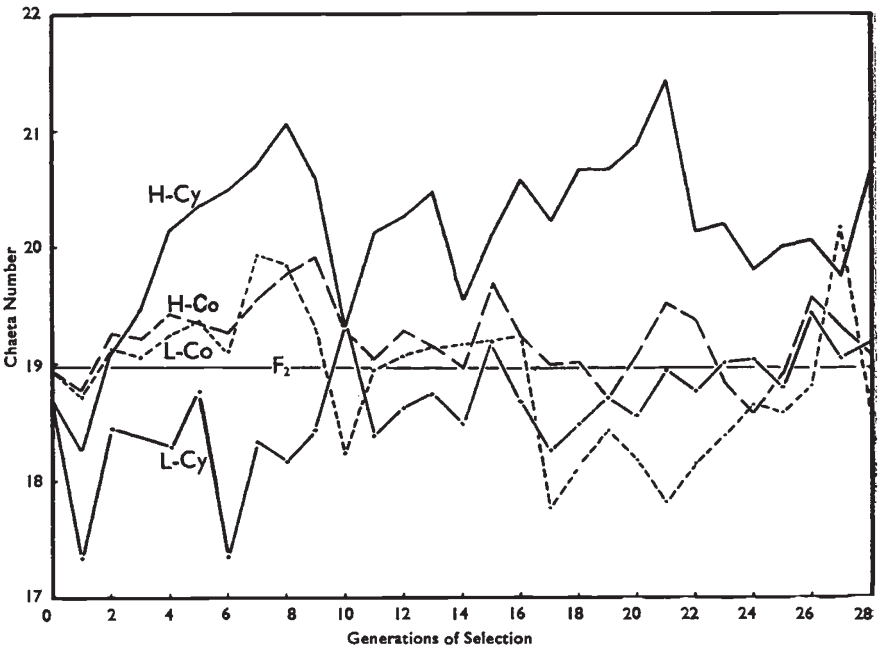

FIG. 6.—The gross number of chaetæ (A) in the four lines of the first selection experiment. H, L, Cy and Co as in fig. 4.

it must still remain conjectural, for neither these lines nor any other showed similar behaviour again, at a time when further analysis would have been possible, and no opportunity has presented itself of attempting to induce retrogression by the experimental manipulation of the environment.

One further point remains to be mentioned about these four lines. The absolute chaeta numbers (A) are plotted in fig. 6, from which it will be seen that although the high and low cyclical lines differed, the high line having a markedly higher absolute number, no corresponding difference is to be seen between the chaeta numbers of the two closed lines. Evidently a difference in V is not always accompanied by a corresponding difference in A : the one is not a simple

secondary effect of the other. Indeed even in the cyclical series an analysis of covariance shows that the difference between the V in the high and low lines cannot be accounted for by reference to the relation shown between this variance and A within the lines. Thus these four selection lines bear out the conclusions from the regression analyses of the previous section.

(b) Relaxation of selection

At the S-22 generation two new lines were started, one from each of closed high and low lines respectively. No selection was practised in these new lines, but in other respects they were maintained in the

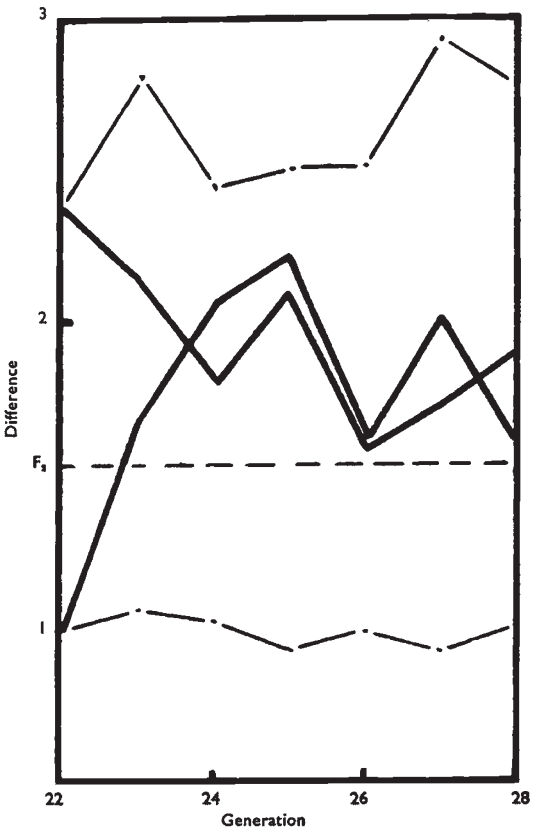

FIG. 7.—The effect on mean square difference of relaxing selection (heavy lines) in the first selection experiment. Relaxation began at S-22. The selection lines (light) are also shown for comparison.

same way as those from which they sprang. In two generations the two new lines had come together in respect of their mean square chaeta differences (fig. 7), albeit at a level slightly above the average value shown by F_2s. Thereafter the unselected lines continued together, with that from the low line slightly, and probably insignificantly, above its fellow.

The small excess of the level at which they stabilised over that of F_2 should probably not be emphasised ; the significant feature is that they came together, and did so in only two generations, despite the twenty-two generations of selection in opposing directions to which their ancestors had been subjected. Clearly the selection had not succeeded in rendering the high and low lines homogenic for the combinations of genes producing the more extreme, and therefore favoured, phenotypes. In fact, genic combinations similar in effect to those of the F_2 from which the selection lines arose must have persisted in these lines, or at least combinations from which recombination could readily build up others similar in effect to those of F_2. Given that the combinations of more extreme effect also gave the correlated response of reduced fertility, they would be held in the high and low lines themselves only by the selection, and would be eliminated from, or at least sharply reduced in frequency in, the new lines where, by the relaxation of selection for chaeta difference, fertility would become the capital character and take charge of events.

The relaxed low line did seem to show a slightly higher fertility than its selected ancestor, but no obvious improvement occurred in the relaxed high line. The fertility results do not, therefore, supply quite the confirmation one could have wished for this interpretation, but they are not incompatible with it, for even in the case of the relaxed high line a difference in fertility may easily have been present large enough to change the genotype yet sufficiently small to be obscured by the relatively large sampling errors to which the present estimates of fertility are inevitably subject.

(c) *Crosses between the high and low lines*

Reciprocal crosses were made at S-18 between the high and low selection lines of the closed part of the experiment. F_1s and F_2s were raised and new high and low selections were started separately from the F_2s of the reciprocals. These were maintained by the same methods as the parent high and low lines except that only two cultures

TABLE 8

V in Crosses between high and low selection lines

		F_1		F_2	
		Females	Males	Females	Males
Crosses	H×L	2·18	2·03	2·83	1·45
	L×H	1·43	0·98	1·95	1·80
Parents	H	2·15	2·92	2·65	2·00
	L	1·12	1·02	1·40	1·25

were used in each generation of each line instead of the three of the parent lines.

The most striking feature of these crosses was the difference between the reciprocal F_1s and F_2s. The values of V in F_1 and F_2 are set out for males and females separately in table 8, together with corresponding figures for flies of the parent lines raised at the same time.

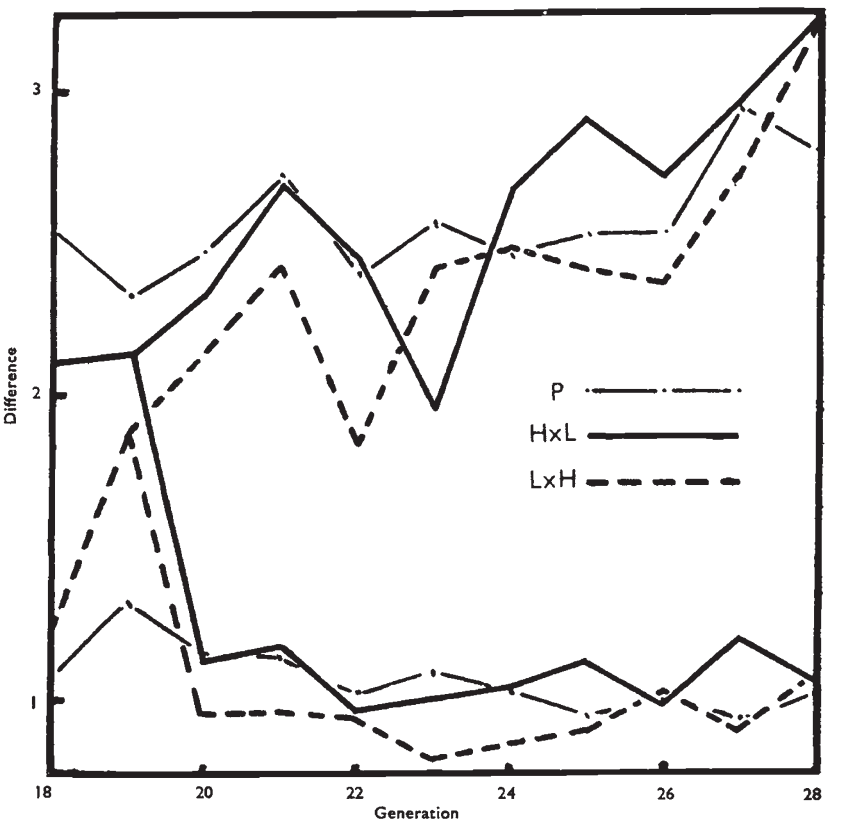

FIG. 8.—Mean square difference in the crosses between the high (H) and low (L) selection lines in the first selection experiment and its behaviour under selection from these crosses. The parent selected lines (P) are shown for comparison. The crosses were made between flies from S-17, so that F_1s appear at S-18, F_2s at S-19 and the derived selection lines from S-20 onwards. H×L and L×H each give rise to an F_1, an F_2 and two reciprocal lines, one selected for high and the other for low V.

The difference between the reciprocal crosses is clearly shown in this table, as it is also in fig. 8, where, however, the mean of the sex values is plotted. The crosses fall nearer to the parent lines from which the mothers were taken, so that two possible explanations offer themselves : that the reciprocal differences may be due to effects of sex-linked genes or that they may be due to an effect of the mother in partially determining the phenotype of her offspring by extra-

nuclear means. If sex linkage is the cause, the difference in F_1 should be between the males from the reciprocals, and in F_2 between the females. Such differences do exist (table 8) but there are similar, though admittedly smaller, differences between the females of F_1 and the males of F_2 where, on the basis of simple sex linkage, we should not expect them. Thus, while sex linkage may play a part, and indeed we have already had reason to postulate sex-linked genes (Section 2 (*b*)), it seems unlikely that the whole of the difference between the reciprocals can be fairly attributed to the effects of genes borne on the X chromosome.

We must therefore keep open the possibility of a maternal effect, though on the present evidence such an effect could hardly be regarded as proved. If maternal effect there should be, it cannot be of a kind traceable to permanent plasmagenes in the cytoplasm, for the difference between reciprocals is much smaller in F_2 than in F_1, and becomes even smaller in later generations. True, the offspring, whether in high or low line, of $L \times H$ never quite reach the same average value of V as the corresponding descendants of $H \times L$; but the discrepancy is no greater than occurs between sister lines within $H \times L$, and may reasonably be ascribed to the accidents of sampling in taking parents from the original heterogenic high and low lines for the crosses. Such small differences are inevitable under these circumstances and indeed Mather and Harrison describe similar cases where no question of maternal effect enters in.

Thus, the present maternal effect, if genuine, is more likely to be ascribable either to some direct somatic influence of mother on the development of the egg, an influence exerted before laying and comparable to uterine effects in mammals or maternal effects on eggs in birds ; or to a relatively long lived, though not fully permanent, cytoplasmic entity initially produced by the genes and possibly having some capacity for reproducing itself, though not to such an extent as to maintain it in the long run where the corresponding genes are absent. This latter explanation, which has been shown to apply in other cases in snails, *Drosophila* and elsewhere (Mather, 1948*a*), would obviously account not only for the reciprocal difference, but also for its gradual diminution and eventual disappearance.

The difference between the reciprocals is not, however, the most important feature of these crosses. The two reciprocals agree in responding to selection so quickly that the levels of the parental lines both high and low are reattained by only two generations selection, though it took at least five generations of selection to achieve those levels in the original building up of the parent lines. This is the same phenomenon as was observed by Mather and Harrison (1949) in selecting for abdominal chaeta, and the explanation is presumably the same. The first selection is effective by building up, through recombination, new linked polygenic combinations of more extreme

effect, which once built up, tend to behave as units or " effective factors " in inheritance. Following crossing of the H×L selection lines, these combinations segregate out as effective units, with the result that the genotypes of the parental lines reappear almost at once, so permitting selection to restore the parental levels much more quickly than they were reached when first built up. It would thus appear likely that the genetic system controlling stability in develop-

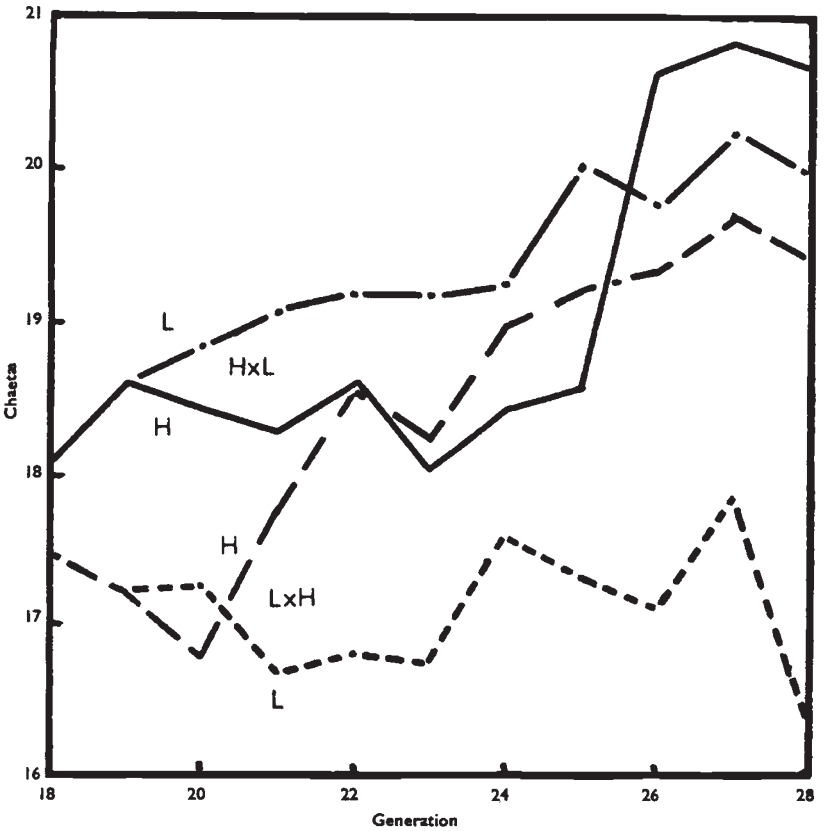

FIG. 9.—Chaeta number in the crosses H×L and L×H and their derived lines selected for high (H) and low (L) mean squares differences. Compare with fig. 8.

ment is similar in properties to those which Mather and Harrison discussed.

One further point needs mention before leaving these crosses. The absolute numbers of chaetæ in them and the selection lines to which they gave rise are shown in fig. 9. Though the high and low lines from H×L and L×H clearly resemble both one another, and the parent H and L lines, in V, they depart widely in A, thus emphasising once again the lack of dependence of difference on absolute number.

(*d*) *The second selection experiment*

A second selection experiment was started, again from the F_2 of the cross O × S, and proceeded to S-13 before it had to be terminated. This experiment was divided into two parts, in one of which each culture was raised from a single pair of parents and in the other from four parents, two of each sex on the pattern of the first experiment. Three lines were maintained in each part, one by high selection (H), one by low selection (L) and the third by the use of unselected parents (U). For this third line the parents were taken at random : they were not chosen as being near to the mean of the family. Thus the unselected line offers a picture of the effect that drift can have. In the high and low lines selection was practised at the same rigour as in the first experiment : in the double pair lines the extreme two flies were taken from twenty counted of each sex in each culture, and in the single pair lines the extreme fly of the first ten counted, although a further ten were then always counted to bring up to twenty the total from which was found the value of V in that culture. Two cultures were set up in each generation of each line, the mating system being of the cyclical type described, except of course where one culture failed. The time spent by the parents in each culture bottle was the same as in the first experiment.

The results of this second experiment are set out in figs. 10. In general, these results confirm those of the first experiment, and show no especially remarkable feature. After hesitant starts, the selection lines of single and double pair matings made progress, the low line responding more rapidly in both cases. Both low lines stabilise fairly soon near a mean square difference of 1. The single pair high line rose to nearly 3 at S-7 and stayed just below that value until the end of the experiment. The double pair high line rose until S-10 when it had obtained a value of rather more than 3, though it fell somewhat thereafter. The two unselected lines fluctuated, the double pair line somewhat more sharply than its single pair counterpart, but in both cases they came to occupy positions between the corresponding selection lines in the later part of the experiment after the high lines had showed their responses. Evidently, as indeed could hardly have been doubted, it is not reasonable to ascribe the behaviour of the selection lines to drift.

The fertility of the single pair lines fell a very little during the course of the experiment, perhaps due to the slight inbreeding which would go on even under the mating system used. The fall could in any case hardly be correlated response to selection for it occurred as much in the unselected line as in the selected. The double pair lines showed no clear evidence of a fall in fertility. It is perhaps surprising that fertility should hardly be affected in this second selection experiment while falling so strikingly in the selection lines of the first. The difference may be due to the absence from the

second experiment of the very sharp responses seen in the early generations of the first, responses which were not equalled even in the latter generations of the second. Albeit that these gains in the first experiment were substantially lost after a few generations, as already discussed, the effect on fertility seems already to have been established (fig. 5). In any case, it should be a characteristic of correlated response due to linkage that it will appear or not appear according to the nature of the linkages and the recombination which breaks them. Such a difference would, however, be troublesome to explain by a correlated response due to pleiotropic action.

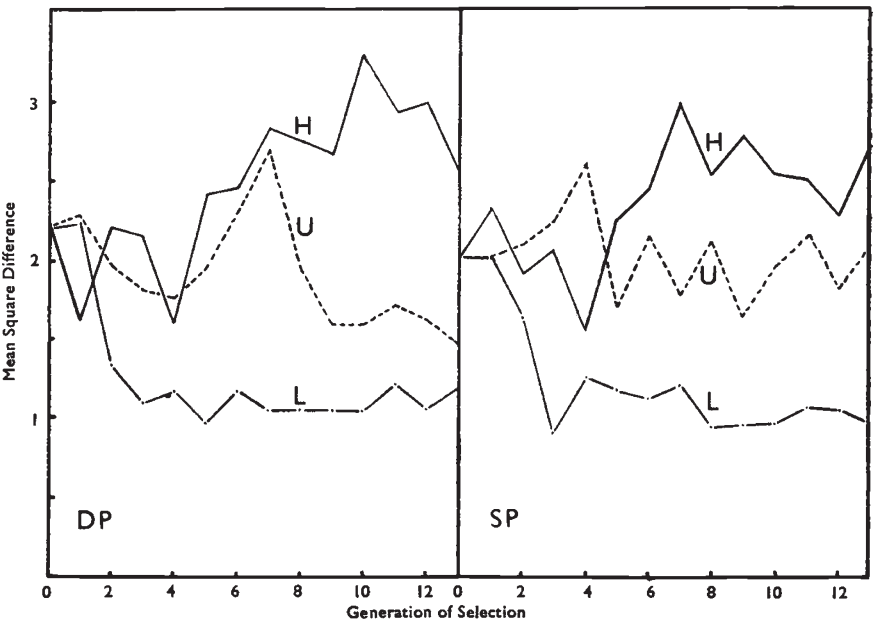

FIG. 10.—The effect of selecting for mean square difference in the second selection experiment. H indicates selection for high V, L selection for low V, and U the unselected lines. The double pair mating (DP) and single pair mating (SP) parts of the experiment are shown separately.

One last point remains to be made about this second experiment. In both single and double pair selections the value of A in the unselected line departed more from the high and low lines than did these two selection lines from one another. Thus, once again, we can see how the bilateral difference can be separated from gross chaeta number, so that change in the one cannot be a mere secondary expression of difference in the other.

4. THE GENETICAL STRUCTURE OF CONTROL

These results, from both inbreeding and selection experiments, leave no doubt that there is a heritable component in the control of the difference between the two sides of *Drosophila melanogaster* in this

cross between the O and S lines. The genic structure of this component is, however, less obvious. The very complexities of the character, stability in the development of an individual, virtually precludes the observation of simple segregations except such as would have the grossest effects. There is an indication that the X chromosome carries at least one gene affecting the character, in that the males of reciprocal F_1s and the females of their F_2s show some difference (table 2). At the same time, there seems also to be an autosomal element, in that all the classes of F_1 and F_2 offspring have lower bilateral variances than do their two parent lines. Again, selection produces lines with higher values of V than the parents, so that genotypes capable of transcending in their effects that of the parents can be obtained, presumably by recombination. Furthermore, when selection is relaxed, these lines revert to the levels of expressions characteristic of the unselected crosses, so that these selection lines cannot be homogenic : they must each carry a number of homozygous types, differing from one another and from the parents, or, more likely, they must be heterozygous. And if the latter, they cannot be heterozygous in the same way as the F_1, for they differ in expression from it, one selection line showing a much higher V and the other a lower.

Beyond seeing that inheritance is most likely complex, we cannot proceed with full confidence. We cannot even set a minimum to the number of genes, or effective factors, in the system. Nonetheless, it is worth noting that the general behaviour of the character in this cross resembles that of characters which can be shown to be under the control of a polygenic system, such as the number of abdominal chaetæ analysed by Mather and Harrison (1949) and Harrison and Mather (1950). Both are characters which vary in wild type flies, and which can confer fine shades of adjustment on those flies. Both are characters in which selection can easily bring about expressions transcending those of the parental lines, or, in other words, both are characters for which the genotype carries potential variability. Selection for the bilateral difference is perhaps less easily, or at least less strikingly, effective than selection for abdominal chaetæ, even in the upward direction where there is no technical limit set to its rigour ; but even in this connection, it should be noted that in the cyclical high line of the first experiment, selective advances were still in progress at S-28. The difference between the two characters may thus lie more in the difficulty of releasing the potential variability than in its quantity.

It is worth noting too that following the H × L and L × H crosses, selection restored the parental levels of the bilateral difference in only two generations, whereas these levels themselves took at least five generations of selection to establish from the original crosses of O × S and S × O. Though less marked, this is as we have already

observed, reminiscent of the difference in speed of response to selection between the offspring of the original crosses and the offspring of crosses between selected lines in Mather and Harrison's experiments ; a difference which they showed to be most reasonably attributed to the effects of linkage. The slow advance under selection in the last few generations of the high cyclical line adds its weight to the evidence for linkage effects. We also have a similar relation of the selected character to fertility, in that when selection for the bilateral difference was relaxed, the character reverted to the unselected level in both high and low lines.

Control by a polygenic system of the kind which Mather and Harrison discussed will thus account for the properties of the bilateral difference in the crosses and the selection lines. It will also account for the difference in the expression of this character between the O and S lines and their immediate crosses. Where polygenic variability expresses itself in wild individuals, the balance of the genic combination comes to be adjusted by natural selection, so that in outbreeding species, such as *Drosophila melanogaster*, inbred lines have a different and usually less advantageous expression of the character than do crossbreds. In the present case, a higher bilateral difference, or lower stability, would presumably be less advantageous, so that the higher variances of the inbred lines, O and S, could be regarded as manifestations of inbreeding depression, an explanation which Mather (1946 and 1950) has already suggested for the higher variability of floral characters within individuals of inbred lines of *Primula sinensis*.

A remarkable feature of the present results is that the variance is lower in F_2 than in the F_1 of the cross. Two explanations might be advanced. As Dr J. M. Thoday has pointed out to me, the genic balance of the F_2 individuals from a wide cross might resemble those of flies from wild populations more closely than would the balance of F_1. Wild individuals will necessarily be homozygous for a proportion of the genes varying in the population, since the maximum proportion of heterozygotes which outbreeding can give will, in general, exceed by little, if any, the proportion resulting from random mating. Thus the F_1 of a wide outcross might be too heterozygous to give the best genic balance, so that its F_2 would have a more advantageous expression of the character.

The second possible explanation is that the bilateral difference may be partly a maternal character and hence influenced by the balance of the mother's genotype as well as by that of the individual itself. The lesser variance of F_2 would on this view reflect the better genic balance of F_1. The difference between the F_1s of the $H \times L$ and $L \times H$ crosses also suggests such a maternal effect, as we have already observed. Our present results, however, offer no way of deciding finally between these explanations.

5. STABILITY IN DEVELOPMENT

We have discussed stability as it is manifested by the differences shown by a bilateral structure within individuals, because such a character is technically convenient for the investigation. Properties in respect of stability will, however, affect the variation between individuals in the expression of single as well as bilateral structures. Properties of stability are less easy to investigate by comparisons between individuals, however, because such comparisons must reflect both segregation for genes and the action of external agencies affecting the average expression of the character, as well as the effects of genes controlling stability round that average.

A comparison of F_1s with their parent lines, where these latter show but little genetic variation within themselves as compared with the genic differences between them, and where all are grown under a comparable range of conditions, can nevertheless provide relevant evidence. The variation in flower morphology between the plants of an F_1 from the cross *Petunia axillaris* × *violacea* is less than the variation between the individuals of each parental stock, even on a scale where the average expression in F_1 is almost at the mid-parent value (Mather, 1949). This finding has been extended by Robertson and Reeve (1952) to certain bodily measurements of *Drosophila*, where F_1s are less variable between individuals than are the parent inbred lines, when a " coefficient of variation " is used to allow for differences in mean expression. In both cases, the F_1s, though heterozygous, will be as homogenic as their parents, and since the individuals of both parental and F_1 families will be showing the effects of environmental agencies, we must conclude that the individuals of F_1 are less responsive—less buffeted about—by these agencies. The genotypes of the F_1s give a more stable development better buffered against upset by outside factors.

The bilateral differences we have observed in the present experiments may reflect the effects of local differences in external conditions affecting the sides of the fly. No evidence was, however, obtained of the differences between cultures that one might perhaps expect to find, at least within the parent lines, if the bilateral difference in the character were in large measure dependent on, and an expression of, differences in external conditions to which the two sides of the fly have been exposed. It would seem likely, therefore, that within the fly the bilateral differences reflects the effects of internal upsets in development rather than external inequalities ; and that the greater stability of some of the generations and lines displayed a greater genotypic capacity of these flies to correct their own internal accidents.

All these species, *Petunia*, *Primula* and *Drosophila*, which we have observed to display greater stability in the more crossbred individuals,

are outbreeding forms. As we have already pointed out, this is understandable if greater stability is selectively advantageous, so that it would characterise those cross-bred genotypes which had been exposed to natural selection and whose balance would therefore have been adjusted to produce just this effect, rather than the more inbred genotypes which must be rare in the wild. Two consequences would follow on this view. In the first place, we should expect smaller differences in stability between inbred and cross-bred families of naturally inbreeding species, though some differences may remain as relics of a past ancestry of outbreeding. Observations on naturally inbreeding plants, at present not available, will be needed to take this point further.

The second consequence is that we should expect the levels of stability achieved in respect of different characters to vary with the selective properties of the character. Thus, while gross differences in the manifestation within an individual of almost any bilateral character could hardly fail to be deleterious, some instability leading to small bilateral differences in a character such as number of sternopleural chaetæ, would be unlikely to bring a serious disadvantage to the fly; and indeed we have seen that some bilateral variation remained even in lines selected for its reduction. This is most probably true of a great many characters of the individual. Wing size and shape are, however, likely to be more critical, for even quite small differences between the wings on the two sides would be expected to have a marked effect on the properties and power of flight. The variation in wing length shown by an inbred line of *Drosophila melanogaster* has been analysed by Reeve and Robertson (1953) who find that the coefficient of variation (*i.e.* ratio of standard deviation to mean length) ascribable to asymmetry is 0·48 per cent. In the F_2 of the cross between Oregon and Samarkand the $\overline{V}$ when averaged over reciprocals and sexes was 1·6843, or on the basis of single sides 0·8422. The standard deviation due to asymmetry is thus 0·918, which when compared with the single side average of 18·98 chaetæ (table 1) gives a coefficient of variation of 4·84 per cent. Although the F_2 shows the lowest asymmetry for sternopleural chaeta number, the coefficient of variation is 10 times as high as that found for wings.

In discussing stability from the point of view of the effect of rectification of upset (whether arising from external or internal cause), we must not lose sight of possible causes of increase of the bilateral difference other than the mere reduction of ability to cope with such upsets. Where difference reflects only the ability or lack of it to adjust upsets, an increase in the average difference between the sides merely marks an increase in the general variation of their behaviour. But if the two sides interact with each other, by physiological means which at present we need not discuss, so that when on the average the character was high in expression on one side it was correspondingly

low on the other, a large average difference between them could be associated with smaller chance upsets on the individual sides than would be the case if interaction was absent. Such an interaction is of course most obvious when one side is regularly the larger, so that a consistent bias exists between them ; but it might also exist without leading to a bias of this kind, where either side could take the lead, the other then following in its expression, by virtue of the interaction.

An interaction of this kind, unfixed in respect of side, would be impossible to detect by bias, for insofar as each side had an equal chance of taking the lead, it could not lead to an average excess of one side over the other, nor could it be detected by a consideration of variation in the expression on a given side, for this would reflect not merely the variation intrinsic in development once the course of the side in question had been laid down, but would also contain a component reflecting the uncertainty that the side would follow the one course or the other. It should, however, be detectable from the properties of the bilateral difference when measured without taking sign into account. When sign is neglected, the average difference no longer measures bias, and should rise *pari passu* with the mean square or root mean square difference, if both merely reflect the variation due to upset ; but if an interaction comes into play between the sides, the mean should be increased disproportionately.

Such an interaction would give rise to what Timoféeff-Ressovsky (1943) has called *antisymmetrical manifestation*, for which he was unable to select effectively in the case of his *vti* gene. There is, however, some evidence that this relation has been favoured in our lines selected for high difference between the sides. In fig. 11 the standard deviation of the difference is plotted against the mean difference, both taken neglecting sign, for the parents O and S, the F_1-F_{10} generations of the inbreeding experiment, and the H, L and U lines of the selection experiments. The reciprocal F_1 and F_2 crosses are plotted separately as are the F_3-F_{10} generations from the two inbreeding experiments, in order to bring out the error variation to which the points are subject. Four H and four L points are shown, as found from the last ten cultures raised in each of the four H and L lines, two from each selection experiment. The two unselected (U) points are also shown from the second selection experiment.

The points fall into three groups, the parental, inbred and unselected, the low selection and the high selection. The parental, inbred and unselected group of points and the group of four L points fall very nearly on a common straight line passing through the origin, as would be expected if the standard deviation changed proportionately with the mean. The standard deviation, and with it the variance, is lower than the mean, but there seems to be no reason to anticipate it to be otherwise, as there is no ground for expecting these differences to follow a Poisson distribution.

When, however, we turn to the four H points, while they too agree sufficiently well with a linear relation among themselves, they clearly fall on a line different from that describing the parent, inbred, unselected and low points. The mean is higher for a given standard deviation or variance, just as would be the case if selection had favoured the rise of an interaction between the sides. Either the existing genetic system gives a new developmental relation beyond a certain point past which selection has carried it, or perhaps more likely a new genetic

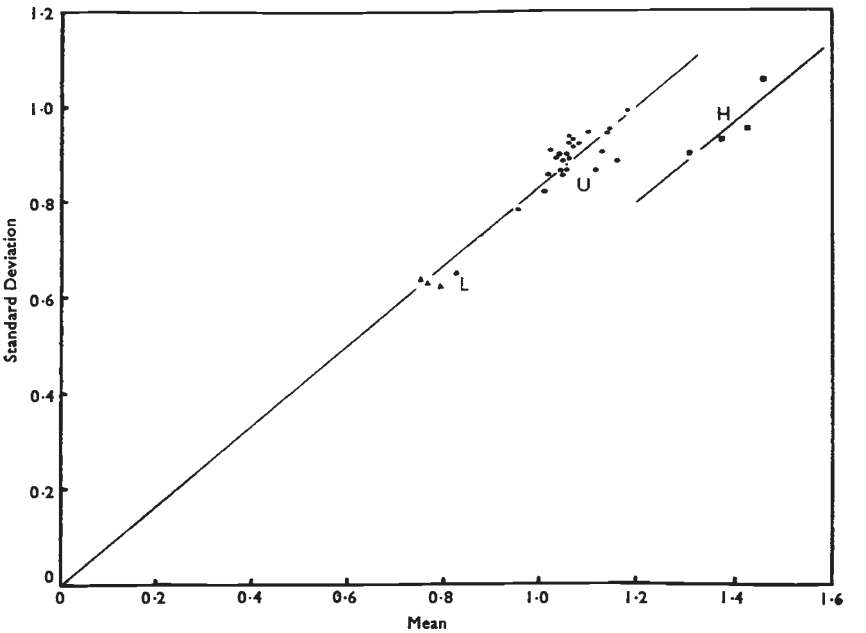

FIG. 11.—The standard deviation of the difference between the sides plotted against the mean difference where both are found neglecting the sign of the difference (*i.e.* neglecting whether the difference was in favour of left or right). The four low selection lines, two from each experiment, are represented by the four points in group L, and the four corresponding high selection lines in group H. The U, or unselected group, includes points for the parent lines, O and S (1 point per parent), for their reciprocal F_1s and F_2s (1 point per reciprocal per generation), for the generations F_3-F_{10} in the two inbreeding experiments considered separately (1 point per generation per experiment) and for the two unselected lines of the second selection experiment (1 point per line). The lines are suggested regression lines, that for the U and L groups being drawn to pass through the origin and that for the H group being drawn parallel to its fellow.

system has been established which gives this new relation. However this may be, the relation in the H group appears to differ from that in the L and U groups in that the two sides follow different paths in development, which side following which path seeming to be a matter of chance, but each side varying no more by upset from its path, once this is laid down, than did the sides round their common path in the ancestral flies.

The difference which appears to have become thus fixed in these experiments is small, but an extension of the process would presumably give a situation such as is observed, for example, in male fiddler crabs. In these animals, I am kindly informed by Dr I. Gordon of the Natural History Museum, the claw is always much larger on one side than on the other, but the large one is equally often on right as on the left. In *Uca mordax* Dr Gordon has counted a ratio of 41 : 34 for the large limb on the right and left respectively, in *U. tangeri* a ratio of 10 : 13, and in *U. inversus* 14 : 16 among the animals available at the museum. Though the animals ascribed to *U. mordax* and *U. inversus* might conceivably be of mixed specific origin, those of *U. tangeri* are certainly not.

Once a system had developed which leads to a marked bilateral difference, though one which was equally often displayed in favour of the one side as the other, it would not seem difficult for a mechanism, whether environmental or genetic, to grow up which determined the regular favouring of one side in the asymmetrical development. Quite a trivial difference should be able, at an early stage of what would appear to be so finely balanced a process, to tip it regularly in one direction. The result would then be a biased asymmetry of the kind with which we are so familiar, and which might well have sprung up in this way from an unfixed or unbiased asymmetry. There seems to be an example of such fixation among the hermit crabs, where, Dr Gordon tells me, it is usual for whole families or sub-families to show a bias towards a particular side, which is, however, the right in some of these groups and the left in others.

We have been considering the laying down of the two (or more) channels of adjusted development within a single individual, but, of course, the cases where development of whole individuals may follow one or other of the possible adjusted paths implies the same capacity of the basic genotype to offer these paths as alternatives. In these cases, however, the result will be, not an asymmetry within the individual, but a polymorphy between individuals, of the kind we commonly see in relation to crossbreeding devices like dicœcy or heterostyly, or in other characters such as the " phase " difference in locusts. The switching system may depart in these cases from that which operates in asymmetry, and will in general be expected to come to depend on a genetic segregation, or a distinct environmental factor. But even so the successful operation of the system must require the selective adjustment of the common genotype to offer the alternative channels of adequate development, an adjustment which we have seen broken down by overwide crossing or intense inbreeding in various plants and animals (Mather, 1948*a*), and whose building up under selection we may now have observed in its very early stages in *Drosophila*.

6. SUMMARY

The canalisation of development leading to characteristic differences between individuals and between parts of the same soma requires not only that initially small differences between cells and parts of cells can be built up to give large effects, but also that similar differences appearing in other cells or at other times can be reduced to relative ineffectiveness. The part played by the genotype in securing this stability in development can be investigated by the study of asymmetry and its variation in bilateral structures which show little or no difference between the average expressions of the two sides.

The number of sternopleural chaetæ may differ markedly between the two sides of an individual *Drosophila*, but there is only a very small difference between the sides when averaged over a number of flies. Furthermore, this small bias varies with sex, genotype and culture conditions.

The difference between the sides is more variable (and development hence less stable) in the Oregon and Samarkand inbred lines than in the F_1s and F_2s of their crosses. Two inbreeding experiments, started from these F_2s and carried on to F_{10}, gave evidence of segregation of genes affecting the stability of development as revealed by variation in asymmetry. Some evidence appeared of a sex-linked component in this genetic control. No evidence was obtained of environmental differences in stability between cultures. Natural selection within cultures appears to favour a higher degree of stability than that shown by the parent lines. The differences in stability could not be regarded as springing solely from differences in overall number of chaetæ. In fact, the relation of variation in asymmetry to variation in overall chaetæ number itself appears to change with genotype.

Two selection experiments, started from the same cross, confirmed the genetic control of stability by establishing differences between lines selected for high and low expressions of asymmetry. The differences between the high and low selection lines were themselves not large, but selection appeared still to be effective when the longer experiment had to be terminated after 28 generations. Again the change in asymmetry could not be referred to change in overall number of chaetæ. One of the experiments gave evidence of a correlated response in fertility and of the building up of linked polygenic combinations by the selection practised.

Though it was impossible to establish complete proof, the genetical system mediating stability appears to be polygenic in nature. There may also be a maternal effect. The level of stability appears to depend on a genic balance, itself the product of natural selection, in the species of *Drosophila*, *Primula and Petunia* in which it has been investigated. The properties of asymmetry should thus vary between inbreeding and crossbreeding species (a comparison on which we have

no information at present) and between characters of the same species, as indeed appears to be the case.

In the lines selected for increased asymmetry there is evidence of an interaction having arisen between the two sides of the fly in development, such as could produce the type of extreme, but directionally unbiased, asymmetry of the male fiddler crabs ; and which, by the likely next step of directional fixation, could give rise to the directionally biased asymmetry of the hermit crabs.

Acknowledgments.—I am indebted to Miss B. Loverock for much assistance with the experimental work on *Drosophila*, and to Miss D. M. Knight and Mr K. B. Parry for help in the extraction of the records for analysis. My gratitude is also due to Dr I. Gordon of the Natural History Museum for the information about asymmetry in fiddler and hermit crabs, and for permission to quote it. Dr Gordon must, of course, be absolved from responsibility for the interpretation placed on this information.

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