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Regular responses to selection

I. DESCRIPTION OF RESPONSES

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1. INTRODUCTION

A delayed response to selection may be said to occur when a line suddenly increases its rate of response without the population's size or the proportion of individuals selected having been changed. Sometimes the acceleration may occur in a line which has not shown any response to selection in the immediately previous generations. Such responses to selection provide a challenge, for they cannot be simply interpreted in terms of changes in the frequency of additive genes.

For such an acceleration to occur, the genetic variation of the line under selection must either increase or become in some way more effective. Either new genetic variance must be produced in the line, or genetic variants hitherto present but inviable or ineffective must be rendered viable, or effective on the character under selection, through changes in the genetic background or environmental conditions. If new genetic variance resulting from mutation or recombination is involved, its origin also might result from changes of genotype previously produced by the selection, or from special environmental conditions occurring just before the delayed response.

Some extensive selection experiments that have been under way since November 1953 have produced a number of similar accelerated responses, and seem to throw light on this problem generally and also on the more general problem of the factors limiting the responses of populations to selection.

In the present paper the actual responses of the lines will be described and some of the general possibilities will be discussed. Attempts to elucidate the nature of the genetic changes involved will be described in a later paper or papers.

2. MATERIAL AND CULTURE METHODS

The experiments were started primarily with a view to determining whether correlated responses might lead to the degeneration of a 'normal' phenotype, if its maintenance by natural selection was minimized by the use of mutant genes preventing its development. The character complex chosen was normal wing development, and it was suppressed by the presence of *vg* homozygous in one line and *dp* in another. After selection the normal wing type should be recoverable by crossing the lines, unless the gene complexes upon which its development depends have been

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significantly altered. This aspect of the experiment will be discussed elsewhere, and is merely mentioned here to explain the presence of the mutant genes in the lines to be described.

Selection began in one line homozygous for *vg* and in another homozygous for *dp*. Each had been rendered heterozygous at other loci by outcrossing the relevant mutant stock to an Oregon inbred strain and segregating the mutants. These two lines form the starting material for the experiment and all the other lines to be described derive from them. Selection began in F_3. The character selected for was high sternopleural chaeta-number in all the lines except two which were back-selected. Assay of a line in any generation was made by counting the chaetae on both sides of twenty flies of each sex from each of four single-pair cultures. The four single-pair cultures represented four separate female sublines maintained as a single population by the rotational mating system described in Thoday (1958*a*, and in Table I of 1958*b*).

From any such twenty flies assayed, the four with highest chaeta-numbers were set up in four cultures and given corresponding mates from the culture with which they were to be crossed. The best of these four cultures (that with highest parental chaeta-numbers) was intended to carry on the line. The other three were set up lest the best fail. To minimize the loss of female sublines, four further flies were selected at random from the flies surplus to the twenty counted and set up with mates from the appropriate culture. These 'mass' cultures were later set up with six pairs when some lines became difficult to maintain and some loss of female sublines occurred.

The lines were maintained with a generation every three weeks as described in Thoday (1958*a*). The flies were pre-mated in vials, allowed 96 hours to lay eggs in the culture bottles, and the offspring were collected for 4 consecutive days morning and evening, the sexes being separated to ensure virginity and the maintenance of the mating system.

Thus, except when all four single-pair cultures of a female subline failed, the system ensured that the population size would always be four pairs of flies.

In some generations, especially the earlier, crosses were set up between the lines. These will not be discussed here. Some discussion of them was given in Thoday (1955).

In generation 55, so many lines caused difficulty that all were set up without selection from the 'mass' cultures. The fact that the assays were due on Christmas Day mitigated the disappointment this would otherwise have given.

Mass (no selection) lines were taken from the lines from time to time. These are maintained by six-pair transfers, which are made every 3 weeks so that the number of generations of a mass line corresponds with that of the line from which it was derived. The results of the assays of these mass lines are listed in Table 1.

3. RESULTS

Fig. 1 illustrates the mean chaeta-numbers of most of the lines. Their behaviour will be described under headings which are the line designations.

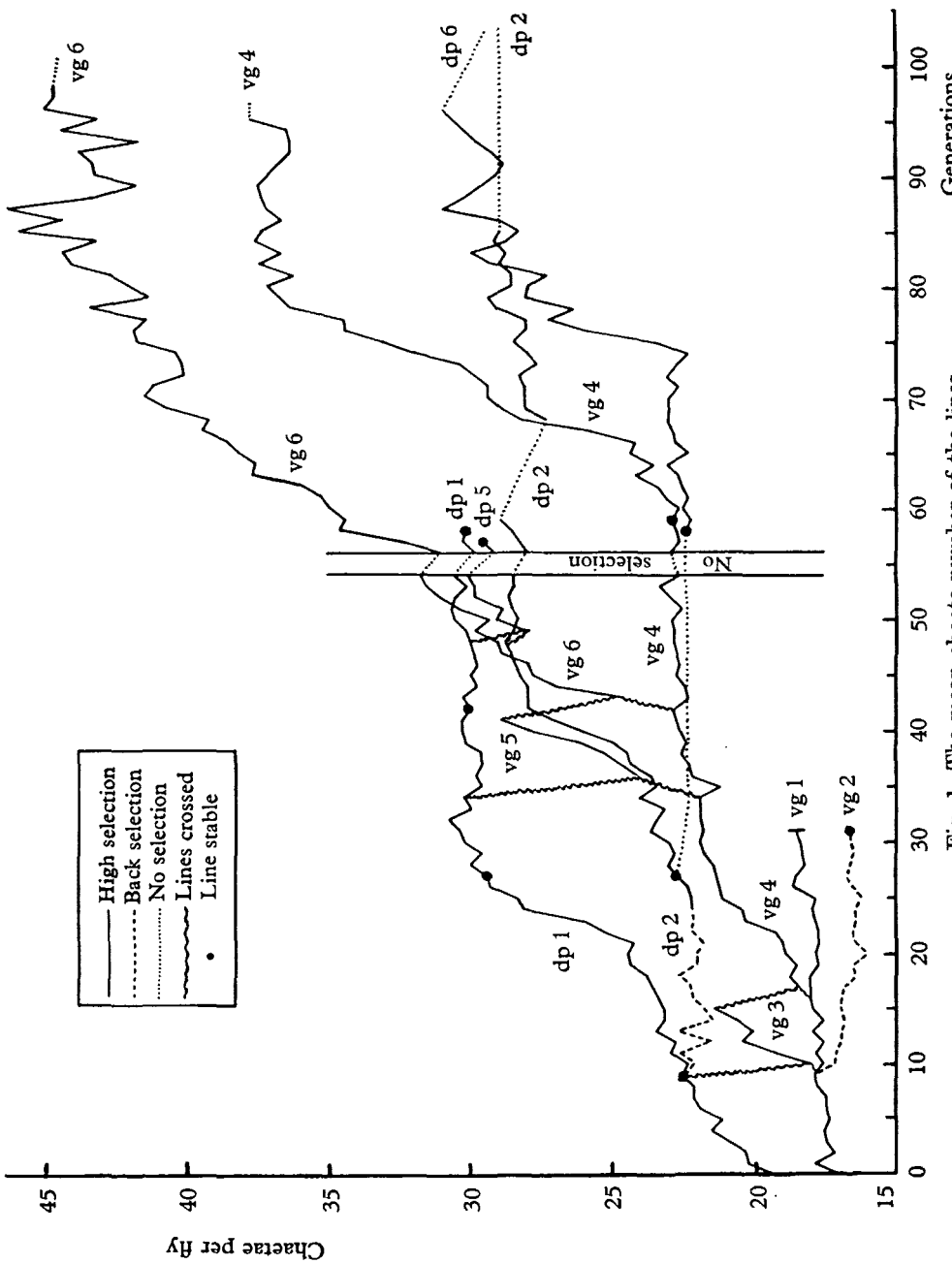

Fig. 1. The mean chaeta-number of the lines.

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Table 1. *Mean chaeta-numbers of the mass (no selection) lines*

Line	Generation extracted	Mean at extraction	Mean when assayed in generation 34	58	67	78	96
dp 1	27	29·2	29·0	30·3	29·7	29·2	29·9
	42	29·8				29·2	29·6
	58	30·0				28·8	30·0
dp 2	27	22·6	22·4	22·4	22·9	22·9	21·6
	52	28·7		28·8	25·5	22·2	22·2
dp 5	57	29·5				29·0	28·6
vg 4	59	22·8				22·4	21·0
	69	29·0				23·8	23·3
	80	37·4					34·1
	91	36·9					35·1
vg 6	59	34·2				28·9	27·3
	63	37·6				34·3	35·0
	79	41·5					39·6
	85	45·9					40·0
	93	41·8					43·2

(*a*) *The Dumpy lines*

Line dp 1

This is the line in which the accelerated response first occurred. (It was discussed briefly in Thoday, 1955.) Initially 19·6 chaetae, the mean of this line rose at a steady but decreasing rate until it reached 24·2 chaetae in generations 20 and 21. It then rose very rapidly to 28 chaetae in generation 24 and more slowly thereafter to a plateau at a little below 30 chaetae. At its plateau the line gave difficulty. Many single-pair cultures failed and soon after generation 40 all except one of the original female sublines were lost. (When a female subline was lost, it was replaced by using an extra culture from one of the surviving female sublines.) The line was terminated in generation 58 when a mass (no selection) line was established from it. This mass line was assayed in generation 78 when it had 29 chaetae showing the line to be stable.

Another mass (no selection) line had also been established from dp 1 in generation 27. (See Table 1.) This retained a mean of over 29 chaetae. The line dp 1 was therefore stable as soon as it reached its plateau, despite its infertility at the plateau when subjected to continued selection. Another mass line was taken out at generation 42 with similar results. All these mass lines fell slightly but none have fallen below 29 chaetae.

Line dp 1 is the line which showed evidence of containing in one of its component female sublines a cytoplasmic factor tending to increase chaeta-number by about 0·39 chaetae (Thoday, 1958*b*). Subsequent to generation 40 only this female sub-

line survived. The cytoplasmic effect, though definite, is slight and need not worry us here.

Line dp 2

Line vg 1 (see below) proved remarkably resistant to selection, and to test whether this was a result of homozygosity, a low chaeta-number line was taken from it in generation 9. At the same time a corresponding line was taken from dp 1, and this is the line to be called dp 2.

Despite the facts that dp 1 had originally only 19·6 chaetae, and that dp 1 must be presumed heterozygous for genes affecting chaeta-number in generation 9 (otherwise it could hardly have continued to respond to high selection), dp 2 proved stable against low selection. Low selection failed altogether to reduce the chaeta-number of dp 2 significantly, and it remained for fifteen generations a little above 22. At generation 24 it was again subjected to selection for high chaeta-number, to determine whether it was resistant to selection for high as well as for low chaeta-number. Shortly after this a mass (no selection) line was established from it, which has been stable ever since. Line dp 2 began to respond to forward selection immediately, and thereafter its behaviour was extraordinarily similar to that of dp 1. Fig. 2 illustrates this similarity. The only difference between the two lines is that dp 2 has never reached quite as high a chaeta-number as dp 1: its plateau was approximately 28·5 chaetae. This difference is hardly surprising, especially in view of the fifteen generations of back-selection in the dp 2 line. Selection ceased in generation 59 but the line continued to be maintained by the standard mating system using the 'mass', not single-pair, cultures. It was assayed again in generation 67 and, as its chaeta-number had fallen a little, selection was reintroduced. The mean recovered. This line appears less stable than dp 1.

In generation 52 (mean 28·7) when dp 2 had reached its plateau, a mass (no selection) line was taken out. This was assayed in generation 58 and its chaeta-number proved to be 28·8 (Table 1). dp 2 was therefore presumed stable, hence the relaxation of its selection in generation 59 referred to above. This generation 43 dp 2 mass line was assayed again in generation 67 when it had 25·5 chaetae, and in generation 78 by which it had fallen approximately to the original value of dp 2 during the back-selection period. It was assayed again in generation 96 but had not fallen further. dp 2 may therefore be described as metastable: it was stable for at least six generations, but capable, under some circumstances at least, of losing all the phenotypic results of selection, that is of falling again to the stable level of 22 chaetae.

Line dp 2 therefore presents us with three remarkable phenomena. First, its resistance to back-selection and subsequent response to forward-selection demonstrates that an evidently heterozygous line can resist back-selection despite recent response to forward-selection, a fact relevant to theories concerning genetic inertia or genetic homeostasis, and to explanations of asymmetrical responses to selection (*cf.* Falconer, 1955). During its period of back-selection, the line showed what might be called genetic homeostasis in reverse.

Second, the response of dp 2 when forward selection was reinstituted was almost

identical with that of dp 1. Whatever made the accelerated response possible was retained, despite back-selection. The accelerated responses in dp 1 and dp 2 occurred after the same number of generations of forward-selection, at the same mean chaeta-number, and at the same time of year (January). Almost exactly 12 months separated the two accelerated responses. This fact may be irrelevant, but should not be forgotten. January is, for example, a period of low humidity in a constant-temperature room and such environmental factors may not be ineffective.

The third phenomenon is the metastability of dp 2, which must be taken into account when explanations of the accelerated responses are considered.

Line dp 5

(Lines dp 3 and 4 will be described in another paper.) Line dp 5 was established by crossing dp 1 and dp 2 in generation 48. All four cultures of each parent line were involved in the cross. The line was maintained until generation 57 and showed no sign of rising above dp 1. dp 1 and dp 2 must therefore be very similar genetically and the accelerated response must have had similar causes in both. At generation 57 a mass (no selection) line was set up from dp 5. This was assayed in generation 78 and had 29 chaetae. The line seems therefore to be stable like dp 1.

Line dp 6

Line dp 6 was established in order to test whether the accelerated response that had occurred in dp 1 and dp 2 could be repeated. It was taken at generation 58 from the mass (no selection) line that had been set up at generation 27 from dp 2. It was therefore initiated forty-nine generations and close to two years after dp 2 was taken from dp 1. Extra precautions were taken to reduce any possibility that this line might be contaminated from the others. It was handled in a different part of the laboratory at all stages of virgin collection, etc., and was treated as independently of the others as facilities would permit.

This line showed less readiness to respond to selection than its predecessors. Nevertheless, after almost the same number of generations of forward selection, and at about the same time of year, it showed what seems clearly to be the same sort of response. This time the response began at a rather lower chaeta-number and seemed more sudden, but the parallel between it and that of the other two lines (Fig. 2) is again remarkable. The end-result is a mean chaeta-number similar to those of dp 1 and dp 2. It seems clear that the accelerated response is a regular and reproducible phenomenon. dp 6 became very infertile as soon as the response occurred and is now difficult to maintain. A high proportion of the single-pair cultures fail in each generation.

(*b*) *The Vestigial lines*

Line vg 1

This, the first vg line, was remarkably resistant to selection. Selection in fact seems to have picked out developmentally unstable genotypes rather than high chaeta-number genotypes (Thoday, 1955) and it seems that the line lacked effective

genetic variation for chaeta-number. This seems surprising in view of the fact that vg 1 was started with *vg/vg* flies newly segregated from an outcross. Other lines, established in exactly the same way from the same parental stocks, have responded well to selection (Dinsley, 1960) and it seems that ill-luck was in-

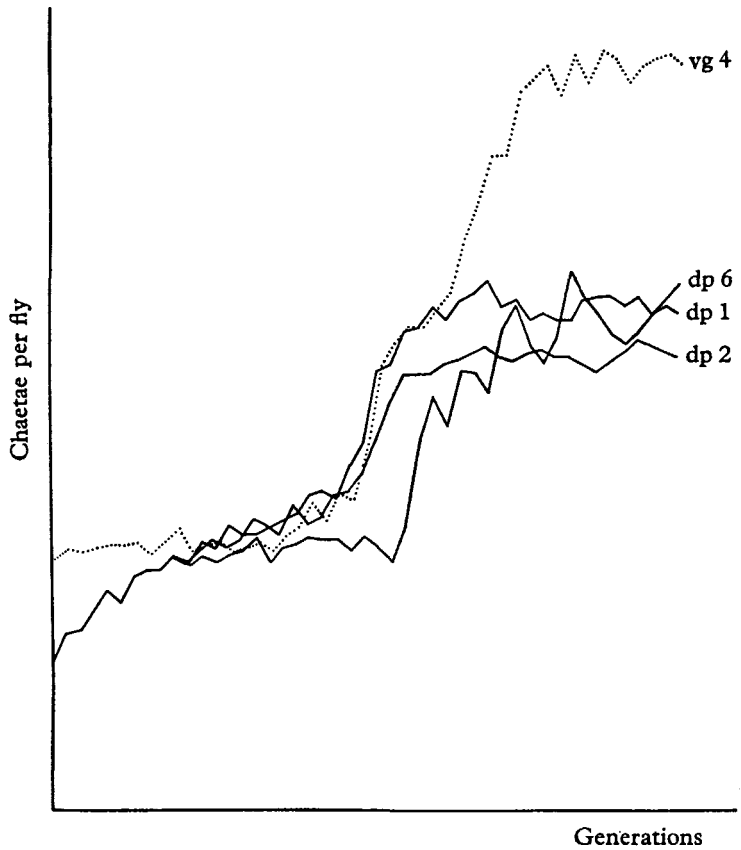

Fig. 2. The accelerated responses. The solid curves show the similarity of response of dp 1, dp 2 and dp 6. The curve for dp 2 is plotted by deleting the generations of back-selection: that for dp 6 by deleting these generations and the generations of mass culture. The dotted curve shows the response of vg 4 plotted so that its accelerated response coincides with those of dp 1 and dp 2. All lines are plotted to the same ordinate scale.

volved in the establishment of vg 1. vg 1 was extremely infertile by generation 31, and even the mass (no selection) line set up from it was lost.

Line vg 2

This was a back-selection line taken from vg 1 at generation 9 to test the possibility that vg 1 lacked usable genetic variance affecting chaeta-number. It responded but the response was slight and consistent with the view that vg 1

lacked useful variance. It was terminated in generation 31 and a mass (no selection) line established. This has survived and is more or less stable.

Line vg 3

This line was set up with a view to obtaining a vg line that would respond, by transferring genes from dp 1 to vg 1. An F_1 was made by crossing all female lines of dp 1 and vg 1 in generation 8. Two of the four crosses were of each reciprocal type. *vg/vg* segregants were taken from each of the four crosses to establish the four female sublines of vg 3. Line vg 3 responded to selection immediately, but characteristics of the thoraces of the flies it produced soon indicated that the line was rapidly becoming homozygous *dp*. Males were therefore taken at random, progeny tested to *dp/dp* females and also mated to *vg/vg* females of line vg 1. Males which produced no dumpy offspring in the progeny test and were also successful in producing offspring by the *vg/vg* females, provided the first generation of a new line vg 4.

Line vg 4

This line responded steadily to selection for ten generations, but then the rate of response decreased until it seemed to be reaching a plateau in generations 30–34. It did in fact continue to rise slowly thereafter, but, as it seemed to be approaching a plateau, a new cross to dp 1 was made in generation 34 to establish line vg 5 and later vg 6. These new lines will be described before description of vg 4 is continued.

Line vg 5

This line was initiated from F_2 *vg/vg* segregants by crossing vg 4 and dp 1 in generation 35. dp 1 had by then reached its plateau. vg 5 responded rapidly to selection as if it had acquired whatever had caused the accelerated response in dp 1. Because of the experience of line vg 3 which acquired *dp* genes from dp 1, vg 5 was treated to give vg 6 in the same way as vg 3 had been treated to give vg 4. There was, however, no evidence that vg 5 had acquired *dp* genes. It seems that the distinction between dp 1 and vg 1 in generation 8 involved a significant proportion of genes linked to *dp* (or an effect of *dp* itself on chaeta-number, which would be compatible with the initial means of vg 1 and dp 1), but that, by generation 34, dp 1 had such effective chaeta-producing factors not linked to the *dp* locus that selection did not pick out the *vg–dp* crossovers in line vg 5.

Though this test showed vg 5 to be free of *dp*, it was nevertheless thought better to continue with vg 6. vg 5 was continued until generation 49, by which time it had reached 29·7 chaetae but had been caught up by vg 6.

Line vg 6

This line, initiated at generation 43, responded to selection like vg 5, showing at first a response similar to, but a little more rapid than that of dp 1 and 2 in their periods of acceleration. Thereafter it has responded at a rate which is slower but nevertheless seems phenomenal. This response continued for forty-four generations with only slight deceleration. The mean chaeta-number of this line has been 46

and is now about 44, which is higher than the mean reached with the aid of radiation in the lines described by Scossirolli (1953). Fertility is now very low, though unselected subcultures do well.

Line vg 6 clearly shows that the two lines vg 4 and dp 1 contained different genes that could be combined to give remarkable advances in mean chaeta-number. There seems little reason to doubt that line dp 1 contributed the results of its accelerated response (but see vg 4 continued, below). This might be expected to raise the mean of vg 6 to about 30 chaetae. But there remains the further 14 chaetae raising the mean from 30 to 44 to be accounted for. These must result from the conbination of genes from dp 1 with genes from vg 4, and normally one would conclude that different genes were fixed in the different lines and that simple recombination permitted the favourable genes from each line to be exploited. That this is not the whole truth is clear from the later behaviour of vg 4.

Line vg 6 shows, however, that between them, lines dp 1 and vg 4 contain the genes necessary to raise chaeta-number to 44, though dp 1 is certainly stable at 30 chaetae, and vg 4, at 22 chaetae, seemed very little responsive to selection for high chaeta-number. The line suggests that hybridizing 'improved' strains, even when one of the strains used is comparatively poor compared with the other in regard to the 'improved' character, may sometimes be well worth while.

A mass (no selection) line was taken out of vg 6 at generation 59 when the mean was 34·2 chaetae. This mass had 29 chaetae when assayed in generation 78. A further mass was taken from vg 6 at generation 64 (mean 37·6) and had 34·3 chaetae when assayed in generation 78 (see Table 1). That vg 6 was unstable at these times is to be expected in view of the rapid responses it was then showing.

Line vg 4 continued

Line vg 4, as has been indicated above, responded very slowly after generation 30. There was a rise in mean of 1 chaeta over the next twelve generations which took it to about 22·8 chaetae at generation 42. It was still 22·8 chaetae eighteen generations later at generation 60. In the light of its subsequent history this is a very limited response to selection and a very long period of stability against selection. vg 4 was stable at generation 59, for a mass (no selection) line taken out then had not altered in chaeta-number when assayed in generation 78.

In generation 61, vg 4 began once more to respond, passed 24 chaetae in generation 65 and then showed a very rapid response to 28 chaetae in generation 78. Once again we have a very rapid response, similar to that in dp 1 and dp 2, as soon as the line passed 24 chaetae. This time, however, though the response showed signs of slowing down as the level of 30 chaetae was approached, a new acceleration followed and further rapid response occurred. Until generation 78, when the line reached a mean of 36·4 chaetae, the response was more rapid than that of vg 6. Response then slowed abruptly and a plateau was reached at a little over 37 chaetae.

The accelerated response shown by vg 4 is the most remarkable of all. vg 4 had, of course, the opportunity to acquire genetic material from dp 1 in generation 9, and clearly did acquire such material as is shown by its early behaviour as compared

with that of vg 1. This genetic material must be presumed responsible for vg 4's accelerated response once its mean reached 24 chaetae. This occurred at a time of year entirely different from that in which dp 1, dp 2 and dp 6 produced accelerated responses, and seems to indicate that the time of year is irrelevant and that the coincidence of season of the dp accelerations depended solely on the initiation of forward-selection from a little over 22 chaetae at similar times of year in each of these three lines.

The behaviour of vg 4 taken in conjunction with that of vg 6 throws light on the sources of genetic variation that have permitted these two lines to reach such high chaeta-numbers.

It was suggested above that the response of vg 6 indicated that dp 1 and vg 4 contained different genes that could be recombined to produce higher chaeta-number genotypes. The subsequent history of vg 4 makes it clear that the essential factor that vg 6 obtained from dp 1 was that or those responsible for the accelerated response of dp 1 which took dp 1 to about 30 chaetae. Much of the remainder of the genetic variation that raised chaeta-number to 44 in vg 6 must have come from within vg 4. But it must have been held in vg 4 in some unexploitable form, for vg 4 showed negligible response to selection for twenty-four generations after vg 5 was made by crossing vg 4 and dp 1, and eighteen generations after vg 6 was made by crossing vg 4 and vg 5. The fact that vg 6 caught up vg 5 (see p. 168) also implies the importance of sources of variance in vg 4.

Yet again vg 4's later history shows that this variance could be rendered exploitable by changes within vg 4 itself, and that it was in these circumstances more rapidly exploitable as if the cross with dp 1 in the origin of vg 6 had involved a loss of some of the potentialities of vg 4, though of course it presumably involved the gain from dp 1 of the sources of variance that raised vg 6 from 37 to 44 chaetae.

Experiments designed to analyse the genetic differences now distinguishing these lines, the results of which will be published later, indicate that vg 4 now contains a genetic 'factor' the same as or similar to that responsible for the accelerated responses in dp 1 and dp 2 (dp 6 has not yet been tested). It seems that a similar or the same 'factor' was produced in vg 4 as that transferred into vg 6 from dp 1. It follows, therefore, that vg 4 must have carried, throughout its history, the capacity to produce this 'factor', and also some source of potential genetic variance that could only be exploited after the genotype had been changed by the introduction of this factor. To account for the rapidity of the rise of vg 4 from 30 to 37 chaetae, it must have been a very powerful source of potential variation.

The behaviour of vg 4 seems most important from the point of view of selection theory, for it implies that genetic variance must sometimes be of a kind that has to be exploited in a particular order. Hence we must presume that it is possible to lose genes in the early history of a selection line, that might be most valuable in its later history. Crossing an improved variety to the unimproved stock from which it originated, and then reselecting, might in these circumstances be a profitable operation.

A mass (no selection) line taken from vg 4 in generation 69 (mean 29·0) had fallen

to 23·8 chaetae when assayed in generation 78. vg 4 shows the same instability as vg 6, though the rate of fall of the vg 4 mass seems to be higher than that of the two vg 6 masses. Later mass (no selection) lines from vg 4 and vg 6 were relatively stable (Table 1).

4. DISCUSSION

Description of the lines in previous pages has covered a number of points. This discussion of the results will be confined to consideration of the accelerated responses, mainly those accelerated responses which occurred in the related lines dp 1, dp 2, dp 6 and vg 4 when they reached means of about 24 chaetae and rose thereafter in a few generations to 28 chaetae or more.

There seem in principle to be four possible classes of explanation that might account for these accelerated responses: contamination, mutation, gene-interaction and recombination. Explanation might involve combinations of some of these.

(i) *Contamination*

It is always difficult to rule out contamination as a possible cause of any particular genetic change in an experimental population. To rule it out absolutely is impossible. Contamination, however, seems most unlikely to be the cause of the accelerated responses that occurred in these lines, for the reasons which follow.

(*a*) The lines are normally handled with care. Collection of virgins, setting up cultures, etc., is always done so that dp and vg lines are handled alternately. Accidental transfer of flies from one line to another would therefore be most likely to involve contamination of vg lines with *dp* flies or dp lines with *vg* flies and would immediately be detected. *dp* flies have never been found in the cultures of any vg line (except of course vg 3), neither have *vg* flies been found in any dp line. Crosses between the dp and vg lines still produce wild-type wings, but neither wild-type flies nor any other contaminants have ever been found in any culture. Further, line dp 6 was handled with extra precautions (see p. 165) and these did not prevent it showing the accelerated response.

(*b*) There were no other high sternopleural chaeta-number stocks of *dp* flies in the laboratory when dp 1 showed its acceleration, which indicates that the dp 1 acceleration cannot have been caused by contamination. dp 1 is the only possible source of contamination for dp 2, dp 1 and dp 2 for dp 6, and vg 6 seems the only possible source for vg 4. (Certain other high vg lines were being maintained independently by Miss Dinsley, but it does not seem plausible to regard these as a source for contamination of vg 4. They were maintained in different parts of the culture room and handled in a different laboratory.) Of these possibilities, the contamination of vg 4 by vg 6 can virtually be ruled out on internal evidence. Until generation 67, assays of vg 4 never gave a fly with more than 31 chaetae. Assays of vg 6 never gave a fly with less than 32 chaetae after generation 63.

(*c*) Far more cogent evidence is provided by experimental contamination. This has been carried out using dp 6 just before it showed its rise and deliberately contaminating a culture with a fly from dp 1. The effect of contamination was

immediately detectable. The contaminated culture had a mean chaeta-number of 26·3, whereas the other three cultures had means of 22·7, 22·4 and 22·3. Repetition by contaminating dp 6 with dp 2 gave similar results. This evidence seems to rule out such contamination altogether as a source of the responses.

(*d*) In any case, it would be odd if contamination always occurred when a line approached 24 chaetae, but never occurred at other times!

The possibility of contamination has been discussed to a length that may seem unnecessary. It does, however, seem most important to show that contamination is not a plausible hypothesis, and hence that we are dealing with a regularly reproducible phenomenon that depends only on changes within the lines themselves.

(ii) *Mutation*

Mutation is a possible source of the variation that permitted the accelerations of response to selection. However, simple mutation of the type normally considered in genetic theory does not seem a plausible postulate for the following reasons:

(*a*) The mutation rates that would have to be postulated are high. The number of gametes tested in the different lines before the accelerated responses occurred are known, and are lower than would normally be required before a particular mutant turned up. If it be assumed that the same mutation was involved in dp 1 and dp 2, for which there is some evidence (p.165), and dp 6, the joint mutation rate is 2×10^{-4}, which is definitely high.

(*b*) 'Random' mutation in the lines should produce the same response to selection as should contamination, that is one culture should suddenly increase in mean, and this did not occur. Against this it might be argued that the mutant gene when it first arose may have been either less effective on chaeta-number (e.g. less dominant), or less viable, until further selection had picked out modifiers that increased its effects or improved its viability. These are possibilities for or against which evidence may be obtained later. The evidence so far obtained suggests that the accelerated responses depend on a factor in the left arm of chromosome III which is perfectly effective and adequately viable when removed from the background of dp 1. The viability modifiers, etc., would therefore have to be linked to the mutant locus, and we would be involved in mutation *and* recombination in explaining the responses.

(*c*) Ordinary 'random' mutation could hardly account for the fact that the accelerated responses all occurred when the lines had reached the same order of number of chaetae.

The most cogent of the arguments above is (*c*). It seems that, if mutation were the source of the accelerated responses, the mutation would have to be one which only occurred when selection had so changed the genotype of the line that the level of 24 chaetae was approached. This would be possible only if we were to accept one of the three following hypotheses. First, that the mutation regularly occurred before 24 chaetae were attained but was dominant lethal or dominant sterile before this. The mutation rate would then have to be very high and the hypothesis would not account for the viability of the extracts at lower chaeta-numbers. Second, that by the time the critical chaeta-number was reached, developmental stability had

fallen to a low level (see Lerner, 1954; Thoday, 1958*a*) and that this led to a loss of control of gene-reproduction in the line and, hence, to an overall increase of mutation rate, so that the probability of effective mutation to high chaeta-number genes was greatly increased. This hypothesis might account for the results and for the continued responses of vg 4 and 6 beyond the 30-chaetae level, but it seems highly improbable that the very high mutation rates that would be required should occur. The number of gametes tested *after* 22 chaetae had been reached and before the major responses is only 2400 in dp 1, 2240 in dp 2, 2560 in dp 6, and 4640 in vg 4. The third hypothesis is that of directed mutation. The responses can be accounted for in terms of mutation if we suppose that the physiological situation in 24-chaetae lines causes a change in gene-production of a kind that results in the appropriate mutant genes. This is the same speculation as that to which Waddington (1958) was led as a result of finding the same gene in different lines with similar histories of selection. The hypothesis would account formally for the present responses, but it seems undesirable to invoke it until all alternatives can be eliminated.

(iii) *Gene Interactions*

It would be possible to account for the accelerated responses in terms of gene interactions. A line would on this view have to be heterozygous for alleles either neutral in their effects on chaeta-number in a genetic background giving a chaeta-number below the critical value, or homozygous lethal in such a background. Selection of other genes would be responsible for the rise of chaeta-numbers to the critical value at which complementary action of these other genes and the hitherto neutral pair would lead to one of the two neutral alleles becoming a very effective chaeta-number increaser or viable when homozygous if lethal before. Put in another way, the background genes would be chaeta genes 'in their own right' but after the critical chaeta-number had been reached would become powerful modifiers of the 'key' locus, modifying either its effects on chaeta-number or on viability.

Such gene interactions must occur, for they seem necessary to explain the response of the line vg 4 above 30 chaetae. But it seems most unlikely that they can account for the main delayed responses from 24 to 30 chaetae in dp 1, dp 2, dp 6, and vg 4.

Assays have been made that seem successfully to locate the 'factor' responsible for the accelerated response in the *se–cp* region of linkage group III. These assays have been made on foreign backgrounds, some of which give means lower than 21 chaetae for the flies possessing the 'factor' in the heterozygous state. To account for these assays, the modifiers would have to be closely linked to the key factor so that, like the 'random' mutation hypothesis, this hypothesis would also have to invoke recombination as a factor in the origin of accelerated responses.

(iv) *Recombination*

Recombination could account for the responses, provided that it could be supposed that the lines all carried linked repulsion-phase complexes of genes affecting chaeta-number, such as Mather (see 1943, 1953) has demonstrated occur in wild flies and in laboratory stocks. Breakage of the linkage and the production of

coupling-phase recombinants would provide the new source of effective variance exploited in the accelerated response. Cistrans position effects might or might not be involved. The frequency of the responses in terms of gametes tested (p. 171) is not high in terms even of tight linkage. There is already some evidence (Thoday, 1960) that the 'factor' in chromosome III is a complex involving more than one locus. This evidence is consistent with a recombination hypothesis, such as that to which Sismanidis (1942) was forced when he wished to account for some regularities in his results.

Such recombination would also have to be restricted in such a way that it was only likely to occur when a line approached 24 chaetae. This, however, seems not impossible for there are two mechanisms that might give such results.

The first mechanism does not involve change of recombination frequency with changing chaeta-number. We postulate a pair of tightly-linked loci affecting chaeta-number such that the population contains some individuals heterozygous $+-/-+$, the chromosome $+-$ giving a slightly higher chaeta-number than the chromosome $-+$. We postulate that $+-$ is relatively rare and that $-+$ is relatively common. The frequency of heterozygotes will be low. Selection will then raise chaeta-number by raising the frequency of $+-$, and will consequently raise the frequency of heterozygotes. As the frequency of heterozygotes is raised, so the probability of picking up the rare recombinants only heterozygous females can produce will rise, and the probability of obtaining recombinants will rise with mean chaeta-number. A certain regularity in the relation of the accelerated response to the course of selection would result. It does not, however, seem probable that this mechanism would provide a degree of regularity comparable with that found in the experimental lines. The populations have only four females and four males as parents per generation. The lowest possible frequency of the less frequent chromosome is therefore 1/16. The lowest probability of a heterozygous female would then be 1/2 and the probability of there being at least one recombinant in the forty offspring of the heterozygous female that are assayed would be $(1-(1-r)^{40})/2$, where r is the recombination frequency in females. The highest possible frequency of heterozygous females is 1 per pair of parents, and the probability of there being at least one recombinant among their 160 assayed offspring would be $1-(1-r)^{160}$. When r is 0·1 or more, the two probabilities are 0·49 and almost 1·0. When r is 0·05, they are 0·44 and 0·99, and with r 0·01 they are 0·17 and 0·80. Unless, therefore, r is quite small there seems insufficient scope for change in the relative probability of recombinants occurring to account for the regularity of the response, unless for some reason heterozygous $+-/-+$ males were much more likely to be selected than the corresponding females. This hypothesis, however, cannot be ruled out, especially if we consider that some viability interactions may also be involved.

The second explanation invokes the known capacity of change of genotype to change recombination frequencies and recombination spectra.

Rees (1955) has shown that meiosis is relatively uncontrolled in Rye suffering from inbreeding depression and we may expect similar consequences from the unbalance to which selection gives rise. Further, the well-known effect of inversion

heterozygosity on recombination in other chromosomes not only involves increased frequencies of crossing-over, but relatively large increases in crossing-over in regions where crossing-over is normally rare (Schultz & Redfield, 1951). Such changes might result in the production of crossover types in a line approaching 24 chaetae, which had not occurred at all, or had occurred only rarely, in the previous history of the line. The dominant effect of the crossover chromosomes would account for the rise to 24 chaetae, and the rise to 28 would be due to their homozygous effect.

The difficulties of the recombination hypothesis lie in explaining the maintenance of the repulsion phase heterozygosity for so long in populations as small as those used in this experiment, and especially in the back-selection phase of dp 2. They would have to be fairly tightly linked and would have to have effectively the properties of balanced lethal systems. We have, however, clear evidence from dp 2 in its back-selection period and from vg 4 that the lines did maintain heterozygosity. Relationally balanced systems such as Mather (1943, see also Mather & Harrison, 1949) has discussed, involving overlapping complexes of chaeta-number and 'fertility' genes, could have the required properties, and the merits of this hypothesis render if attractive.

It may seem that the metastability of line dp 2 argues against recombination as the source of the gene-complex responsible for its rise to 28 chaetae. If, as seems possible and is not contraverted by the assays of the lines that have so far been done, the line at 28 chaetae was homozygous for the 'factor' we presume to have been produced by recombination, then we would have no explanation other than mutation for its loss. We would then be forced to invoke recombination as responsible for the building of the 'factor' and mutation as responsible for its loss. This, however, seems reasonable. A single mutation can 'destroy' all the zygomorphic pattern of an *Antirrhinum* flower, though its evolution must have involved many selective steps. Likewise a single mutation, affecting only part of the pattern which we call a major-gene, can destroy the enzyme-producing capacity of the whole. Now that we know that genes of specific effect are highly complex patterns composed of smaller genic units (e.g. Benzer, 1959; Pontecorvo, 1959) we are surely forced to the view that they must have been built up by stages in evolution. That they can be broken down by single mutations is not incompatible with this, and provides clues only to their structure not their origin.

We therefore consider that the results are most plausibly explained in terms of recombination. Further study of the chromosomes that can be extracted from the lines may provide evidence permitting critical tests of this hypothesis.

SUMMARY

1. Several lines of *Drosophila melanogaster* have been selected for increase of sternopleural chaeta-number.

2. Three lines of the same origin, dp 1, dp 2 and dp 6, showed remarkably similar patterns of response involving an accelerated response from 24 to 28 chaetae and a plateau at about 30 chaetae. A line formed by crossing two of these was not responsive to selection, suggesting that the two parent lines were genetically similar.

3. A fourth line, vg 4, related to these others, showed a similar accelerated response but continued to respond beyond 30 chaetae, reaching a plateau at about 37 chaetae.

4. A further line, vg 6, set up by crossing two 'improved' lines, one with 30, the other with 22 chaetae, reached 46 chaetae and suggests there may sometimes be merit in selecting from the hybrids of 'improved' strains.

5. It is argued that the event making possible the accelerated response is probably a recombinational event.

6. The line vg 4 clearly demonstrates that some of the genes that may be exploited in a selection experiment have to be exploited in a particular order because of gene interactions.

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