

# GENETIC VARIABILITY, TWIN HYBRIDS AND CONSTANT HYBRIDS, IN A CASE OF BALANCED LETHAL FACTORS

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## THE PROBLEM OF FACTOR VARIABILITY AND THE CASE OF BEADED WINGS

In numerous breeding experiments there is positive evidence that the factors concerned undergo no sensible fluctuation, nor sensible contamination during segregation. But, unfortunately for a clear and simple proof or disproof of the generality of these principles, Mendelian theory demands, and experiment has proved, that not infrequently multiple factors and other complications quite consistent with factor constancy

should be met with, that lead to results superficially similar to those which would arise from factor variability or contamination, although not really due to such a cause. Consequently, unless apparently irregular results have been subjected to a rigorous factorial analysis, they are equally reconcilable either to an explanation consistent with, or to one contradictory to, the "genotype conception." But the fact that these particular results cannot be used as arguments for factor constancy does not mean that they are arguments against it, and valid evidence *against* factor constancy could not be secured except by analyzing such results and proving that factor fluctuation is the only (or the more probable) explanation.

As a matter of fact, whenever a factorial analysis of the questionable cases was possible and has been made, evidence of the presence of the multiple factors or other complicated genetic phenomena to be expected on the strict genotype conception has always been obtained, and in no case has it been found possible to show that the factors underwent fluctuating variation or contamination. Of course, this furnishes "presumptive evidence" for all such cases, and, taken together with the positive evidence for factor constancy previously referred to, tips the scales heavily on this side. Opponents of the latter view have thus found it necessary hitherto to base their argument on the questionable cases in which factorial analysis was impossible or lacking, and they have not admitted that these cases really supply an argument for neither view. Accordingly it seems necessary to find more typical cases of the above sort which can be dissected, and to expose the factor mechanism there involved if we are ever to reach a decision that will be accepted by both sides.

The case of beaded wings in *Drosophila* appears to be particularly favorable for this purpose, from the point of view of both contending parties, because its inheritance seems most markedly "non-Mendelian," and because it exists in the organism best suited for a rigorous factorial investigation. Beaded was the third variation discovered by MORGAN in these flies, and it and truncate from the start formed the two great unconformable cases that quite refused to fit in with the ideal *Drosophila* scheme of things. The following are some of the more important peculiarities of this character and of its mode of inheritance.

(1) In the first place, the character is irregular in its manifestations. It consists of a sort of lopping off of the edge of the wing, which may be evinced at any point and to almost any extent (see figure 1). In some flies only a small piece of the margin seems gone; in others the

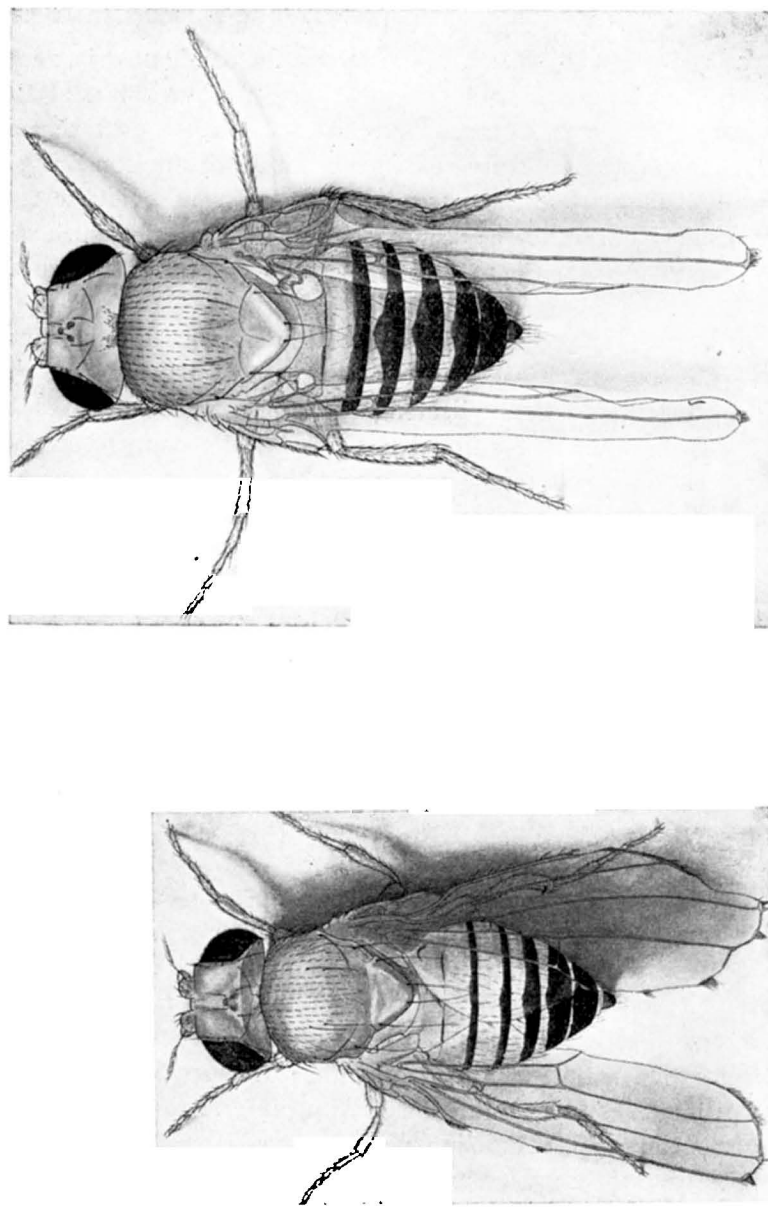


FIGURE 1.—Flies from "pure" beaded stock; an "average" beaded on the left, an "extreme" beaded on the right.

margin may be cut into at intervals—if this happens along the outer edge of the wing it results in the “beaded” appearance, on account of the breaking up of the marginal vein; in still other flies a large part of the border of the wing seems to have been cut away, along either a straight or a wavy line, and at either the inner or outer edge, or both. The exact nature and amount of the deformity is seldom the same in the two wings of the same fly, although a good deal of correlation does exist. These differences between the two wings cannot of course be ascribed to heterozygosis, unless we accept the extremely dubious doctrine of somatic segregation; they are therefore an indication either of factor inconstancy or of “somatic” (non-genetic) fluctuability. Whether or not the differences between the wings of different flies is due to one of the latter causes could not be decided; however, by these observations alone.

(2) (a) In the second place, when MORGAN bred beaded flies together, he obtained, besides the beaded of various types, some flies with wings entirely normal, and for years he was unable, by selection, to secure a strain consisting entirely of beaded flies. (b) This latter variability was proved to be not merely somatic, for it was found that the normals produced in this stock gave, on the average, a much larger proportion of normal flies among their offspring than did the beaded flies. (c) The tendency towards normal on the whole overbalances that towards beaded, for it was found, when the beaded stock was allowed to run for many generations in mass culture without selection or supervision, that the beadedness tended to disappear from it more and more, i.e., there was reversion towards normal.

If we believe in factor constancy, these genetic variations so continually occurring in the beaded stock must be referred to a different source from the somatic variations between the two wings of the same fly, and in order to account for them we would have to suppose that beaded flies for some special reason are always heterozygous, like Andalusian blue fowl or like yellow mice. If, on the other hand, we believe in factor fluctuation, all of the above results, of both preceding paragraphs, are directly explained on the simple basis of a variability in the factor for beaded itself, and it is then further to be noted that the results would fall into line with the widespread notion that factors vary more in the reverse direction—regressing towards their ancestral condition—than in the original direction of variation.

(3) (a) Not only the variation from beaded to normal, but also the variation in the intensity of “beading” was shown to be at least partly

genetic, because MORGAN was able by selection to obtain stocks differing distinctly and consistently in the degree of their beading; in fact (b) he succeeded, by selection, in bringing the extreme and the average intensities of this character both far beyond their original points. These facts too can be covered by the one assumption of factor inconstancy; denying this interpretation, we must add to our previous apparently unrelated assumptions of somatic variability and persistent heterozygosis the assumption of multiple factors.<sup>1</sup>

(4) The climax came when, after very many generations of the above selection work, a stock pure for beaded was, in some inexplicable way, finally obtained. The result came almost suddenly, as though a certain critical point in the "strength" of the factor had been passed, owing to the long selection; or, to be more specific, as though the beaded flies were now so far removed from their normal ancestors both in number of generations and in intensity of character that reversion to the latter no longer took place. In this more stable stock the character was on the whole well developed, and although it was still somewhat variable its range of fluctuation was not as wide as before, and it seldom approached very closely to the normal type. This result, like the others, falls naturally in line with the view of factor variability; the alternative view would require still another postulate, apparently disconnected with the previous ones,—namely, that a new factor or factors had appeared in the stock, which somehow did away with, or obscured, the tendency to "reversion."

To sum up, all the different peculiarities evinced by the character beaded point consistently to the explanation of factor inconstancy, and, *neglecting the evidence against such a conclusion afforded by the great body of work in genetics*, it would seem by far the most probable interpretation here; to avoid it, at least four apparently unrelated assumptions, two of them seemingly unusual to a high degree, are necessary.

An elaborate study of the character, beaded wings, was carried out several years ago by DEXTER (1914). His extensive and exemplary investigations have, besides confirming points mentioned above, brought to light a number of further facts which have an important bearing on the problem. He has found, firstly, that the character is markedly affected by various environmental conditions; drought, for example, tends to inhibit its appearance. These effects, if they are really exerted on the factor itself, must be but temporary, as succeeding generations, if brought up under the original conditions, will exhibit the character in its origi-

<sup>1</sup> Or else multiple allelomorphs.

nal form. In other words, there exists in beaded stock a sort of variation that is purely somatic, which is caused by external influences. Secondly, DEXTER found that beaded is linked to pink eyes and to ebony body color, and a factor for beaded must therefore lie somewhere in the third chromosome, as do the factors for both these characters. In some cases, however, he found that an intensifying factor was also present in the beaded stock, and this factor proved by its linkage relations to lie in the second chromosome. Hence there are at least two pairs of factors concerned in the character beaded, one chief factor in chromosome III, and one accessory factor in chromosome II. Two of the four postulates which I have shown to be requisite for an explanation of the beaded case on the theory of factor constancy were thus proved by DEXTER to be true: these are the postulates of "somatic" (non-genetic) variability and that of multiple factors. This would seem to be rather a strange coincidence if the results, or part of them, are really due to factor variability. DEXTER accordingly concluded that the explanation of the peculiar behavior of beaded was to be found not in factor variability, but in complications of a different kind.

There was as yet, however, no experimental basis for the other two suppositions which the adherent of factor constancy must make,—namely, that the stock was at first necessarily heterozygous, and that later a new factor or factors appeared, that somehow obscured or did away with this heterozygosis. Moreover, these latter postulates were, after all, much more unusual than the other two, and so the more important part of the problem still remained with only a vague and entirely hypothetical explanation. It is true that DEXTER had found that the intensifying factor in chromosome II was not present in all the beaded flies which he examined, and that on the basis of this he had suggested, as an explanation of the difficulties in question, that perhaps this intensifier might act as a lethal, like the factor for yellow in mice. For if this were true, it would be impossible to obtain stock pure for the intensifier, and since flies without the latter might sometimes be normal in appearance, an all-beaded stock could never be obtained. But the mere fact that the intensifier was not present in all beaded flies examined affords very little evidence for the assumption that it is lethal, and a serious objection to such an assumption might perhaps be found in the fact that in some strains at least of the pure stock that was finally obtained, all of the flies are beaded to a high degree, as if the intensifier was present in all.

These difficulties and contradictions gave an air of unsoundness to the

whole argument for factor constancy in the case of beaded wings. Opponents of the genotype idea, had they come across the case, would undoubtedly have urged with much conviction (for themselves at least) that the discovery of an intensifying factor and of somatic variability did not by any means clear up the chief uncertainties of the case, nor weaken very much the argument which this case, taken by itself, unquestionably provided for factor variability.

#### PRELIMINARY CROSSES AND FORMULATION OF A WORKING HYPOTHESIS

The present work will for the most part be described in its original sequence, as this mode of presentation will probably provide the reader with the best working knowledge both of the way the phenomena in question interfere with "regular" Mendelian results, and of the genetic methods used to unravel them.

The investigation originated in connection with some linkage experiments. As previously mentioned, beaded had been shown by DEXTER (1914) to lie in the third chromosome, and, more recently, STURTEVANT had found it to be about 15 units from sooty body color, on the other side of sooty from pink. As the writer was engaged in constructing a map of this chromosome, it was desirable to determine the linkage of beaded more exactly. With this object in view a fly from the "pure" beaded stock—the stock in which all individuals were always markedly beaded—was crossed to a fly of stock which I had made up for the study of linkage, and which contained in its third chromosomes the factors for sepia eye color ( $s_e$ ), spineless integument ( $s_s$ ), kidney-shaped eye ( $k$ ), sooty body color ( $e^s$ ), and rough eye ( $r_o$ ). The  $F_1$  flies should contain the latter factors in one of their third chromosomes, and beaded in the other. As, according to DEXTER's statement, beaded is dominant, but only partially and irregularly so, and as the other mutant factors concerned are all recessive, we should expect that the  $F_1$  flies would appear normal in their body, eye, and bristle characters, but that some (not all) would show a moderate amount of beading in the wings. This result was in fact obtained. (The cross is shown in the first two lines of diagram I.) Two of the beaded  $F_1$  females and one normal-winged  $F_1$  female were then back-crossed to the homozygous multiple recessive stock,  $s_e s_s k e^s r_o$ ,<sup>2</sup> in order to obtain the linkage data.

<sup>2</sup> If a horizontal line does not appear in the formula of an individual, it may be taken for granted that the latter is homozygous for all factors represented. In cases where a horizontal line appears, however, the compositions of the chromosomes received from the two parents will be shown on different sides of the line. Only mutant factors will be represented and the presence of the normal gene may then be assumed in every case where its mutant allelomorph is not shown.

The counts from the two  $F_1$  females with beaded wings were quite unexceptional. They showed the recessive factors to be linked with each other here in the order in which I had previously found them to lie (this is the order in which they are given above), and the strengths of the linkages also agreed with those previously found to be usual (MULLER 1916). Furthermore, these factors showed the amount of linkage with beaded which was to be expected on the basis of STURTEVANT'S previous results, though the numbers I secured at this time were not large enough to include crossovers between beaded and rough, its nearest neighbor, and so to determine the position of beaded accurately.

The results from the other  $F_1$  female—the one with normal wings—were so different that for a long time I failed to regard them seriously, and was persuaded that some mistake must have been made in the matings.

In the first place, the linkage was of a different type from that shown in the other counts—no crossing over was observed to have taken place between any of the loci except *sepia* and *spineless*, the characters of  $s_s$ ,  $k$ ,  $e^s$ , and  $r_o$  always appearing together. This result recalls the effects produced in the presence of the factor  $C'$ , a brief account of which may accordingly be given here. It had been discovered by STURTEVANT that the percent of crossing over between pink ( $p$ ) and ebony ( $e$ ) is usually much lower than between pink ( $p$ ) and sooty ( $e^s$ ), although sooty is an allelomorph of ebony, and he had attributed this effect to a factor (which we may call  $C'$ ) in the ebony stock that, according to his results, is rarely or never separated from the ebony factor. A similar factor in the second chromosome also was shortly afterwards discovered by STURTEVANT (1915), and in the latter case he worked out the details of the effects produced. Subsequently to this, I found that the third chromosome factor,  $C'$ , or a factor like it, is present not only in ebony flies, but also in flies of the mutant stock "spread" ( $s_d$ ), and, furthermore, that the third chromosome factor exerts a different amount of influence on the different parts of this chromosome, causing a very pronounced lowering of crossing over in the right-hand end, near sooty, where it itself is located, and having less and less influence at increasing distances from its own region. In the section of the chromosome between  $s_e$  and  $p$ , which lies furthest from it, crossing over is affected comparatively slightly. But when spread flies were crossed to ebonies, the  $F_1$  females (presumably homozygous for  $C'$ ) showed no reduction in crossing over at all, but rather an unusually large amount of crossing over; that is,  $C'$  produces a decrease of crossing over only



DIAGRAM I.

P<sub>1</sub> Beaded female from "pure" stock crossed by  $\frac{s_e s_s k e^s r_o}{s_e s_s k e^s r_o}$  male.

F<sub>1</sub> Beaded flies  $\left(\frac{B_d'}{s_e s_s k e^s r_o}\right)$  and normal flies  $\left(\frac{C' b_d^?}{s_e s_s k e^s r_o}\right)$ .

P<sub>2</sub> 2 beaded females of above type were back-crossed, i. e.,

$$\frac{B_d'}{s_e s_s k e^s r_o} \text{♀} \times \frac{s_e s_s k e^s r_o}{s_e s_s k e^s r_o} \text{♂}$$

P<sub>2</sub> 1 normal female of above type was back-crossed, i. e.,

$$\frac{C' b_d^?}{s_e s_s k e^s r_o} \text{♀} \times \frac{s_e s_s k e^s r_o}{s_e s_s k e^s r_o} \text{♂}$$

F<sub>2</sub> Expected count from back-cross, in which there appeared ordinary amount of crossing over, and beaded flies in usual proportions.

F<sub>2</sub> Unexpected count, in which there was no crossing over, and no flies appeared beaded; flies thus of two kinds only:

$$\frac{C' b_d^?}{s_e s_s k e^s r_o} \text{ (normal appearing) and } \frac{s_e s_s k e^s r_o}{s_e s_s k e^s r_o}$$

$$P_3 \frac{C' b_d^?}{s_e s_s k e^s r_o} \text{♂} \times \frac{D_i' p s_s}{p s_s} \text{♀}$$

F<sub>3</sub> Flies of four expected classes obtained, namely:

$$\frac{p s_s}{s_e s_s k e^s r_o}, \frac{D_i' p s_s}{s_e s_s k e^s r_o}, \frac{p s_s}{C' b_d^?}, \frac{D_i' p s_s}{C' b_d^?}$$

P<sub>4</sub>  $\frac{D_i' p s_s}{C' b_d^?}$  crossed together to obtain homozygous  $\frac{C' b_d^?}{C' b_d^?}$ , i. e.,

$$\frac{D_i' p s_s}{C' b_d^?} \text{♂} \times \frac{D_i' p s_s}{C' b_d^?} \text{♀}$$

F<sub>4</sub> A few normals.

many deformed  $\frac{D_i' p s_s}{C' b_d^?}$

and a few  $\frac{D_i' p s_s}{D_i' p s_s}$

P<sub>5</sub> Normals bred together

deformed bred together

deformed pink spineless bred together.

F<sub>5</sub> Same result as from deformed flies, proving parents to have been

$$\frac{D_i' p s_s}{C' b_d^?}$$

no  $\frac{C' b_d^?}{C' b_d^?}$  obtainable.

Count similar to generation above.

P<sub>6</sub> All flies allowed to cross indiscriminately.

No offspring at all.

F<sub>n</sub> Similar result in every generation.

when it is in *heterozygous* condition (MULLER 1916). The same sort of results had been found in STURTEVANT'S experiments with the similar factor in the second chromosome (STURTEVANT 1917).

It will be seen that the unusual linkage relations shown in the back-cross of the non-beaded  $F_1$  female are similar to those occurring when  $C'$  is heterozygous—crossing over is lowered in the same regions and to the same extent in both cases. And we may anticipate by saying that later generations showed these effects to have been produced here also by a definite factor, lying in the third chromosome, and located somewhere near *sooty*. This factor, which may for the present be called  $C'_b$ , had never been met with in the  $s_e s_s k e^s r_o$  stock, and it was therefore probable that it lay in the chromosome derived from the beaded stock, as indeed the later results proved to be true.

The second remarkable feature of the count from the normal-winged  $F_1$  female is that not one offspring with beaded wings was produced from this fly; if the factor for beaded was present at all here it must therefore have been completely recessive, either on account of absence of the intensifier or for some other reason. But absence of the factor for beaded itself, or of the intensifier either, in the  $F_1$  female, seemed contra-indicated by the high degree of beading in all the flies of the parental beaded stock. As for the other mutant factors— $s_e$ ,  $s_s$ ,  $k$ ,  $e^s$ , and  $r_o$ —these appeared with their expected frequencies.

It was now important to determine whether the factor for beaded was merely recessive and "latent" in these flies, or really absent. To do this it was necessary to obtain flies homozygous for the chromosome derived from beaded stock, and to see whether they showed the beaded character. The normal-appearing offspring of this back cross were heterozygous for this chromosome, which we may temporarily call " $C'_b b_a$ ?", they had received from their father the chromosome containing  $s_e s_s k e^s r_o$ . Their composition therefore was  $\frac{C'_b b_a?}{s_e s_s k e^s r_o}$  (see  $F_2$  in diagram). It should be possible to obtain homozygous  $\frac{C'_b b_a?}{C'_b b_a?}$  by crossing these heterozygotes to each other, but any normal-appearing  $\frac{C'_b b_a?}{C'_b b_a?}$  individuals produced in such a cross might not be distinguishable from the heterozygotes produced at the same time, and so it would not be possible to tell with accuracy what proportion of the  $\frac{C'_b b_a?}{C'_b b_a?}$

flies (if any) showed the beaded character. A special scheme of crossing was accordingly followed, in order finally to get a count in which the  $\frac{C_b' b_a?}{C_b' b_a?}$  flies would be identifiable through some other character than beaded; in such a count, then, the number of these homozygotes could be accurately determined, and the proportion of beaded among them could be found out by inspection. The dominant character deformed eye (factor  $D_f'$ ) was chosen as the means of identification. Flies were made up in which a chromosome containing  $D_f'$  was substituted for the one containing  $s_e s_s k e^s r_o$ ; on crossing the  $\frac{C_b' b_a?}{D_f'}$  flies so obtained to

each other the homozygous  $\frac{C_b' b_a?}{C_b' b_a?}$  progeny would be distinguishable from the other flies, because they alone would *not* show the dominant deformed. The ratio of beaded among them could then be readily ascertained.

The following were the actual operations performed in accordance with this plan. Normal-appearing male flies of the composition  $\frac{C_b' b_a?}{s_e s_s k e^s r_o}$ , which had resulted from the back cross, were crossed to

females from a stock of deformed eye, pink eye, and spineless integument, which I had previously made up for the study of linkage (see  $P_3$  in diagram). The stock was not pure for deformed, but since this factor is a dominant (though rather irregularly), it was possible to pick out, among the offspring of the cross, those flies which had received a  $D_f' p s_s$  chromosome from their mother. Half of such offspring must have received from their father the  $s_e s_s k e^s r_o$  chromosome—their composition would be  $\frac{s_e s_s k e^s r_o}{D_f' p s_s}$ ; the other half must have received

$C_b' b_a?$ , and their composition would be  $\frac{C_b' b_a?}{D_f' p s_s}$ . The former, be-

ing homozygous for spineless, were distinguishable from the latter, which were of course perfectly normal except in regard to their deformed eye. The  $\frac{C_b' b_a?}{D_f' p s_s}$  offspring were then mated to each other

(cross  $P_4$ ) in order to get the flies ( $F_4$ ) homozygous for  $C_b' b_a?$ . It is in this latter cross that these homozygous  $\frac{C_b' b_a?}{C_b' b_a?}$  offspring should be dis-

tinguishable, for the most part at least, from the other types of offspring, because of the presence in almost all the others of the factor deformed, which is usually dominant. (Theoretically, deformed might not occur in quite all the heterozygous flies, on account of crossing over, but such discrepancy due to crossing over should be very slight, because the presence of  $C_b'$  would prevent almost all crossing over in the regions containing the factors under consideration.) The results of the cross

( $F_4$ ) should accordingly be nearly 1  $\frac{D_f' p s_s}{D_f' p s_s}$  (appearance  $D_f' p s_s$ ):  
 2  $\frac{D_f' p s_s}{C_b' b_a?}$  (appearance  $D_f'$ ): 1  $\frac{C_b' b_a?}{C_b' b_a?}$  (appearance normal or

beaded). The  $\frac{C_b' b_a?}{C_b' b_a?}$  flies would thus be distinguishable from the others, and the proportion of beaded appearing in them could be determined.

But again the results played hide-and-seek with the experimenter. Strange to say, no homozygous  $\frac{C_b' b_a?}{C_b' b_a?}$  were produced at all, and the object of the experiment was apparently defeated. The very fact that this class of flies did not appear, however, seemed to afford an important clue to the constitution of the  $C_b' b_a?$  chromosome, and hence of the beaded stock, as will appear presently. But not only did the homozygous  $\frac{C_b' b_a?}{C_b' b_a?}$  flies fail to appear in this count; the other types of homozy-

gotes expected, namely the  $\frac{D_f' p s_s}{D_f' p s_s}$ , for the most part failed to appear also. Thus the offspring of this cross were nearly all of the heterozygous type  $\frac{D_f' p s_s}{C_b' b_a?}$  like their parents; they showed deformed eye,

in varying intensity, but no other mutant characters (see  $F_4$  of diagram). A very few, however, had extremely deformed eyes, and also showed the pink and spineless characters: these must have been homozygous for the  $D_f' p s_s$  chromosome,  $\frac{D_f' p s_s}{D_f' p s_s}$ . A very few others

also appeared, that were perfectly normal, but breeding tests on some of the latter proved these to have been really heterozygotes in which deformed had failed to dominate, for they produced deformed-eyed off-

spring. Now when heterozygous flies of the generation which has just been described were crossed with each other, they again gave results like the preceding one, and this was continued generation after generation. The mixed stock was then allowed to run in mass-culture without supervision or selection of parents for over six months (over 14 generations), and at the end of that time it was still in the same condition as before.

It is true that the frequency with which the heterozygotes manifested deformed varied greatly, apparently in response to environmental influences, and that the proportion of homozygous  $D_f' p s_s$  also varied markedly. Still, the great bulk of the flies was of the type expected for the heterozygotes; homozygous  $C_b' b_a?$  were never found to be produced, and the number of homozygous  $D_f' p s_s$  did not tend to increase as it would in any ordinary case in which the heterozygotes and one class of homozygotes were allowed to interbreed in every generation. The stock thus approached the condition seen in "constant hybrids."

Various non-Mendelian interpretations of the constant hybridism—such as blending or imperfectly alternative inheritance seemed now in order, and the fact that these phenomena appeared in flies descended from beaded stock might appear to strengthen much more than ever before the view that beaded also was not a strictly Mendelizing character. It was now possible only by means of certain very special assumptions to hold on to the strict Mendelian point of view; nevertheless this was rigorously followed and the interpretation which was thus arrived at proved indispensable in investigating and explaining the further developments.

On the view that Mendelian inheritance was taking place, the absence of most of the  $D_f' p s_s$  homozygotes could best be explained by assuming that there was a "semi-lethal" factor in the  $D_f' p s_s$  chromosome, which usually killed the fly in which it was homozygous, although not invariably. Support was found for this latter assumption in the weakness and infertility of the homozygous  $\frac{D_f' p s_s}{D_f' p s_s}$  flies which did appear, and actual proof of it was obtained when it was found that the expected proportion of homozygous  $\frac{D_f' p s_s}{D_f' p s_s}$  are hatched if environmental conditions are very favorable; it was further found that this semi-lethal effect is produced by the factor for deformed itself. From this it was not a long step to the assumption that absence of all the

homozygous  $\frac{C'_b b_a?}{C'_b b_a?}$  flies also was due to a factor which exerted a lethal effect when homozygous—in this case, however, the factor must be completely lethal, and it would necessarily be a different factor from the semi-lethal in the  $D'_f p s_s$  chromosome—otherwise the heterozygous  $\frac{D'_f p s_s}{C'_b b_a?}$  flies would die too as they would be pure for the lethal.

On the Mendelian view, then, the  $C'_b b_a?$  chromosome, which came originally from the beaded stock, probably contained a lethal factor. It might here be imagined that all three of the peculiarities that were found in this chromosome were due to one factor,—that (1) when homozygous this factor killed the fly, and when heterozygous (2) it reduced the amount of crossing over, and (3) inhibited beadedness so far as to make beaded recessive. But this would seem a peculiar coincidence of effects for one factor, and it seemed more likely that the lethal effect (1) and the effect on crossing over (2) were due to two different factors, and that the absence of the beaded character (3) was due merely to the lack of the factor for beaded (i.e., to the presence of its normal allelomorph instead). A theory of the inheritance of beaded was then formulated on the basis of this conclusion in regard to the  $C'_b b_a?$  chromosome, and by using as an analogy the above postulated mechanism of constant hybridism in the  $\frac{D'_f p s_s}{C'_b b_a?}$  stock. Although looking far-fetched at

first sight, upon further consideration it appeared to the writer most probable, and was used as the working hypothesis for the investigations subsequently to be described. The theory was as follows:

(1) The factor for beaded is itself lethal when homozygous, or, if not directly lethal, it at least makes the homozygous beaded flies of one sex or both sterile. Not only would this general postulate account for the early difficulty in getting pure stock, and for the reversion to normal which occurred in the early stock when selection was discontinued, but it would also explain why a non-beaded chromosome was found even in the apparently pure beaded stock that was evolved later. For on this theory it would be impossible ever to secure a homozygous race of beaded flies, and the seemingly pure stock must really be a heterozygous stock in which, by some mechanism, non-beaded flies are prevented from appearing. The nature and origin of this latter mechanism will next be considered.

(2) During the course of the selection experiments that were under-

taken to get pure stock, there must have appeared by mutation, or have been introduced by crossing, into the flies in one or more of the bottles, a lethal factor lying in the third chromosome that contained the normal allelomorph of beaded, and located in its right-hand portion. Therefore in this bottle not only would the flies homozygous for beaded die, as before, but those homozygous for the normal allelomorph of beaded would now die also, *except* in the cases in which this normal allelomorph had become separated by crossing over from the new lethal factor. The bottle containing this new lethal would hence give a much lower proportion of normals than the other bottles, and so it would be selected to supply the parent flies for the next generation.

(3) In the same chromosome in which the lethal factor arose, the factor *C'* also must have been present, or must have arisen subsequently by mutation. Thus crossing over between the new lethal and the normal allelomorph of beaded would be prevented, and all flies homozygous for the normal allelomorph of beaded would die. Such a stock would give an even higher percent of beaded than that described in (2) and would, therefore, have been perpetuated in the selection experiments when it was found. In this stock, then, only flies heterozygous for beaded could appear, and these would breed true to their peculiar type because all homozygotes of both classes would now die. Even in this stock, however, all the flies would not show the beaded *character*, if the stock was not pure for the intensifier.

(4) When, in the course of selection, the intensifier which lies in the second chromosome was introduced, or arose by mutation, flies containing it would be chosen, on account of their greater degree of breeding. There was no reason to believe that the intensifier was a lethal, and the constant hybrids above discussed might hence become pure for the intensifier, if they were not so already; thus the flies of the final stock would *all* show beadedness in rather marked degree. In the process of getting the stock pure for the intensifier the average intensity of the beading would have been increased, and this would account for the success of the selection experiments in this respect.

The further history of the beaded case has consisted in a substantiation of this theory in all its details, together with the finding of correlative facts. Besides this, considerations will be given which may make more comprehensible the reasons for the concurrent appearance of so many peculiarities in one case, and the general bearings of the findings will be discussed.

## SUBSTANTIATION OF THE HYPOTHESIS, AND CORRELATIVE EXPERIMENTS

(1) *Existence of two kinds of chromosomes in beaded stock verified*

The first requirement, in the testing of the hypothesis, was to confirm the finding of a non-beaded, lethal,  $C'$ -bearing chromosome in the "pure" beaded stock. Several beaded flies were therefore taken from this "pure" stock, and each was outcrossed separately to a fly from some non-beaded stock.

The first cross was of a beaded female by homozygous  $s_e s_s k e^s r_o$  male. The  $F_1$  flies consisted of: beaded, 16♀, 11♂; not beaded, 24♀, 13♂. The second cross was of the same nature and resulted in beaded, 12♀, 11♂; not beaded, 13♀, 7♂. Although the beaded mothers were decidedly infertile, nevertheless these counts are large enough to give a strong indication of a 1 : 1 ratio.

The third cross was of a beaded male by  $\frac{s_e D' p s_s k e^s r_o}{s_e p s_s k e^s r_o}$ . The mutant factors in the female used all lie in the third chromosome and all are recessive except  $D'$ .  $D'$  is the symbol for the dominant mutant factor called "dichaete," which causes the wings to be spread and which reduces the number of spines on the thorax. In this cross the  $F_1$  flies should all look normal except with regard to the maternal character *dichaete*, which half of them should possess, and the paternal character *beaded*, which is under investigation. The results were 50 beaded (including 31 *dichaete*) and 62 non-beaded (including 24 *dichaete*).

The results of these crosses, which have since been amply corroborated, would ordinarily be taken to mean that the beaded parent was heterozygous, since approximately half the offspring were beaded in each case. Yet it was possible that the difference between the normal and beaded offspring was merely somatic, and due to the varying dominance of *beaded*, especially since the beaded offspring in all the crosses were of various grades, merging into normal, and were all of a much less marked type than that of their beaded parents. Moreover, two other crosses, of beaded by black-bodied flies, gave a very much lower proportion of beaded in  $F_1$  than did the above crosses.

To determine whether the normal  $F_1$  offspring were really different genetically from the beaded, and contained a non-beaded, lethal,  $C'$ -bearing chromosome, eight *dichaete* females from the third count—four beaded and four non-beaded—were back-crossed separately to  $s_e s_s k e^s r_o$  males. The results are shown in table 1. The classification in regard to kidney is not given, as the determination of this character is rather



TABLE I

Crossing over between :	Characters shown	A	B	C	D	Total A-D	E	F	G	H	Total E-H
(No crossing over)	{ Beaded Normal $s_e D' s_s e^s r_o$	53 8 32	13 7 7	11 5 4	14 3 14	91 23 57		1 78 52		27 29 28	1 184 145
1. $s_e$ and $D'$	$s_e$ { Beaded Not beaded $D' s_s e^s r_o$	9 11	3 2	0 0	4 2	16 1 15	18 14	8 8	5 3	5 2	36 27
2. $D'$ and $s_s$	$s_e D'$ { Beaded Not beaded $s_s e^s r_o$	3 1 8	1 1 5	2 0 4	5 0 5	11 2 22	5 5	8 7	2 0	1 3	16 15
3. $s_s$ and $e^s$	$s_e D' s_s$ { Beaded Not beaded $e^s r_o$	7 0	1 2	1 1	4 0 1	13 0 4				1	0 0 1
4. $e^s$ and $r_o$	$s_e D' s_s e^s$ { Beaded Not beaded $r_o$	3 5 12	2 0 5	1 1 3	3 0 8	9 6 28					0 0 0
5. $r_o$ and $B_d'$	$s_e D' s_s e^s r_o B_d'$ (Others of this class not distinguishable)	1	0	1	0	2					0
1; 2	$s_e$ and $D'$ ; $D'$ { Beaded Not beaded $D'$ and $s_s$ $s_e s_s e^s r_o$	1 1 1	0 0 1	0 0 0	0 0 0	1 1 2	2	0	1	0	0 0 2
1; 3	$s_e$ and $D'$ ; $D' s_s$ { Beaded Not beaded $s_s$ and $e^s$ $s_e e^s r_o$	0 0	1 1	0 0	0 0	1 1					0 0
1; 4	$s_e$ and $D'$ ; $D' s_s e^s$ { Beaded Not beaded $e^s$ and $r_o$ $s_e r_o$	1 1				0 1 1					0 0 0
2; 3	$D'$ and $s_s$ ; $s_s$ { Beaded Not beaded $s_s$ and $e^s$ $s_e D' e^s r_o$					0 0 1					0 0 0
2; 4	$D'$ and $s_s$ ; $s_s e^s$ { Beaded Not beaded $e_s$ and $r_o$ $s_e D' r_o$	2 3	0 2	1 2	1 0	4 0 7					0 0 0
2; 5	$D'$ and $s_s$ ; $s_s e^s r_o B_d'$ (Others of this class not distinguishable)				1	1					0
3; 4	$s_s$ and $e^s$ ; $e^s$ { Beaded Not beaded $e^s$ and $r_o$ $s_e D' s_s r_o$	1	0	1	0	2 0 0					0 0 0
1; 3; 4	$s_e$ and $D'$ ; $s_s$ and $e^s$ ; $e^s$ and $r_o$ $s_e e^s B_d'$ (Others of this class not distinguishable)	1				1					0
1; 4; 5	$s_e$ and $D'$ ; $e^s$ and $r_o$ ; $r_o$ and $B_d'$ $s_e r_o B_d'$ (Others of this class not distinguishable)				1	1					0
Totals		166	54	38	68	325	174	128	66	63	431

uncertain, and of little consequence for the present purposes. The counts from the beaded mothers have been designated as A-D, and those from the four which did not show beaded in their wings as E-H.

One non-beaded female (I) which had not received the factor  $D'$ , and which was therefore of composition  $\frac{s_e p s_s k e^s r_o}{C_b' ? b_d'}$  was also backcrossed to  $s_e s_s k e^s r_o$  males. It gave the following count. Non-crossovers: beaded 0, normal 81,  $s_e s_s k e^s r_o$  55; crossovers: between  $s_e$  and  $s_s$ — $s_e B_d'$  0,  $s_e$  30,  $s_s k e^s r_o$  20; between other loci, 0.

The results tabulated demonstrate that the  $F_1$  females fell into two distinct genetic classes, corresponding with the phenotypic classes non-beaded and beaded. From the five non-beaded  $F_1$  females no beaded offspring at all were produced, except one fly with wings slightly cut into at the tip, and this one exception was proved by later breeding tests to be due to a mutation in a different factor for beaded than that under investigation, lying in a different chromosome. From the four beaded  $F_1$  females, on the other hand, beaded flies were produced in the expected numbers, and the factor for beaded here concerned was shown, by its linkage relations to be the ordinary one. Secondly, the five non-beaded  $F_1$  females all agreed in having practically no crossing over in the portion of the third chromosome to the right of  $s_s$ . They therefore contained the factor  $C'$ , or a similar factor. The four beaded  $F_1$  females, however, all produced a considerable number of crossovers (in proportions which will be discussed later).

The genetic difference between the  $F_1$  females in regard to the factor for beaded must have been due to heterozygosis in the  $P_1$  "pure beaded" fly, and since the distribution of  $C_b'$  among the  $F_1$  females was completely correlated, negatively, with the distribution of the beaded (being exactly opposite to it), the factor  $C_b'$  too must have been derived from the "pure beaded"  $P_1$  fly (not from the  $s_e D' p s_s k e^s r_o$   $P_1$  fly), and it must have lain in that chromosome of the beaded fly not containing the dominant beaded.

Since the non-beaded  $C_b'$ -bearing chromosome had thus persisted in the beaded stock for several months after the preliminary crosses (pp. 429-431) were made, it was clear that this chromosome was not a mere transient intruder in the race. Moreover, it will be recollected that all three  $P_1$  beaded flies which were reported in the present section as having been outcrossed to multiple recessive females gave similar counts in  $F_1$ , and so all three of them were probably of the same heterozygous composition as the one whose daughters ( $F_1$  females) were tested.

(2) *Crossover frequencies of the two chromosomes; position of beaded*

Before proceeding with a further inquiry into the composition of the "pure" beaded stock, the linkage ratios obtained in the back cross of these  $F_1$  females should be considered in more detail. It will be seen that the counts obtained from the beaded females conform in orthodox fashion to the law of linear linkage. The data clearly show that the factors are linked in line in the order  $s_e D' s_s e^s r_o$  (omitting consideration of beaded for the moment), and that crossing over involves a breakage of this chain of factors usually at only one, and very rarely at more than two points, as in the author's previous experiments with factors in this chromosome. The following are the percents of crossing over shown here between adjacent loci, as compared with the percents previously observed, which are placed in parenthesis:  $s_e D'$  13.2 (10);  $D' s_s$  16.3 (13.5);  $s_s e^s$  7.1 (11.5);  $e^s r_o$  18.4 (20). The agreement is as close as would be expected for a count of this size, considering the evidently poor viability of certain classes, as indicated by the discrepancy in size between contrary classes like  $s_e D' s_s$  and  $e^s r_o$ , which should be equal.

The number of coincident crossings over which took place in various positions, as compared with the number which would be expected if crossings over did not interfere with one another, were as follows: In regions 1 and 2 (i.e., between  $s_e$  and  $D'$  and between  $D'$  and  $s_s$ ), 5 (7); in 1 and 3, 3 (3); 1 and 4, 4 (7.9); 2 and 3, 1 (3.8); 2 and 4, 11 (9.8); 3 and 4, 3 (4.3). This list includes all the combinations of crossings over, considered two at a time, which occurred in both the double and the two triple crossovers. It will be seen that the observed are almost always somewhat less than the "expected" values, i.e., that interference does occur here, just as in the case of the other two chromosomes in which linear linkage has been found. So far as they go, the figures indicate also that the interference is greater between positions that lie closer together in the chromosomes. For there were a total of 9 cases of coincident crossing over between adjacent regions (1 and 2, 2 and 3, 3 and 4), as compared with the expected number 15.1, whereas in regions more widely separated (1 and 3, 1 and 4, 2 and 4), there was a total of 18 observed cases, as compared with the expected number 20.7. The proportion which the observed formed of the expected values, i.e., the coincidence ratio, was in the first case 60 percent, in the second 87 percent. This increase of interference accompanying increase of distance has been observed in chromosomes I and II also.

In determining the percent of crossing over of beaded with the other factors, only the offspring having beaded wings can furnish reliable data, since the non-beaded are a heterogeneous class, containing among them many genetic beaded in which the beaded character failed to manifest itself. This is to be expected because only half the offspring in this cross could have received the second-chromosome intensifier of beaded, and even these must have been heterozygous for it.

Most of the non-beaded flies that are recorded in the table in the same bracket with the beaded are accordingly genetic beaded, but as we cannot be sure of the exact number of genetic beaded in any of these cases, only those flies actually showing beaded should be considered; here we can be sure of the genetic constitution. Of the 153 beaded offspring, only 4 (or 2.7 percent) had rough eyes; this shows that the locus of beaded is approximately 2.7 units from that of rough. That it is to the right<sup>3</sup> of rough is shown by the fact that only one of the crossings over between rough and beaded involved also a separation between rough and sooty, which is directly to the left of rough. Thus the factor for beaded occupies at present a terminal position in a map of the third chromosome, being about two units farther to the right than rough, which is itself to the right of all the other factors so far studied.

The above results are from the  $F_1$  females A, B, C and D. The counts from E, F, G, H and I, the non-beaded  $F_1$  females, gave a total of 617 flies, with the following percents of crossing over (the percents found in previous experiments involving  $C'$  are shown in parenthesis):  $s_8 s_8$  24.8 (20.9),  $s_8 e^8$  0.2 (0.2)  $e^8 r_0$  0.0 (0.0). Of these 617 flies, 431 were in counts from the females carrying the factor  $D'$ ; these gave the percent of crossing over for the distance  $s_8 D'$  as 15.3, and for  $D' s_8$  as 7.9 (11.6). There were 3 double crossovers in this count, to be compared with an "expectation" of 5.2; a coincidence ratio of 60 percent is not unusual for such a distance.

### (3) *The lethal effect of the chromosome containing $B_d'$*

The fact that all the "pure-stock" flies were beaded in character and that nevertheless all those tested proved to be heterozygous for a dominant beaded at least, in itself indicated strongly that homozygotes of both types died before reaching maturity, or, in other words, that both types of third chromosome in this stock carried a lethal factor. Further

<sup>3</sup> Which end of the chromosome shall be called right and which left is a purely arbitrary matter, but once this has been decided upon for any two factors the case in regard to the others in that chromosome is of course determined.

tests were made, however, to determine these points more certainly and to analyze the chromosome more in detail. Proof of the lethal effect of the  $B_d'$ -containing chromosome will be given first.

Table 1 shows that in the back cross of  $F_1$  beaded females one spineless kidney sooty rough beaded fly was produced by crossing over. Its composition must have been  $\frac{p s_s k e^s r_o B_d'}{s_e s_s k e^s r_o}$ . This individual (a female)

was crossed to a fly from a stock with spread wings, which contains the factor  $C'$ . The beaded offspring of this mating accordingly had the composition  $\frac{(s_e p) s_s k e^s r_o B_d'}{s_d C'}$  (some of them contain  $s_e$  and  $p$ ,

others do not). Practically no crossing over will occur in such flies between the factors to the right of  $s_s$ , and if the flies are interbred with each other all such factors on either side of the horizontal line may therefore be considered *en bloc*.<sup>4</sup> The expectation, so far as these factors is concerned, would be 1 pure spread: 2 beaded or normal-appearing heterozygotes of composition similar to the above: 1 homozygous for  $s_s k e^s r_o B_d'$ , which latter would show all these characters (except, possibly, beaded and kidney). The observed count from an intercross of this sort was as follows: spread 50, beaded and normal 154 (consisting of 93 beaded and 61 normal, and including 23 with sepia and 1 with pink eyes),  $s_s k e^s r_o B_d' o$ . The beaded were of very different intensities, of course, owing to the independent segregation of the intensifier.

It is quite evident from this result that with the introduction of  $B_d'$  into the  $s_s k e^s r_o$  chromosome the latter has become lethal, inasmuch as all flies homozygous for this chromosome now fail to appear, whereas ordinarily there is no difficulty in securing homozygous  $s_s k e^s r_o$ . That this lethal effect must be due to the factor for beaded itself, or some factor very close to it, is shown by the fact that the lethal  $s_s k e^s r_o B_d'$  chromosome was the result of a crossing over between  $r_o$  and  $B_d'$ , and so it could have contained only the very tip of the chromosome derived from beaded stock, namely, just that part in which lay the factor  $B_d'$  itself.

<sup>4</sup> To facilitate cross reference and the following of pedigrees all the mutant factors present in a fly are shown whenever its genotypic constitution is represented, no matter whether or not all of these mutant factors are essential for the purpose of the particular cross in question. The presence of additional mutant factors will not lead to confusion, however, if the distribution of the group of factors in each chromosome is thought of *en bloc*, as it may be in most cases, instead of in terms of the individual factors.

Several crosses similar to the above were also made, and yielded similar results. Thus a sooty beaded fly from the back cross of  $F_1$  beaded females was crossed to a fly from the  $\frac{D'_f p s_s}{C'_b l}$  stock discussed in the previous section. (The letter  $l$  is used to designate the lethal factor which this chromosome is assumed to contain.) Two  $\frac{e^s B'_d}{C'_b l}$  offspring were then mated to each other and produced 60 offspring with various degrees of beaded, 33 normals, and no sooties. The junction of the  $B'_d$  with sooty had prevented the homozygous sooty from appearing. In another instance a spineless kidney sooty beaded fly from the back cross was mated to  $\frac{D'_f p s_s}{C'_b l}$ , and the  $\frac{s_s k e^s B'_d}{C'_b l}$  offspring, when interbred, gave 89 beaded (of different degrees), 14 normal, all other types 0. By crossing spread flies with  $s_e D' p s_s k e^s B'_d$  flies derived from the back cross given on p. 438, offspring of the types  $\frac{s_e D' p s_s k e^s B'_d}{s_d C'}$  and  $\frac{s_e s_s k e^s B'_d}{s_d C'}$  were obtained, and on mating the latter to each other the characters of  $s_s$ ,  $k$  and  $e^s$  were again conspicuous by their absence. In each case  $B'_d$  prevents recessive factors that are joined with it from being homozygous and manifesting themselves.

The factor  $B'_d$  is, therefore, similar to the factor for yellow in mice, in that it has two distinct effects—a visible “superficial” effect, wherein it is dominant, and a lethal effect, in regard to which it is recessive.

#### (4) Analysis of the $C'$ -bearing chromosome

The experiments on which a more exact determination of this chromosome was based began with a cross of beaded female by  $s_e s_s k e^s r_o$  male, which gave results like those of the similar crosses already reported, that is, about half of the  $F_1$  flies beaded and half normal-winged. To check the composition of the original beaded fly further, three of the beaded  $F_1$  females were bred with their beaded brothers. All of them gave  $F_2$  counts of the predicted type, showing the usual amount of crossing over, and the expected proportion and distribution of beaded (including one crossover between  $r_o$  and  $B'_d$ , of type  $s_e s_s k e^s r_o B'_d$ ).

It was the non-beaded  $F_1$  flies, however, which contained the  $C'_b$ -bearing chromosome now under consideration. Two of the normal-winged  $F_1$  females were therefore chosen also, and they were bred to

their normal-winged brothers. As this was not a back cross to a non-beaded stock, but an interbreeding of the  $F_1$ , there was a chance here for beaded to appear if it were really present in recessive condition in the  $C'_b$ -bearing chromosome, and if this chromosome could be obtained homozygous. Since beaded did not appear, one or both of these premises must be incorrect. The  $F_2$  count consisted of normal 45,  $s_e s_e k e^s r_o$  7,  $s_e s_e k e^s r_o$  5,  $s_e k e^s$  1, beaded 0. These results, it will be seen, also give a confirmation of the previous ones in showing the presence of  $C'_b$ , for, whereas the factors in the left-hand portion of the chromosome cross over almost as freely as when  $C'$  is absent, those to the right (near  $C'$ ) cross over hardly at all.

Crossing over was not entirely prevented in the extreme right-hand end, however, for the one  $s_e k e^s$  fly must have resulted from a crossing over between  $e^s$  and  $r_o$  (as well as coincidentally in the left-hand end, between  $s_e$  and  $s_e$ ). The appearance of this one crossover was a fortunate circumstance, for it provided a dissection of the non-beaded  $C'_b$ -bearing chromosome of beaded stock which is now under analysis. It was important, therefore, to study the properties of this crossover chromosome, to find out whether it contained  $C'_b$  and whether it contained a lethal factor.

In order to find out whether it contained  $C'_b$ , the  $s_e k e^s$  crossover fly, which was a male of genetic composition  $\frac{s_e k e^s C'_b? l?}{s_e s_e k e^s r_o}$ , was crossed to a beaded female, and one of the daughters, which proved to be of composition  $\frac{s_e k e^s C'_b? l?}{B'_d}$  was back-crossed to homozygous  $p s_e k e^s r_o$ .

The count, as regards  $s_e$ ,  $e^s$ , and  $B'_d$ , consisted of non-crossovers  $s_e k e^s$  47, not spineless or sooty 46 (25 of these showed beaded); crossovers between  $s_e$  and  $e^s$  -  $s_e$  12 (including 4 phenotypic  $B'_d$ ),  $e^s$  7; between  $e^s$  and  $B'_d$  -  $s_e e^s B'_d$  7, the contrary class to the latter, namely the normals, are included with  $B'_d$  non-crossovers that failed to show  $B'_d$ , as these two classes are alike phenotypically. The high percent of crossing over here shown excludes the possibility of  $C'_b$  having been present.

The original  $\frac{s_e k e^s C'_b? l?}{s_e s_e k e^s r_o}$  male was also bred to a  $\frac{s_e D' p s_e k e^s r_o}{s_e p s_e k e^s r_o}$  female. A daughter was chosen which had received from her father the chromosome in question and from her mother a chromosome con-

taining  $D'$ . Hence her composition was  $\frac{s_e k e^s C'_b ? l ?}{s_e D' p s_e k e^s r_o}$ . This fly

was back-crossed to a  $p s_e k e^s r_o$  male. As all the offspring were homozygous for  $s_e k$  and  $e^s$ , and none were homozygous for  $s_e$ , the distribution of only  $D'$ ,  $p$  and  $r_o$  could be followed. The result was: non-crossovers  $D' p r_o$  12, normal (for characters considered) 26; crossovers between  $D'$  and  $p$ — $D' 2$ ,  $p r_o 2$ ; between  $p$  and  $r_o$ — $D' p 17$ ,  $r_o 25$ ; double crossovers  $D' r_o 1$ ,  $p 1$ . Here too, then, the frequency of crossing over proves that the factor  $C'_b$  was not present. This freeness of crossing over was also shown when a female from the above brood, of composition

$\frac{s_e D' s_e k e^s C'_b ? l ?}{p s_e k e^s r_o}$ , was back-crossed to a  $s_e s_e k e^s r_o$  male. A

sample count with regard to  $s_e$ ,  $D'$  and  $r_o$  gave: non-crossovers —  $s_e D' 13$ ,  $r_o 21$ ; crossovers between  $s_e$  and  $D' - D' 2$ ,  $s_e r_o 3$ ; between  $D'$  and  $r_o - s_e D' r_o 8$ , normal (for characters considered) 13, double crossovers  $D' r_o 0$ ,  $s_e 2$ .

It is therefore clear that the original  $s_e k e^s$  crossover fly did not contain  $C'_b$  even though it had received that part (the right-hand end) of the  $C'_b$ -bearing chromosome which carried the normal allelomorph of rough.  $C'_b$  therefore must lie to the left of the locus of rough.

Crosses were also made to determine whether the  $s_e k e^s$  fly had received a lethal factor. For if the supposed lethal effect of the  $C'_b$ -bearing chromosome of the beaded stock were due to a different factor than the effect on crossing over, it was quite possible that the crossover fly had received the lethal in spite of its not having received  $C'_b$ . From the

above cross of  $\frac{s_e k e^s l ?}{s_e D' p s_e k e^s r_o}$  female by homozygous  $p s_e k e^s r_o$  male

(the second cross of the preceding paragraph but one) two  $s_e D' p s_e k e^s l ?$

$\frac{p s_e k e^s r_o}{p s_e k e^s r_o}$  male offspring were chosen. A glance at the formulae

of the mother will show that these males must have resulted from a crossing over somewhere between  $p$  and  $r_o$ . It is evident also that if this crossing over had occurred to the right of the hypothetical " $l$ ", these flies, which contained only the right hand end of the  $s_e k e^s l ?$  chromosome would necessarily fail to have the lethal factor, but if the crossing over had occurred to the left of  $l$ , they must contain it, provided that such a factor existed in the parent fly. The subsequent crosses showed that one of the two males lacked the supposed lethal factor, but



that the other male contained it. A lethal factor must consequently have been present originally, and the first of the two males was thus a crossover to the right of  $l$ , the second a crossover to the left of it.

The evidence that  $l$  was absent in the first male was obtained by crossing it to a female of composition  $\frac{s_s k e^s B_d'}{C_b' l?}$ ;  $C_b' l?$  here represents a non-beaded chromosome derived from pure beaded stock. Now, if the supposed lethal were present in the  $\frac{s_e D' p s_s k e^s l?}{p s_s k e^s r_o}$  male it would also be present in the  $C_b' l?$  chromosome of the female (as in the former fly it would have been derived from a prototype of the latter chromosome). All flies resulting from the union  $\frac{s_e D' p s_s k e^s l?}{C_b' l?}$  would in that case die; only the other three possible combinations could survive, namely,  $\frac{s_r D' p s_s k e^s l?}{s_s k e^s B_d'}$  (appearing  $D' s_s e^s B_d'$ ),  $\frac{p s_s k e^s r_o}{C_b' l?}$  (appearing normal), and  $\frac{p s_s k e^s r_o}{s_s k e^s B_d'}$  (appearing  $s_s k e^s B_d'$ ). But all four types appeared, in nearly equal numbers. Hence there was no lethal factor "l" in the father.

The other  $\frac{D' p s_s k e^s l?}{s_s k e^s r_o}$  male, on the other hand, gave different results. It was crossed to a beaded female from pure stock, and therefore presumably of composition  $\frac{B_d'}{C_b' l?}$ . If lethal is present in both parents only half as many dichæte as non-dichæte flies should appear, for those zygotes of composition  $\frac{D' p s_s k e^s l}{C_b' l}$  will die. The flies which survive will be dichætes of composition  $\frac{D' p s_s k e^s l}{B_d'}$  (appearing also beaded in most cases) and non-dichætes of the two types  $\frac{p s_s k e^s r_o}{C_b' l}$  (normal!), and  $\frac{p s_s k e^s r_o}{B_d'}$  (beaded). The count was  $D'$  6 (including 4 beaded); not dichæte 28 (including 12 beaded). As the count was

scarcely significant, owing to the infertility of the beaded female, the crosses were carried further. One of the  $\frac{D' p s_8 k e^s l?}{B_d'}$  female offspring was mated to a pink ebony male (containing  $C'$ , as do all ebony flies). A son of composition  $\frac{D' p s_8 k e^s l?^s}{p e C'}$  was then crossed to a  $\frac{s_8 k e^s B_d'}{C_b' l?}$  female (derivation shown on p. 443). The count from the latter mating was  $\frac{D' p s_8 k e^s l?}{C_b' l?}$  (appearing  $D'$ ) o,  $\frac{D' p s_8 k e^s l?}{s_8 k e^s B_d'}$  (appearing  $D' s_8 k e^s B_d'$ ) 26,  $\frac{pe C'}{C_b' l?}$  (normal-appearing) 35,  $\frac{p e C'}{s_8 k e^s B_d'}$  (appearing  $e-e^s B_d'$ ) 34.

This result was not due to lack of  $D' p s_8 k e^s l?$  sperm, or to their inviability, since approximately the expected number of  $D' s_8 k e^s B_d'$  offspring appeared. Neither could it have been due to selective fertilization, for in that case the third class (normals) would have been doubled, since all  $C_b' l?$  eggs would have been fertilized by  $p e C'$  sperm. It must be concluded, therefore, that all expected classes of gametes were formed, and that fertilization was normal, in that all expected classes of zygotes were formed also. The first class of individuals, therefore, must have died before completing their development. That is, they contained a lethal factor.

It is accordingly established, in the first place, that the non-beaded,  $C_b'$ -bearing chromosome present in pure beaded stock does contain a lethal factor. It should be noted that although this factor, when homozygous, is lethal in its effect, yet, when heterozygous, it produces no visible character differing from the normal. It is established, secondly, that this lethal factor is a different gene from the factor  $C_b'$ , lying in a different locus, for the two became separated from each other by crossing over when the  $s_8 k e^s$  fly ancestral to the father of the above count was produced. The fly received the lethal, but not  $C_b'$ . Thirdly the lethal factor, which we shall call  $l_{III 1}^6$ , must lie to the right of  $C_b'$ , since the  $s_8 k e^s$  fly containing it had received the right-hand end of the originally  $C_b'$ -bearing chromosome.

<sup>5</sup> Ebony ( $e$ ) is an allelomorph of sooty ( $e^s$ ) and the heterozygote  $\frac{e^s}{e}$  is intermediate in appearance between homozygous ebony and homozygous sooty.

<sup>6</sup> The Roman numeral III designates the chromosome in which the lethal lies; the Arabic number 1, which is to distinguish it from other lethals in the same chromosome, is chosen because this is the first lethal found in that chromosome.

A scheme of crossing had meanwhile been undertaken in order to determine the position of  $l_{III1}$  more exactly. From the mating (described

on p. 447) of  $\frac{D' p s_s k e^s l_{III1}}{B_d'}$  female by  $p e C'$  male, 19 offspring were

chosen which contained both  $e^s$  and  $B_d'$ , and which were hence crossovers between the latter factors. Each of these flies was tested for the presence of  $l_{III1}$ , in order to find in what proportion of cases  $l_{III1}$  had remained with  $e^s$ , when  $e^s$  and  $B_d'$  crossed over, and so to determine the position of  $l_{III1}$  with respect to  $e^s$  and  $B_d'$ .

18 of these 19 flies showed the character of  $D'$  and were therefore of composition  $\frac{D' p s_s k e^s l_{III1} B_d'}{p e C'}$ . They were tested for  $l_{III1}$  by being mated

to flies of composition  $\frac{s_s k e^s r_o}{C_b' l_{III1}}$ . Now in this cross the zygotes

formed at fertilization would consist of equal numbers of the following four types: (1)  $\frac{D' p s_s k e^s l_{III1} B_d'}{C_b' l_{III1}}$  ( $D' B_d'$  in appearance), (2)  $\frac{p e C'}{C_b' l_{III1}}$

(normal-appearing), (3)  $\frac{D' p s_s k e^s l_{III1} B_d'}{s_s k e^s r_o}$  (appearance,  $D' s_s k e^s B_d'$ ),

(4)  $\frac{p e C'}{s_s k e^s r_o}$  (appearance,  $e^s-e$ ). There would be, besides, in case the

tested parent were a female, a small number of crossovers (some of sorts phenotypically like those above), but these would be negligible in number because of the presence of  $C'$ . The differentiation between counts in which the tested parent contained the lethal and those in which it did not would lie in the absence from the former counts of all flies of class (1) (except for the few possible crossovers that resemble this class phenotypically).

The tests showed that 8 of the offspring (4 males and 4 females) contained the lethal, and 10 (4 males and 6 females) lacked it. A typical count from a male which carried the lethal was as follows:  $D' B_d' o$ , normal 27,  $D' s_s k e^s$  30 (including 25 showing  $B_d'$  also),  $e^s-e$  32. Besides these 18 crossover flies, one double crossover (a male), which did not contain  $D'$ , was also tested, in the same way. It proved to have the lethal. In all, then, in the 19 cases of crossing over between the loci of  $e^s$  and  $B_d'$ ,  $l_{III1}$  remained with sooty 9 times and crossed over from it 10 times. This shows that  $l_{III1}$  is nearly half way from  $e^s$  to  $B_d'$ , and so

almost exactly midway between  $e^s$  and  $r_o$ . The order and approximate distances of factors in this region are therefore  $\frac{e^s \quad l_{III 1} \quad r_o \quad B_d'}{10 \quad 9 \quad 2}$ , with the factor  $C'$  somewhere between  $e^s$  and  $l_{III 1}$ , or else slightly to the left of  $e^s$  (other experiments prove it to be to the right of kidney).

As  $l_{III 1}$  is approximately 12 units from  $B_d'$ , a race of composition otherwise like that of the pure beaded stock, but lacking  $C_b'$ , would gradually revert to normal, for in every generation 12 percent of the chromosomes produced by the heterozygous  $\frac{B_d'}{l_{III 1}}$  females would be crossovers. Half of these crossover chromosomes would contain both  $l_{III 1}$  and  $B_d'$ , the other half would contain neither of these mutant factors. Individuals receiving the latter, normal, chromosome would multiply at a greater rate than the others, both on account of their greater fertility, and because not so many of their offspring would be killed before reaching maturity.

Even in the  $\frac{B_d'}{C_b' l_{III 1}}$  stock (the "pure" race), however, a normal chromosome should *very rarely* be produced by crossing over, since, as we have seen in one of the crosses above, a crossover between the loci of  $e^s$  and  $r_o$  was found among the offspring of a female heterozygous for  $C_b'$ . This occurrence of a normal fly would be so infrequent in pure beaded stock that it would naturally be classed as a "reverse mutation" by an observer ignorant of the mechanism of inheritance of beaded.

In order to put this expectation to the test, the writer examined a large number of flies of the pure beaded stock; all of them were markedly and unquestionably beaded except one, which had perfectly normal wings. This fly, when outcrossed, gave no beaded offspring in  $F_1$  or in any subsequent generation. Tests of its offspring showed that it had been heterozygous for  $l_{III 1}$  but homozygous for  $C_b'$ ; thus its composition had been  $\frac{C_b'}{C_b' l_{III 1}}$ . The  $C_b'$  chromosome could not have arisen by one

step from the chromosomes in a  $\frac{B_d'}{C_b' l_{III 1}}$  female, for this would have required double crossing over between  $C_b'$  and  $B_d'$ , which would be next to impossible in the presence of  $C_b'$ . We must therefore infer that a  $C_b' B_d'$  chromosome was first produced by crossing over between  $C_b'$  and  $l_{III 1}$ ; this chromosome must have entered a female which received  $C_b'$

$l_{III1}$  from its other parent. In the  $\frac{C_b' B_d'}{C_b' l_{III1}}$  female so constituted the homozygous condition of  $C_b'$  would render crossing over frequent, and in such a female a chromosome with  $C_b'$  alone could easily be formed, by crossing over between  $l_{III1}$  and  $B_d'$ . In the production of the normal-winged fly that was observed, the latter chromosome must have entered an egg that received  $C_b' l_{III1}$  from its other parent. Here, then, is a case in which the  $C_b'$  factor has been obtained separate from  $l_{III1}$  by crossing over; this forms a complement to the other case, in which  $l_{III1}$  was obtained separate from  $C_b'$ .

The preceding case also settles another question which was raised concerning the  $C_b'$ -bearing chromosome, i.e., whether it contains a recessive factor for beaded, or merely the normal allelomorph of beaded ("not-beaded",  $b_d'$ ). On account of absence of the lethal from one of its chromosomes, the original  $\frac{C_b'}{C_b' l_{III1}}$  fly was able to survive, although homozygous for that part of the  $C_b'$ -bearing chromosome which contains the locus of beaded (i.e., the extreme right-hand portion). As the fly nevertheless did not have beaded wings, there could have been no recessive factor for beaded at this locus, but only the normal allelomorph, "not-beaded."

#### (5) *Origin of the factor $C_b'$*

Having determined the mechanism of constant hybridism in beaded stock, it now became of interest to discover, if possible, the origin of the factors therein involved. It seemed probable that the factor  $C_b'$  present in the non-beaded chromosome of pure beaded stock was the same as the factor  $C'$  present in ebony flies and in spread flies, for (1) it produced about the same amount of decrease in crossing over, (2) this effect was exerted on the same regions of the chromosome in all three cases, (3) the factor itself, so far as its position could be ascertained, lay in the same locus (near sooty) in all three stocks, (4) all three races of flies containing it were genetically related, since both the ebony and the spread mutants were derived from beaded stock.

Still another test was possible. It will be remembered that when spread and ebony stocks were crossed together, the hybrid flies, having  $C'$  or a similar factor in both chromosomes, gave a large proportion of crossovers. If these factors are the same as the  $C_b'$  present in the non-beaded chromosome of pure beaded stock, hybrids containing the  $C'$

from beaded stock in one chromosome and the  $C'$  from one of these stocks in the other should also give a high percent of crossovers, unlike most hybrids containing the  $C'$  from beaded stock. Three hybrid females of this kind were tested. They were of composition  $\frac{peC'}{C_b'l_{III1}}$  (produced by a cross of  $\frac{peC'}{peC'}$  to  $\frac{D_f'ps_s}{C_b'l_{III1}}$ ; the  $C_b'l_{III1}$  chromosome in the latter stock having of course been originally derived from the pure beaded race). On being back-crossed to  $\frac{peC'}{peC'}$  they gave the following count: non-crossovers, normal 128,  $pe$  52; crossovers,  $p$  59,  $e$  49; thus there were 40 percent of crossovers, instead of 0 to 5 percent, the usual amount between these factors in crosses heterozygous for  $C'$ . It may, then, be regarded as nearly certain that the factor  $C_b'$  in the pure beaded stock is the same as that which I have called  $C'$ , present in the spread and ebony flies.<sup>7</sup> These results, it may be mentioned, explain the early finding by DEXTER of a surprisingly large amount of crossing over in one of his crosses involving beaded stock.

Since the mutants ebony and spread, which contain the factor  $C'$ , are both descended from the original beaded stock, and since, conversely,  $C'$  has never been found in races not at least partially derived from the beaded stock, it seems highly probable that the  $C'$  in the above mutant races was derived from the  $C'$  present in beaded, even though the mutants in question were at first subjected to some outcrossing, before the above stocks pure for them were secured. But if this is true,  $C'$  must have been present in the beaded race without  $l_{III1}$  being present with it, as the mutants ebony and spread do not contain  $l_{III1}$ , and never could have. For the presence of  $l_{III1}$  in the same chromosome with  $C'$  and ebony or spread would perpetually have prevented the latter factors from becoming homozygous. Moreover, these mutants were found in flies derived from beaded stock long before the "pure" beaded,  $l_{III1}$ -containing race had been established. Hence some, or all, of the non-beaded chromosomes of the early impure beaded stock probably carried  $C'$  before  $l_{III1}$  had appeared in them, and the establishment of  $l_{III1}$  in all the  $C'$ -bearing chromosomes of the race must consequently have occurred a considerable time after the selection experiments on beaded had been inaugurated. Its appearance in them at that time could not have been due

<sup>7</sup> It has since been found that flies having the  $C'$  from spread stock, in addition to the  $C'$  from beaded, give a similarly high percent of crossovers.

to an introduction of it into beaded stock through some cross of the latter to another race of *Drosophila*, for the presence of  $C'$  itself would then have made a transference of  $l_{III1}$  into the chromosome with  $C'$  next to impossible. The lethal must, therefore, have arisen *in situ* by mutation, probably during the progress of the selection experiments. Once having arisen, it is easy to see why the line containing it should have been perpetuated by the experimenter rather than the other lines, because of course this line would produce a smaller proportion of normals. The occurrence of such a mutation is not surprising either, for, as will be pointed out later, there is reason to believe that lethals arise not infrequently by mutation, though they are, in the nature of the case, not ordinarily detected or selected for.

That  $C'$  should have been present so early in the history of the stock is somewhat more unusual, although it is not very uncommon to find factors affecting crossing over. There is no way of telling whether  $C'$  arose in the beaded stock by mutation, or was introduced by crossing. Once present there, however, it would automatically perpetuate itself, even though absent from the  $B_d'$  chromosome, for the latter could never be obtained homozygous.

#### (6) *Modifying factors*

It now remains to consider the possible effect of other factors than the above on the inheritance of beaded wing. The question of intensifying factors in the pure beaded stock may first receive our attention.

The presence of at least one intensifying factor here is proved by the fact that  $F_1$  beaded flies, derived from a cross of pure beaded by almost any other stock, are not nearly as intensely beaded as their beaded parents. This cannot be due to heterozygosis in the chief factor for beaded, for it has just been proved that even the pure stock is heterozygous for this factor. It must therefore be due to heterozygosis of the  $F_1$  flies in one or more intensifying factors, for which the pure stock is homozygous. The homozygosis of the intensifiers in  $P_1$  is further shown by the small variability of the beading in the  $P_1$  and in the  $F_1$  beaded flies, compared with the large variability in  $F_2$ .

The main intensifiers of beaded in question here can not, however, be present in the first (sex) chromosome of this stock, as the  $F_1$  males show the above effects as well as the females. (There appears to be some effect directly due to sex, however, the males being, on the whole, less beaded in crosses). Neither are there any factors markedly intensifying beaded

peculiar to the third chromosome of pure stock. This is shown by the fact that flies with as high an intensity of beading as those in the pure stock may be obtained regularly in a race no part of whose third chromosome except the extreme right-hand end, i.e., the part containing the chief factor  $B_d'$  itself, is derived from the beaded stock. Such a race may be made up from the  $F_2$  of a cross of pure beaded to  $s_e s_s k e^s r_o$ , by breeding together the crossovers of type  $s_e s_s k e^s r_o B_d'$ . It should be stated, however, that the presence of  $e^s$  may here aid to some extent in the manifestation of the beaded character. Of course, all individuals of this race will not show beaded to the marked extent of pure stock, for a race so obtained could not, in any event, be pure for intensifiers; the full intensity of beading appears, however, in about the proportions expected on the supposition that it depends upon a single intensifier, segregating independently of the chief factor, and so not lying in chromosome III.

As an intensifier is present, but lies neither in chromosome I nor III, it is almost certainly in II, for IV is a very tiny group. The present results, then, agree with DEXTER's finding of an intensifier for beaded in the second chromosome. They disprove, on the other hand, his postulate that this intensifier is a lethal, which cannot appear when homozygous (DEXTER 1914).

Crosses were also made to ascertain whether the factor under consideration is truly an "intensifier" or whether it can, when homozygous, give rise to the character beaded by itself—that is, without the contributory activity of the chief factor,  $B_d'$ . This was determined by breeding together the non-beaded offspring of a cross of pure beaded by homozygous  $s_e s_s k e^s r_o$ . Such offspring must have failed to contain the factor  $B_d'$ , having received the  $C' I_{III 1}$  third chromosome instead, yet they must all have inherited a second chromosome containing the intensifier. One-fourth of the  $F_2$  obtained by breeding these non-beaded flies together would hence be homozygous for the intensifier, without containing the chief factor at all. In a count of a large number of  $F_2$  flies obtained in this way not one had beaded wings. The second chromosome factor is, therefore, simply an "intensifier," or "plus modifier" of the chief factor,  $B_d'$ , of chromosome III; it may accordingly be designated as  $I_{B_d}'$ , and its normal allelomorph as  $i_{B_d}'$ . This choice of symbols would indicate that it is a dominant; the dominance is far from complete, however, as has been noted above.

Although there is evidence of only one intensifier in the selected stock, other factors have been found, in other stocks, which also alter the intensity of the beading produced in the presence of the chief factor  $B_d'$ .



For example, DEXTER (1914) discovered that black, even when heterozygous, tends to inhibit beaded, and vestigial, when heterozygous, intensifies it. Counts made in the course of the present work also illustrate these points. Thus, beaded flies homozygous for sooty have been found to have more beading than beaded flies of the same count which were heterozygous for sooty. Again, crosses of pure beaded females by  $s_e s_s k e^s r_o$  males gave  $F_1$  in which presumably all of the flies that had received beaded (i.e., about half of the population) had the inner margins of the wings well cut away; on the other hand, a cross of a pure beaded male by black pink spineless female gave, among 109 offspring, only 3 beaded, and these were very slightly beaded. The difference between these two counts is probably due partly to the presence of sooty in the first cross, but mainly to the presence of black in the second. Nevertheless, when a pure beaded male was crossed to a female containing the factors black, purple, vestigial, arc and speck (all in II), and also pink and spineless (in III), the presence of black did not prevent the appearance of beaded, for it was counterbalanced by the intensifying action of vestigial, and half of the  $F_1$ —presumably all which had received  $B_d'$ —were intensely beaded phenotypically. The rest, lacking  $B_d'$ , were perfectly normal.

The amount of intensification caused by  $v_g$  (vestigial) was compared with that caused by  $I_{B_d}'$  by crossing an intensely beaded male of this count

(having the composition  $\frac{I_{B_d}'}{b p_r v_g a_r s_p p s_s} \frac{B_d'}{b p s_s}$ ) to a  $\frac{b p s_s}{b p' s_s}$  female. The  $p s_s$  offspring (26 in number) of course failed to receive  $B_d'$ , and so none of them showed the beaded character at all. The 37 red-eyed flies with normal spines, on the other hand, must all have received  $B_d'$ , and whether they received  $I_{B_d}'$  or  $v_g$  could be determined by whether they had gray or black body color, respectively. It was found that the 22 grays, which were heterozygous for  $I_{B_d}'$ , consisted of 10 slightly beaded and 12 phenotypically normal, whereas the 15 blacks, which were heterozygous for  $v_g$  (together with  $p_r$ ,  $a_r$ , and  $s_p$ ) consisted of 9 fairly well beaded and 6 slightly beaded flies, in spite of the fact that black acts as an inhibitor of beaded. Thus the heterozygous  $v_g$  (at least when in company with heterozygous  $p_r$ ,  $a_r$ , and  $s_p$ ), is a much stronger intensifier of beaded than heterozygous  $I_{B_d}'$ .

As the intensifier vestigial lies in chromosome II it was thought possible that  $I_{B_d}'$  might be merely a "weaker" allelomorph of vestigial. Vestigial, when homozygous, results in a most extreme reduction or appar-

ent cutting away of the wing, whether  $B_d'$  is present or not, unlike  $I_{Bd}'$  which has been shown above to have no visible effect without  $B_d'$ . If this contrast in results were merely due to a difference in intensity of action of two factors which, being allelomorphs, probably produced their developmental effects by similar reactions, it was quite possible that a "compound" fly, containing  $v_g$  in one chromosome and  $I_{Bd}'$  in the other (and not containing  $B_d'$ ) might show a wing intermediate between vestigial and normal. This would indeed be expected by analogy with the results in other cases of multiple allelomorphs. Flies known to have this genetic composition were obtained as follows: A beaded  $F_1$  male from the cross of pure beaded to black pink spineless was back-crossed to black pink spineless. As  $b$  is in II and  $p$  and  $s_s$  are both in III, the grey pink spineless offspring ( $F_2$ ) must have contained the second-chromosome intensifier but not the third-chromosome factor for beaded;

their composition thus was  $\frac{I_{Bd}'}{b} \frac{p s_s}{p s_s}$ . An  $F_2$  male of this kind was

crossed to a  $b p_r v_g a_r s_p$  female. The gray offspring ( $F_3$ ) of this mating must then have been of the desired composition, containing in one second chromosome  $I_{Bd}'$  and in the other  $v_g$  (together with  $b p_r a_r$  and  $s_p$ );  $B_d'$  of course was absent completely. Among 79 such gray flies, however, not one showed an abnormality of the wing; the black offspring (49) produced in the same count were of course also normal-winged.

If, then,  $I_{Bd}'$  and  $v_g$  are allelomorphs their behavior transgresses the usual rule that the "compound" individual, heterozygous for two mutant factors that are allelomorphic to each other, is intermediate in appearance between the two respective homozygous types. It might be ob-

jected, however, that as in this case one of the homozygotes ( $\frac{I_{Bd}'}{I_{Bd}'}$ )

looked exactly like normal the usual rule did not apply; that the reactions tending to cause wing reduction in the compound  $\frac{v_g}{I_{Bd}'}$  might really be

intermediate in strength between those in  $\frac{I_{Bd}'}{I_{Bd}'}$  and  $\frac{v_g}{v_g}$ , and still not have reached the critical intensity necessary for a *visible* reduction of the wings.

This objection could not apply to a determination made with flies

otherwise like the above, but containing the factor  $B_d'$ , for the chief factor would bring the reactions of  $I_{Bd}'$  tending to cause wing reduction beyond the necessary critical point, as is shown by the fact that  $I_{Bd}'$  does, even when heterozygous, have a decided effect in the presence of

$B_d'$ . The  $\frac{I_{Bd}'}{b p_r v_g a_r s_p} \frac{B_d'}{p s_s}$  flies, produced by crossing pure beaded to

$p_r v_g a_r s_p p s_s$  were therefore compared with the  $\frac{I_{Bd}'}{I_{Bd}'} \frac{B_d'}{C' l_{III 1}}$  flies of pure stock. It was found that the amount of reduction of wing was

not greater in the former flies (compound  $\frac{I_{Bd}'}{v_g}$ ), but was in fact just

about the same in the two cases. This result could not have been due to an inhibition of beaded caused by the  $b$  or other factors present in the compound flies, for we have already seen that even the "handicap" of these other factors does not prevent heterozygous  $v_g$  from having a more intensifying effect on beaded than heterozygous  $I_{Bd}'$  alone. Thus, even in the presence of these factors,  $v_g$  should act as a stronger intensifier than  $I_{Bd}'$  and, if the two are allelomorphs, the compound  $\frac{v_g}{I_{Bd}'}$  should

therefore be expected to give rise to a greater amount of beading than does homozygous  $\frac{I_{Bd}'}{I_{Bd}'}$ . The fact that it does not may therefore be taken as strong evidence that these factors are not allelomorphs, but occupy different loci in the second chromosome.

It would be difficult to make quite certain of this point by using the most conclusive method—that of determining the distances of the  $v_g$  and  $I_{Bd}'$  loci from one another—because there is a strong possibility that the effect on the intensity of the beading, of the other factors which would be used correlatively, would seriously disturb the apparent linkage ratios.

It is now evident that, in the presence of the chief factor,  $B_d'$ , the boundaries of the wings must be subject to modification by many factors which would not otherwise influence them. This modifiability of beaded by various factors is paralleled by its modifiability under environmental influences. Thus, it was often noted in the course of the above experiments that beaded flies developing under drier conditions, such as are prevalent late in the history of a brood, usually show less beading than

those raised under more normal conditions. An example of this effect may be found in the results of the back cross given in table 1, p. 438. In these counts the distribution of the factor for beaded could be almost exactly followed, by noting the distribution of other factors linked with it. Among the flies hatching from the first four bottles there were 136 which, by the linkage criterion, almost certainly carried beaded. 58 of these appeared in the two earliest counts and consisted of 56 flies phenotypically more or less beaded, and only 2 apparently quite normal. 78 appeared in the later counts, that had developed under drier conditions, and these consisted of 50 beaded and 28 phenotypically normal.

DEXTER (1914), who has made an extensive study of the influence of environment on beading, was able, within certain limits, to alter the amount of beading at will by various means.

The fact that so many modifying factors and environmental influences have been discovered, all in the case of a single character, beaded, whereas most characters seem to be fairly stable, should not be surprising, for it is to be expected that some characters<sup>8</sup> will depend, for their development, on structures or reactions that are in general easily modified, whilst other characters depend on processes that are beyond the reach of most agents. The former would of course be subject to influence by numerous modifying factors and external conditions, and the discovery of such modifying factors and conditions would thus be much more likely in their case than in the case of the other, more usual, characters.

For these reasons it follows that with each new discovery of factors or environmental conditions which influence the amount of beading, the probability of the discovery of still other agents which will affect this character becomes not less, but greater, for there is more evidence that the character is of the "modifiable" type. The finding of one modifying factor for beaded hence in a sense explains, rather than makes more surprising, the finding of another, and the discovery of its environmental modifiability further helps to "explain" the finding of the modifying factors. The original postulation of both "somatic" variability and multiple factors in the case of beaded wings accordingly did not amount to the making of two independent assumptions, and the experimental discovery that both are true at once is, from this point of view, not at all remarkable, since it is to be expected that the two phenomena will tend to occur together.

<sup>8</sup> It should be superfluous to mention that the terms character and factor must be rigorously distinguished from one another in the above discussion.

(7) *Other factors for beaded*

In all the cases reported above, the contributory agents can influence the character of the wing only if the chief factor  $B_d'$  is present, so that they may be termed mere "modifying" factors or conditions, dependent on  $B_d'$  for their expression. This fact would indicate that the reactions which determine the edge of the wing when the normal allelomorph of  $B_d'$  is present are more stable, or at any rate not subject to the same influences, as those taking place in the presence of  $B_d'$  itself. Yet it is conceivable that some factors might also be found that would cause similar imperfections in the margin of the wing even without the contributory influence of  $B_d'$  being necessary. These other factors might then be termed factors for beaded in the same sense as the original chief factor,  $B_d'$ , itself. At least one such factor has already been found.

On p. 439 it was reported that one slightly beaded fly was produced among the 128 offspring of a non-beaded female of composition

$\frac{C' l_{III1}}{s_e D' s_s k e^s r_o}$ , crossed to a homozygous  $s_e s_s k e^s r_o$  male. This exceptional "beaded" fly had received from its mother the chromosome

$C' l_{III1}$  since it did not show any of the recessive characters; its composition, then, was

$\frac{C' l_{III1}}{s_e s_s k e^s r_o}$ . The beaded character which it showed

was scarcely of the usual type, as the wings were cut into only at the very tip, yet the fact that the fly was beaded at all seemed contrary to the supposition that the  $C' l_{III1}$  chromosome contained the normal allelomorph of  $B_d'$ . To test this point the fly (a female) was back-crossed to a homozygous  $s_e s_s k e^s r_o$  male. The count, as regards  $s_e$ ,  $s_s$ ,  $e^s$  and  $r_o$ , was as follows: Non-crossovers: normal 14,  $s_e s_s e^s r_o$  16; crossovers between  $s_e$  and  $s_s$ - $s_e$  12,  $s_s e^s r_o$  12; other crossovers 0. The result proves that  $C'$  is present, as was supposed; if, then, the factor  $B_d'$  was present too, it would remain tied to the normal allelomorphs of  $s_s e^s r_o$ , with which it was at the start, for  $B_d'$  lies very close to  $r_o$ . The distribution of the beaded character was, however, quite different from this; there were only 6 beaded flies in all and these were scattered at random among the various classes, the "normal" class including 2 beaded flies, the  $s_e s_s e^s r_o$  2, and the  $s_e$  2, although there happened to be none among the  $s_s e^s r_o$ . This distribution proved that the beaded factor in the present case does not lie in the same locus as  $B_d'$ , and is, in fact, even in a different chromosome. This beaded is dominant, but very irregularly so, since

it does not appear in nearly half of the offspring; its expression, too, is different from that of  $B_d'$ , as it affects only the tip of the wings. It is not, however, a modifier of  $B_d'$ , as its character is shown in the presence of the normal allelomorph of the latter.

This factor or one with a similar effect must also have been present in another cross of the present series in which a mating of a pure beaded male by a female of another stock (homozygous  $B_r' b p_r v_g a_r s_p p s_s$ ) gave in  $F_1$ , not the ordinary count of half "good" beaded and half perfectly normal, that was expected, but 23 good beaded, and 20 other flies, 10 of which were perfectly normal and 10 slightly beaded. This ratio indicates that besides  $B_d'$  there was another, "weaker," chief factor for beaded present in heterozygous condition in one of the parent flies. This factor too must have been a dominant to a certain extent. One of the slightly beaded females of this count was then back-crossed to another stock homozygous for spineless ( $s_e s_s k e^s r_o$ ). There were 43 spineless and 43 spine-bearing offspring; 8 of the former and 3 of the latter were slightly beaded; the rest were normal-winged. As there is certainly no linkage here between the new beaded factor and the normal allelomorph of spineless, the former could not have been located in the third chromosome derived from the beaded stock, although it might have lain in the other third chromosome. The chromosome of beaded stock which these slightly beaded flies had received was then proved to be the  $C'$ -bearing chromosome, not the  $B_d'$ -bearing chromosome, by taking one of the slightly beaded spine female offspring of the last count and back-crossing it to a homozygous  $s_s k e^s r_o$  male. The results of the back cross proved that  $C'$  was present, since there was no crossing over between the factors in question:  $s_s k e^s r_o$  21, including 1 slightly beaded; normal in respect to  $s_s, k, e^s$  and  $r_o$  36, including 8 slightly beaded; other classes 0. The proportion of "beaded" in the two classes furthermore proves that the new factor does not lie in the locus of  $B_d'$ , for the latter would not cross over from  $r_o$  in the presence of  $C'$ .

The frequency with which beaded seems to arise by mutation, also, suggests that various mutant factors can produce the beaded character. DUNCAN (1915) found that among 109 flies with abnormal characters which appeared in a total count of 16,637, 89 were beaded. Probably not all of these beaded flies, however, represented separate mutations which had just occurred, for it is possible for the factor  $B_d'$  to exist for a long time in "latent" condition in a stock, entirely unsuspected and producing no effect upon the wing, if only the intensifiers of the character are absent or inhibitors are present. Its occasional appearance above

the threshold of visibility in such cases might then be wrongly interpreted as due to a new mutation.

Another reason why we cannot accept these figures as proving that beaded really arises oftener than other mutant characters is that, since the factor  $B_d'$  is a dominant, it has a better chance of being detected when it does arise than have most other factors. That is, unlike the majority of mutants, which are recessive, it does not have to depend upon the rare event of being present in both of two uniting gametes in order to manifest itself; it therefore will *appear* to arise more frequently than the others. For these reasons it cannot be concluded with certainty at present, merely because the beaded character arises frequently, that many different factors are likely, by their mutation, to produce this same effect, although we have seen that two at least may do so; neither can it be taken for granted that the factor  $b_d'$  is itself unstable, so as to mutate relatively often to  $B_d'$ . In connection with the former point it should, however, be noted that a number of other mutants besides actual "beaded" are known, that produce a very similar effect upon the margin of the wing, although they cause other effects in addition. Among these factors are included notch, bifid and strap.

#### CONSIDERATION OF RELATED PHENOMENA, AND GENERAL DISCUSSION

##### (1) *The lethal effect of dominant mutants*

The possibility has doubtless suggested itself to the reader that this whole mechanism of beaded inheritance may be merely a highly exceptional case, of no general interest. It is at first hard to believe that it could have any significance, or parallel in other cases. From this point of view it becomes of interest to know how widespread is the occurrence of factors similar in their properties to  $B_d'$ . An examination of other dominant mutant factors of *Drosophila* might be of value here, for it should disclose whether or not it is a mere coincidence that  $B_d'$ , a dominant mutant factor, should, like the factor for yellow in mice, be lethal in its effect when homozygous.

Two dominant mutants which have for a long time been known in *Drosophila* are the sex-linked factors for abnormal abdomen and for bar eye. Unlike  $B_d'$ , however, neither of these factors has been very difficult to obtain in pure stock, and the homozygotes are known to be viable and fertile. It should be noted that these factors are far from completely dominant to their allelomorphs; in fact, the heterozygote in each case is about midway in character between the two types of homozy-

gotes. Data for ascertaining the viability of the homozygote existed in the case of only one other dominant mutant—the factor called notch—which also lies in chromosome I. This factor, which affects the wings somewhat in the same fashion as  $B_d'$ , had first been found by DEXTER (1914) (and called “perfect notch”), and the same factor afterwards arose again by mutation both in stocks of BRIDGES and of the writer. In the case of this factor the results are more encouraging for our present investigation, as DEXTER’S work has proved notch to be a lethal and BRIDGES and the author have each confirmed this finding for the notch which arose in their experiments.

To obtain further data upon the question under consideration, the writer tested the other dominant mutant factors of *Drosophila*. The first one studied was  $D'$  (dichaete). Heterozygous flies were made up of composition  $\frac{s_e D'}{s_e D'}$ ,<sup>9</sup> and these were bred *inter se*. As  $D'$  is only about ten units from sepia most of the homozygous sepia offspring would also be homozygous for  $D'$ , and the count should be about as follows: non-crossovers:  $\frac{s_e D'}{s_e D'}$  (appearing  $s_e D'$ ) 22.5 percent,  $\frac{s_e D'}{s_e D'}$  (appearing  $D'$ )

45 percent, — (appearing normal) 22.5 percent; crossovers:  $\frac{s_e D'}{D'}$  (appearing  $D'$ ) 2.5 percent,  $\frac{s_e D'}{s_e}$  (appearing  $s_e D'$ ) 2.5 percent, — (appearing  $D'$ ) 2.5 percent,  $\frac{—}{s_e}$  (appearing normal) 2.5 percent. Adding

together the phenotypic classes we have  $s_e D'$  25 percent;  $D'$  50 percent; normal 25 percent. The actual count consisted of  $s_e D'$  5,  $D'$  59, normal 32. Thus there is a great deficiency of  $s_e D'$  and a slight deficiency in the number of  $D'$ . Reference to the tabulation of expected classes will show that nine-tenths of the  $s_e D'$  class is composed of homozygous  $D'$  and one-twentieth of the  $D'$  class also. If we subtract these homozygous  $D'$  flies from the expected ratios, we obtain  $s_e D'$  2.5,  $D'$  47.5, normal 25. These are almost exactly the ratios that were actually obtained, and it is therefore evident that homozygous  $D'$  flies cannot live, i.e., that  $D'$  like  $B_d'$  is lethal when homozygous. That the  $D'$  when heterozygous does not lower the viability is indicated by a count obtained from a cross of heterozygous  $D'$  by homozygous normal. If the viability of heterozygous  $D'$  is as good as normal, half the offspring

<sup>9</sup> A blank above or below the line of course indicates that all the factors in the chromosome not represented are normal.



which hatch should be  $D'$  (heterozygous), and the rest normal. The count was  $D'$  73, normal 65.

The dominant factors for star eye and for streaked thorax, which lie near together in chromosome II, were next tested by mating flies containing them to each other and then crossing together the  $F_1$  flies which showed both characters and were therefore of composition  $\frac{S'}{S'_k}$ . The expectation, if there were no crossovers, would be 1  $\frac{S'}{S'}$ : 2  $\frac{S'}{S'_k}$ : 1  $\frac{S'_k}{S'_k}$ . There would, however, be a small amount of crossing over, as judged by the fact that star and streak do not show quite the same linkage values with other factors; hence, in addition to the above classes, there would be a few  $\frac{S'}{S'_k}$ ,  $\frac{S'_k}{S' S'_k}$  and  $\frac{S'_k}{S' S'_k}$ . The count was: star 16, star streak 116, streak 61. The deficiency of stars shows that homozygous star flies die before hatching; the few stars that did appear were expected on account of the production, by crossing over, of a few  $\frac{S'}{S'_k}$ , and also because some of the  $\frac{S'}{S'_k}$  were undoubtedly listed in this class, since heterozygous streak often fails to show the streak character. Here, then, is still another dominant which has a lethal effect when homozygous. Streak, however, turns out to be viable, although the homozygous streak flies have very poor fertility.

It will be recalled that the third-chromosome dominant factor for deformed eye, reported upon in the first section, presents an intermediate condition in regard to the present question; it was found often to be extremely inviable when homozygous, so that in many cases all homozygous  $D'_f$  fail to appear, but in some cases a few, and in still others nearly all of the expected homozygous  $D'_f$  do appear. These are invariably much smaller and weaker-looking than normals or heterozygous  $D'_f$ , however, and have besides an abnormality of the wings not exhibited by the latter, for the wings of these homozygous flies are usually crumpled together and are always very weak and flimsy in texture. The reproductive system too is affected, for offspring cannot be obtained from a mating of  $\frac{D'_f}{D'_f}$  by  $\frac{D'_f}{D'_f}$ . This was found to depend upon a total ster-

ility of the males (the females laid only a few eggs, but these were viable, when fertilized by normal sperm).

The case of truncate flies, which was investigated by ALTENBURG and the writer (see MORGAN, STURTEVANT, MULLER and BRIDGES 1915), presents many similarities to the case of beaded, but it is being dealt with in another paper. Suffice it here to say that the more elaborate tests which were necessary in this case proved that  $T'$  (truncate), also, prevents a fly homozygous for itself from living, although in heterozygous condition it has little noticeable effect except upon the wings, being in this latter respect a dominant factor. The lethal effect in the homozygote is complete.

The intensifiers of beaded and truncate might perhaps be classed as dominants too, since they can exert a noticeable effect when heterozygous. But in this case not only is the effect of the heterozygous factor incomplete, but, as before noted, it fails to appear at all unless the chief factor is present. These intensifiers are not lethal, yet, like  $D'_f$  and  $S'_k$ , they cause a marked reduction of fertility when homozygous.

Omitting these "weak" intensifiers from consideration, and also one or two very recently discovered factors which have not yet been sufficiently investigated, we may now list the dominant mutant factors of *Drosophila* as follows, with regard to their lethal effect when in homozygous condition: viable,  $S'_k$ ,  $A'_b$ ,  $B'_r$ ; partially lethal,  $D'_f$ ; completely lethal,  $N'$ ,  $S'$ ,  $T'$ ,  $D'$ ,  $B'_d$ . (The lethals, it should be noted, are scattered quite at random among the three chromosomes.)

This enumeration proves indisputably that there is a very strong tendency for dominant mutant factors to be lethal, in *Drosophila* at least, and so  $B'_d$  is in this respect rather an instance of the rule than an exception. This fact may seem somewhat surprising, but there are two series of considerations which would furnish ground for expecting such a result.

In the first place, it is likely that lethals are really among the commonest forms of mutants, but they would be discovered much more readily if they were dominant in regard to some visible character than if they were completely recessive, and this would cause the proportion of lethals among the dominant mutant factors to appear to be excessively high, when compared with the proportion among the recessives. Most present-day animals are the result of a long process of evolution, in which at least thousands of mutations must have taken place. Each new mutant in turn must have derived its survival value from the effect which it produced upon the "reaction system" that had been brought into being

by the many previously formed factors in coöperation; thus a complicated machine was gradually built up whose effective working was dependent upon the interlocking action of very numerous different elementary parts or factors, and many of *the characters and factors which, when new, were originally merely an asset finally became necessary* because other necessary characters and factors had subsequently become changed so as to be dependent on the former. It must result, in consequence, that a dropping out of, or even a slight change in any one of these parts is very likely to disturb fatally the whole machinery; for this reason we should expect very many, if not most, mutations to result in lethal factors, and of the rest, the majority should be "semi-lethal" or at least disadvantageous in the struggle for life, and likely to set wrong any delicately balanced system, such as the reproductive system.<sup>19</sup>

Although this conclusion had suggested itself to the writer in 1912 it would manifestly have been very difficult to obtain experimental evidence for it, not only because of the great rarity with which mutations of any sort occur, but more especially because the detection of a lethal mutation, after it has occurred, requires special breeding tests of the particular flies containing the lethal factor. To detect a recessive lethal factor it is necessary not only to mate together two individuals both of which happen to be heterozygous for it, but also to determine what proportion of the offspring that receive a "visible" factor linked with this unsuspected lethal come to maturity. For this purpose it is usually necessary to have both the parents carry, in the same chromosome with the lethal, another recessive, non-lethal factor in heterozygous condition; the deviation from the expected ratio of 3 : 1 will then indicate the presence of a lethal. Hence, it is only in exceptional cases that a recessive lethal will be noticed.

Lethals that are linked with sex, however, will be discovered much more readily than the others, since the progeny of a female containing one of them will exhibit a 2 : 1 instead of 1 : 1 sex ratio (the Y chromosome in the male here takes the place of the lethal-bearing chromosome which the father must carry, since it is not dominant to any mutants in X). All flies the sex ratio of whose progeny has been determined will therefore exhibit their sex-linked lethals. This should result in the discovery of many more recessive lethals in chromosome I (the sex chromosome) than in any of the other chromosomes, although still it does not make the finding of recessive lethals in I nearly as easy as the find-

<sup>19</sup> Consequently, too, the larger the character change caused by a mutation, the greater would be the likelihood of the mutation being disadvantageous or lethal.

ing of other recessive factors there, for mere inspection of a male will reveal any non-lethal sex-linked mutant that it carries. Even with this great handicap, about 17 mutant lethal factors have within the last few years been discovered in chromosome I of *Drosophila*; about 70 other factors, altogether, are known in this chromosome. When we allow for the much greater difficulty of finding lethals than other factors here<sup>11</sup> it becomes evident that *probably the majority, if not the vast majority, of mutants are lethals*. (Of course we do not take into account, in this statement, the unknown number of very "small" mutations, which have so slight an effect that they escape detection. Perhaps these are more numerous than the lethals.)

Experimental evidence for the above conclusion can at present be adduced only in the case of chromosome I, for, as we have seen, the lethals occurring in autosomes can be discovered even much less readily than the sex-linked ones. There is, however, no reasonable ground for believing that lethals arise less frequently in autosomes than in the sex chromosome, and so  $l_{III1}$  is probably merely the first representative found of a large number of similar mutations that are continually occurring,—a number which, if our methods of detection were impartial, would exceed that of non-lethal mutations.

The conclusion that lethals have a much poorer chance of discovery than non-lethals applies, however, only to strictly recessive factors. In the case of dominants, if a factor is dominant only in regard to a visible, non-lethal effect, the probability of its being found will not be decreased by its being lethal when homozygous, for it can be discovered by inspection when in heterozygous condition, regardless of whether or not it can live when homozygous. This fact will tend to result in the finding, among the dominant mutant factors, of much more nearly the true proportion of lethals than among the recessive mutants. Even here, nevertheless, not the full proportion of lethals will be observed, since we

<sup>11</sup> Sex-linked lethals may be discovered only in females which are bred and among whose progeny a sex count is taken; "visible" sex-linked mutant factors, on the other hand, may be discovered in males by inspection. Since for each female that is bred there are produced on the average at least 50 males that are inspected this will seem to make the chance of finding a visible mutant in the X chromosome 50 times greater than that of finding a lethal there; as the female, however, has two X chromosomes, both of which are tested in this process, and each male only one, the chances of finding a visible mutant in X are really about 25 times as great as those of finding a lethal. To find the true proportion of visible to lethal mutations that have *occurred* here we must therefore multiply the number of lethals found by 25. This calculation applied to the above figures makes the occurrence of lethals about *six times* as frequent as that of "visible" mutations.

have excluded from consideration in the above account all factors that are dominant in regard to the lethal effect itself (and not merely for a visible character); as such factors would kill even heterozygous individuals they have no means at all of perpetuating themselves and becoming known by any ordinary methods. We cannot tell how much to allow for factors of this sort. But in view of the unimpeded chance of detection of any dominants which are lethal only when homozygous, and of the great frequency with which lethals occur anyhow, it should not be surprising that such a large proportion of lethals have been found among the dominant mutants of *Drosophila*, as compared with such a small proportion among the autosomal recessive mutants.

It is also possible that not only would relatively more of the lethals occurring among dominant mutant factors be found, but that the excess of lethals observed among dominants is partly due to a greater chance of lethals actually occurring among dominant than among recessive mutant factors. The reasons for such a supposition are as follows:

The mutant factors which have been called dominants in *Drosophila* are not dominants in the same strict sense of the word as most of the normal factors are, in those cases where the normal allelomorphs are called dominant. For in the case of a mutant it has been called dominant if only it produces a conspicuous effect when heterozygous, regardless of whether in homozygous condition its effect is still more marked. Thus, *bar* is called a dominant, but heterozygous *bar* differs almost as much from homozygous *bar* as from normal, and so the mutant factor *bar* scarcely has any more right to be called the dominant in this case than has its normal allelomorph. The same is true of all the other so-called dominant mutants in which the homozygote can be examined; namely, *abnormal*, *streak*, *deformed* and the *truncate* and *beaded intensifiers*. This is in line with and adds significance to the fact that so many more recessives than even partial dominants have been found among the mutant factors of *Drosophila*. The great majority of the mutants, then, are recessive, while the few that are called dominant are very incompletely so.

We may generalize these two findings together in the single statement that, in *Drosophila*, there is a very strong tendency for the normal allelomorph to exert a more powerful influence than the mutant upon the character of the heterozygote; thus the latter inclines to resemble the normal much more closely than the homozygous mutant type. Cases of multiple allelomorphs have shown that two different mutant allelomorphs, on the other hand, tend to exert about equal effects upon the

heterozygote, producing in the latter characters of intermediate grade. These facts are very striking, and undoubtedly of great significance; they might suggest, for example, that mutant factors are usually less active, and that possibly the mutations may often consist in a loss of some portion of the factor structure, but in our present ignorance of factor chemistry the real meaning of the facts must remain hidden. We are at present concerned, however, not with their cause but with their results.

In consequence of this more marked effect which all known mutant factors of *Drosophila* produce when in homozygous than heterozygous condition, it is to be expected that those customarily called dominants will, inasmuch as they produce appreciable effects even when heterozygous, cause especially pronounced abnormalities when homozygous. Homozygous dominants, therefore, would tend to produce more marked effects than homozygous recessives. Now since, for reasons already explained, the abnormalities produced by mutant factors are generally of an injurious nature, it follows that these dominants should be especially injurious when homozygous. A larger proportion of lethals might thus actually occur among these so-called dominant mutant factors than among the recessives.

To sum up, then, the observed excess of lethals among dominant mutant factors may be due both to the greater chance of lethals being detected among the dominants than among the recessives, and also to their actually occurring in greater relative numbers in the case of this class of mutants. It should thus be evident that, from an *a priori* standpoint as well as on the basis of experimental facts, the lethal effect of the dominant mutant  $B_d'$  can in no sense be considered an atypical finding.

### (2) *The balancing lethal*

The chief peculiarities of the beaded case depend upon the co-occurrence of a factor ( $B_d'$ ) of the lethal-dominant type just discussed, with another lethal (in this case purely recessive) in the homologous chromosome. Now, the occurrence of a recessive lethal likewise cannot be considered, in the light of the preceding discussion, an unusual circumstance, as such lethals must be arising not infrequently. Why did just this lethal, so precisely adapted to produce the unusual results of the present case, happen to occur in just this stock, however, whereas lethals are not ordinarily found to be existing at all in usual stocks? There is an especial reason why a lethal should be found in this particular case, and why it should occur near the locus at which  $l_{III}$ , actually lies.

Granting that lethals are really arising by mutation not very infrequently, they would nevertheless tend, one by one, to be eliminated from stocks by natural selection (provided there is an appreciable amount of inbreeding). In the case of the beaded stock this invisible process of mutation and elimination would have occurred too, until a lethal arose in the chromosome homologous to that containing  $B_d'$ , in some locus near-by; this factor, unlike the others, would tend to be selected by the very conditions of the experiment, which was aimed at getting stock producing as low a proportion of normals as possible. Once selected and established, moreover, the lethal would persist indefinitely in the stock by the process of "enforced heterozygosis" previously described. For if the two parents in a certain bottle both had happened to receive this factor (say from a mutant grandparent), this particular bottle would produce an unusually small percentage of normal flies, and since this would be a result desired, the offspring in this bottle would then be selected to continue the beaded race, rather than the offspring in other bottles. All of these offspring would, in such a case, contain the factor in question again, and so the pure race would at one jump be established, and both lethal factors, once selected, would persist indefinitely in the self-perpetuating stock.

Selection for stock pure in respect to one lethal thus automatically tends to result in the establishment of a *balancing* lethal, if I may call it so, in the homologous chromosome. When this happens, a state of enforced heterozygosis is set up which may be regarded as a kind of constant hybridism. Whereas any ordinary heterozygous population, when inbred, will separate more and more into the respective homozygous strains, one of this type must remain always and necessarily heterozygous for the two lethals it contains, except in so far as the "balancing" of the lethals is imperfect, i.e., so far as crossing over between them occurs. It remains heterozygous also for any other factors lying in the same chromosome with either one of the lethals, so long as they too do not cross over from it.

### (3) *Effects of balanced lethals on crosses*

Since in such a race all individuals, though heterozygous, are alike in genetic composition, the stock would seem to be pure, until tests were made upon it by crossing it to other races. Then its heterozygosis would be made manifest by the appearance of "twin hybrids," provided only that the chromosome containing one of the lethals differed from the

other in being dominant in regard to some visible character for which the foreign race was recessive. Thus, in the case of beaded, the chromosome containing  $B_d'$  differs from that containing  $l_{III 1}$  in being dominant for the visible character beaded wings, and so, when the stock is crossed to non-beaded races, twin hybrids—beaded and non-beaded—are produced. On being inbred, the  $F_1$  beaded of course would not breed true, since the  $B_d'$  in them is no longer "balanced" by another lethal; the non-beaded, however, would appear to breed true, if the foreign stock had not differed from the beaded in any visible characters except those carried in the  $B_d'$ -bearing chromosome. The non-beaded  $F_1$  would consequently be "constant hybrids," using the term here in a different sense from that employed in applying it to the beaded stock itself. By introducing other "visible" mutant factors into one of the third chromosomes of the beaded stock, these effects can be duplicated for other characters besides beaded itself.

Thus the factors  $s_8 k e^s$  (and in one lot also  $r_o$ ) were introduced, by appropriate crosses, into the chromosome with  $B_d'$ , making the composition of the stock  $\frac{s_8 k e^s B_d'}{C' l_{III 1}}$ ; this stock perpetuated itself in heterozygous

condition just like the original  $\frac{B_d'}{C' l_{III 1}}$  stock. Since all three (or four)

introduced factors were recessive and heterozygous there was nothing to indicate their presence in the stock, and all the flies were normal in appearance except for the beading in the wings, which was rather weak and uncertain of appearance owing to loss of the intensifiers in making the crosses. A sample of 103 flies taken from this stock gave the following count: much beaded 1, moderately beaded 49, slightly beaded 49, normal 14, flies showing any of the characters of  $s_8 k e^s$  or  $r_o$  0. When this stock was crossed to the homozygous recessive stock  $s_8 k e^s r_o$ , twin hybrids of very different appearance were produced, as expected. Half of the  $F_1$  flies (all those that had received  $B_d'$ ) showed all of the characters of  $s_8 k e^s r_o$ , and most of them showed  $B_d'$  in addition; the other half, which were all non-beaded, looked perfectly normal. The hybrid flies of the first type, when crossed to each other, were constant, i.e., bred true, for all four recessive characters, although they showed segregation for  $B_d'$ . They were therefore "constant hybrids" with respect to the former characters, if we use this term in its customary empirical sense. The normal-appearing class, on the other hand, split up, giving approximately 2 normal to 1  $s_8 k e^s r_o$  in  $F_2$ . Similar re-



sults were obtained with a stock having only  $e^s$  in the chromosome with the  $B_d'$ .

In the stocks like  $\frac{s_s k e^s r_o B_d'}{C' l_{III 1}}$ , which contain  $s_s$  in the chromosome with  $B_d'$ , a very small percent of crossing over is possible between  $s_s$  and the factors to the right of it (about 2 crossovers in 1000 gametes), although practically no crossing over occurs between any of the other loci. By such crossing over  $s_s C' l_{III 1}$  and  $k e^s r_o B_d'$  chromosomes are produced (say 1 of each kind in 1000). If the  $s_s C' l_{III 1}$  chromosome meets in fertilization one of the  $s_s k e^s r_o B_d'$  type, as it has an even chance of doing, a fly homozygous for  $s_s$ , and showing the spineless character, is produced. Thus one individual in 1000 that hatch is spineless; the rest appear normal in all characters but beaded. Here, then, is a stock apparently breeding true, except for the fact that it throws spineless about once in a thousand times. In ignorance of the condition of balanced lethals existing in this stock, and of the not quite complete linkage of  $s_s$  with one of these lethals, the appearance of this character in such small but constant proportions would ordinarily be attributed to mutation.

Similar results were obtained in the case of the factors  $s_e$  and  $p$ , except that, as these lie further to the left of  $C'$  than does  $s_s$ , there was more crossing over, and so a higher percent of "mutations"; in fact, sepia appeared in such numbers that it would rather have been ascribed to the action of multiple factors, by a casual observer. This semblance of mutation with  $s_e$  and  $p$  was also obtained in the case of a stock of composition  $\frac{s_e p s_s k e^s r_o B_d'}{s_d C'}$ , which differs from those above discussed in that

there is no lethal in the  $C'$ -bearing chromosome (though the flies homozygous for  $s_e$ , spread wings, are not as viable as the others). Flies of this type, mated together, should therefore give counts like those of the balanced lethal stock, with the addition of (nearly) 1 homozygous spread individual for every two of the others that hatch. The sample count gave 50 spread and 136 not spread; the latter consisted of 5 well beaded, 35 moderately beaded, 50 slightly beaded, and 49 normal-winged; these were all red-eyed with the exception of 1 pink and 15 sepia-eyed crossovers, in which the respective eye-color mutants had escaped from their bondage to the lethal  $B_d'$ , and so emerged into visibility as though they had just arisen by mutation directly.

(4) *Other cases of enforced heterozygosis*

The correspondence between these findings and those in *Oenothera* is more than curious. *Twin hybridism*, *constant hybridism*, and the repeated production of certain definite types of "mutants" in determinable proportions—any one of these genetic phenomena alone is remarkable, and has been found hitherto only in rare instances; for two organisms both to exhibit any one of them would thus immediately raise a presumption in favor of these organisms having certain special genetic processes in common, yet here we find not one, but the whole concatenation of striking peculiarities present in both forms. The two forms resemble each other also in certain other features of their genetic behavior, which are more difficult of formulation, such as the peculiar ratios in which the "mutants" emerge from crosses with other varieties, and it should be noted that the ratios are just as unusual in the *Oenothera* crosses as in the crosses of beaded. It is difficult in view of all these parallelisms to believe that the two sets of phenomena have not a similar basis, and that the *Oenotheras* do not represent a complicated case of balanced lethals. An interpretation which really amounts to a form of the balanced lethal idea has in fact already been proposed by DE VRIES (1911), for the purpose of explaining his double reciprocal crosses. In order, then, to arrive at a general understanding of the genetic behavior of *Oenothera*, we may not have to introduce a totally new set of notions, but may merely extend the conception that has already been used for the double reciprocal crosses.\* For, as the analogy of the beaded case demonstrates, a mechanism of this same sort will explain nearly all the other types of genetic irregularities observable in the *Oenothera* genus. It will be pointed out later that even abnormalities in chromosome distribution, together with the genetic consequences thereof, may be an indirect result of the balanced lethal condition.

One important difference in detail should, however, be noted, between the results in the two forms: the *Oenotheras* do not give even qualitatively the same results in reciprocal crosses, whereas beaded stocks show only slight differences in reciprocal crosses, that are due to the occurrence of crossing over in one sex only. This difference between the factors contained in the functional sperm and ova of *Oenothera* indicates, as DE VRIES (1911) has suggested, that here the lethals, or some of them, act directly upon the germ cells rather than upon the zygotes.

The above consideration raises the question as to what must be the

\* A beginning is already being made in this direction. See addendum to this paper, page 498.

attributes of balanced lethal factors, and how generally this term may be applied. In order to "balance" each other so as to enforce heterozygosis, each of the two lethals must be responsible for the non-appearance of a different type of homozygote. For this end they must lie opposed in "opposite" (homologous) chromosomes, and must cross over with each other little or not at all. Either one of them may produce its effect either by killing all gametes of one sex which it enters or by killing individuals homozygous for it; if both lethals affect the gametes primarily, they must exert their influence on gametes of opposite sex, one killing the sperm it enters, the other the ova. An intermediate case also is possible, in which one of the lethals kills zygotes whereas the other kills gametes; in this case the lethals would balance no matter which kind of gametes ( $\delta$  or  $\text{♀}$ ) were killed by the second lethal.

Reciprocal crosses seem to indicate that some *Oenothera* species are of the latter type or of the type in which both lethals affect gametes (of opposite sex). On attempting, however, to work out the details of any of the reported series of *Oenothera* crosses, on either of these two schemes, some result is usually found which will not fit in with the specific assumptions. Perhaps the material used to represent the same type of plant in different individual crosses, although it appears similar phenotypically, is really of different genetic composition, or possibly the effect of some of the lethal factors is dependent upon environmental conditions, differences in which would then prevent the results from seeming consistent with each other according to any of the schemes. Then, too, it is likely that the problems are complicated by crossing over, multiple factors, and a balanced lethal condition in more than one pair of chromosomes or regions of a chromosome.

It will take very carefully controlled crosses to analyze the facts with reference to each of these various possibilities. Meanwhile, it seems likely that a condition or conditions of balanced lethal or semi-lethal factors forms the essential part of the explanation. This conclusion seems especially plausible in view of DAVIS'S (1916, 1917) recent work, in which a high and differential mortality existing among zygotes of *Oenothera* is conclusively proved. Especially important, by way of suggesting an explanation for the apparent inconsistencies of earlier results with any scheme of balanced lethals, are the great variations in mortality which he finds to exist, according to environmental conditions; in fact, he has been able to show that certain classes may appear under unusually favorable circumstances which ordinarily are entirely sup-

pressed. The attainment of constant culture conditions, then, is, as he points out, a factor highly important for intelligible results.

In a recent paper DE VRIES (1917) reports the finding of a "mutant" of *Oe. Lamarckiana* which fails to give twin hybrids when outcrossed to other species, but which instead gives them on being bred back to *Lamarckiana* itself. This is just the situation which we should expect to arise occasionally if *Oe. Lamarckiana* really contains balanced lethals, and it is exactly what occurred in the case of the normal-winged fly discovered in beaded stock, described on p. 449. One of the third chromosomes of this fly contained neither  $B_d'$  nor  $l_{III1}$  as it had resulted from crossing over between these two lethal factors, while the other chromosome was an  $l_{III1}$ -bearing chromosome of the type usual in this stock. This normal-winged fly, unlike its parent beaded, on being crossed with flies from non-beaded races did not produce twin hybrids, since it was not heterozygous for any dominant factor; on being crossed back to the beaded stock from which it sprang, however, this fly would have produced twin hybrids, as offspring which received its normal chromosome might have received either an  $l_{III1}$ - or a  $B_d'$ -bearing chromosome from the beaded parent. The *Oenothera* mutant in question, then, may be a similar individual in which the balanced condition that was originally established in the species has been caused to disappear again by crossing over between the lethals. As we shall see later (p. 484), results showing a disappearance of the balanced lethal condition could also be caused by non-disjunction and by doubling (tetraploidy).

If the balanced lethal interpretation of *Oenothera* should hold, the feature of it which would probably be most interesting to the general biologist would not be its explanation of aberrant ratios, and peculiar hybrids, but its disproof of the validity of most of the *Oenothera* "mutations." For the majority of the so-called "mutants" would, in this case, merely consist of *crossovers*, in which recessive factors that had previously been held heterozygous by their linkage to a lethal had broken their bond with this lethal and so had been able to emerge into homozygosis and visibility. As this might happen to a group of linked recessive factors as well as to one factor alone, these "mutants" might usually be departures from the normal type in a considerable number of characters simultaneously, unlike most ordinary mutants, and this is a finding that DE VRIES has often noted in *Oenothera*. It cannot be too strongly emphasized, however, that such disclosures as to the spuriousness of *Oenothera* mutants can no longer in any sense be regarded as a challenge to the mutation idea in general, for the reality of the process

of mutation has been often and conclusively demonstrated in *Drosophila* and other forms. Mutations in *Drosophila* can be distinguished with certainty from the curious counterparts of them which occur in balanced stocks, because in *Drosophila* there is ready means of conducting definitive genetic analyses of the composition of parents and offspring. Thus the mutation theory holds true, even though it may originally have derived its main support from facts in *Oenothera* belonging to quite a different category.

A still more evident instance of balanced factors is Miss SAUNDERS'S (1911, 1913) case of double-flowered stocks (*Matthiola*). Here a race of singles is known all the individuals of which must be heterozygous, for they always give rise to a high percent of doubles when crossed with each other. Homozygosis for singleness is prevented by the death or non-functioning of all pollen grains carrying the factor for single, for it has been found that in crosses of this race to ordinary singles it transmits the (recessive) factor for doubleness to all its offspring, if used as pollen parent, but to only half its offspring, if used as egg parent. There is therefore a factor lethal to pollen which is closely linked with the factor for singleness, or possibly identical with it. This conclusion was first arrived at by GOLDSCHMIDT (1913), who, however, believed that the lethal effect was in some way connected with the factor for sex; this latter postulate is superfluous, as FROST (1915), in his illuminating analysis of the whole case, has pointed out. Miss SAUNDERS herself avoids the conclusion that the single-carrying pollen is killed by assuming that there is a segregation of factors occurring during development, so regulated that all cells destined to form pollen receive only the allelomorph for doubleness; the assumption of such "somatic segregation," however, runs counter to our knowledge of the mechanism of segregation and chromosome reduction. There is, therefore, in the chromosome carrying "singleness," a pollen-lethal factor. In the opposite (homologous) chromosome the factor for double itself, or some factor closely linked with it, acts as a balance to this lethal, for, although this chromosome is not actually lethal, homozygous doubles are sterile. The homozygous doubles, then, cannot be bred, and homozygous singles cannot be produced in this race at all. Hence all plants eligible for parentage in any generation must be heterozygous, and the heterozygous stock will automatically perpetuate itself.

In breeding these balanced singles we are virtually performing a back cross, for the equal numbers of single- and double-carrying eggs will be fertilized exclusively by double-carrying pollen. There should there-

fore be 50 percent of heterozygous singles and 50 percent of pure doubles produced at fertilization. It has been shown, however, that the singles have a lower viability than the doubles (perhaps on account of a slight effect of the lethal factor when in heterozygous condition), and, in accordance with this fact, it is usually found that somewhat less than 50 percent of the plants which come to maturity are singles. This, of course, does not alter the general situation in regard to enforced heterozygosis. SAUNDERS (1911), however, has attempted to explain such discrepancies from the calculated ratios by assuming that there are two factors for doubleness, which are linked together in varying degrees. Although the explanation of differential viability is much more probable, it is not impossible that, in the course of the long selection work whereby breeders endeavored to increase the proportion of doubles, another factor for doubleness may have arisen in the same chromosome and have been perpetuated; but we would also have to assume in that case that the second double, like the first, caused sterility, and that a lethal arose in a locus opposite to it, like the lethal opposite to the first factor for double.

It has been stated that when double-throwing *Matthiola* is used as egg parent in an outcross to ordinary singles, half the offspring receive a factor for doubleness and half do not; in other words, twin hybrids are formed (double-throwing singles and apparently pure singles). Moreover, in other crosses, disturbances in ratios are produced which are clearly owing to the presence of the lethal factor. But the parallel with the *Oenothera* and beaded results goes still further than this, for it is also found that some lines of the double-throwing stocks are eversporting, giving off a small proportion of cream-colored "mutants" in each generation. This circumstance is here admittedly due to the partial linkage of the recessive factor for cream with the single (and therefore with the lethal). The sports are merely crossovers. Here, then, is another illustration of spurious mutation due to a "balanced" condition.

The case of *Matthiola* shows that we may broaden our conception of enforced heterozygosis to include not only balanced lethal factors, but balanced factors which in any way prevent races of both types of homozygotes from becoming established. For this purpose the factors may only produce sterility of the homozygotes. Two such factors might be balanced against each other, or one lethal against one sterility factor. Here the population would be kept genetically *heterogeneous* (not pure for a certain type of allelomorph), although not every individual would be *heterozygous*; all parents, however, would have to be heterozygous.

As sterile individuals are, however, usually a burden on the group, such cases would, under natural conditions, tend to change over into cases of balanced lethals, for, when a lethal factor arose, linked with the sterility factor, it would give the line containing it an advantage. A yet greater departure from the balanced lethal type, in which not even all the parents need be heterozygous but in which the population is still kept heterogeneous, would be a case where one or both of the homozygotes were sterile in one sex only, or sterile only when mated to similar homozygotes. Here pure stock could never be maintained, for though homozygous individuals might be used as parents, they would always have to be crossed to the heterozygous or the opposite homozygous type. Theoretically, two other types of production of enforced heterozygosis also are conceivable, namely, somatic segregation, and selective fertilization<sup>12</sup> between gametes dependent upon the genotype they possess and not upon the genotype of the individual producing them. There is, however, no evidence that either of these processes ever occurs as an event which can in any sense be called normal.

(5) *The reason for the origin of enforced heterozygosis*

It has been shown how the state of enforced heterozygosis must have arisen in the case of beaded-winged *Drosophila*; similar considerations explain the origin of this phenomenon in other artificially bred organisms and also "in nature." The one and necessary condition for its production is the appearance in the species of some beneficial factor which is unable to exist in homozygous stock. By "beneficial" is meant in this connection the property of having survival value under the kind of natural or artificial selection which obtains at the time.

The inability to exist in pure stock may be due to a secondary lethal or sterilizing effect exerted on homozygotes or gametes either by the factor itself or by another factor closely linked with it. Given this sort of factor, there will be a tendency for such stock to be selected as contains the highest possible proportion of it, provided only that the advantage of having the factor is always enough to more than counterbalance the disadvantage of so many individuals being rendered sterile or inviable. In this way it happens that, when a balancing lethal (or even sterilizing factor) arises, as it has a very good chance of doing at

<sup>12</sup> Selective not in the sense that some gametes of an individual are functional and not others, but in the sense that, though all may unite with some kind of gametes or other of a particular individual, they fertilize gametes of different genetic constitution, depending upon what factors they themselves contain.

some time or other if our conclusions regarding the frequency of lethal mutations are correct, the stock containing the balancing lethal in addition to the original "beneficial" factor will be selected rather than that containing the original factor alone. For such stock would maintain a constant output of individuals containing the beneficial factor; in fact all viable or fertile individuals would possess it. The competing energies of this stock, as a group, would thus not be sapped by the continual presence within it of a large number of undesirable individuals that forced a vitiating intra-group competition. The disadvantage of smaller total numbers might easily be compensated for by the fact that it was the "less desirables" which were weeded out.

It is easy to imagine that some time long ago in the history of the *Oenothera* group some dominant factor arose that was of such advantage when in heterozygous condition that it made headway in spite of a lethal effect on homozygotes; the establishment of a balancing lethal was then but a matter of time. In the case of beaded, the "beneficial" factor was of course beaded itself, since the experimenter consciously selected beaded-winged flies to breed from. Artificial selection was no doubt responsible for the case of stocks also, for double flowers are always considered desirable by the fancier. Moreover, there could have been no natural advantage in the flowers' having the factor for double, because it is recessive and so every time it produces its visible doubling effect, it also produces sterility. For a *natural* selection of a beneficial factor which cannot persist in pure stock, the factor must be *dominant* in respect to its beneficial effect (except where sterile workers are of advantage to a community). The case of stocks, then, differs from the others in that the desirable factor is here completely recessive, and it is actually the sterile homozygous individuals which must have been selected for, not heterozygotes in which the factor produced its beneficial without its harmful effect. This being the case, such races of singles would be selected as yielded the highest proportion of the sterile doubles; thus eventually the cultivated singles would be found to contain a balancing lethal.

It should also be pointed out that very long continued breeding from heterozygotes exclusively would in itself tend to result in the establishment of a balancing lethal, even without the special selection, in the way above described, of those strains which produced the smallest proportion of homozygotes among their viable and fertile offspring. For even though a balancing lethal were not specifically selected for, it would become established sooner or later unless it were specifically selected



against, since when any lethal happened to arise in the appropriate position, there would be nothing to prevent its continuance in the race. Hence eventually all individuals would come to contain some balancing lethal.

This argument applies, moreover, not only to a second or "balancing" lethal factor, but also to the appearance of a lethal in the first place. That is to say, if for some reason heterozygotes are always selected as parents, then, even if there are no lethal factors present to begin with, one or more recessive lethals linked with the factor which is being kept heterozygous will eventually come to exist with it in the same chromosome, for there will be no selection against such lethals; meanwhile, in the opposite chromosome the balancing lethal will tend to arise. So, for example, it is likely that if the practice of breeding heterozygous pink *Mirabilis* were kept up for a very long time, even without purposive selection of those families which gave the highest proportion of pink offspring, the race would eventually come to "breed true" to the pink color.

The establishment of lethal factors in cases like the above would simply depend upon the fact that the individuals used as parents would in such cases always have to be heterozygous for any lethal factors that might arise. Lethal mutants that were innocuous in heterozygous condition might then not be selected against, and so they would become established some time or other. Now the same process would also take place not only when heterozygotes are specifically chosen as parents in each generation, but also when organisms are continually out-bred to unrelated lines, for here, although they are not necessarily kept heterozygous in regard to some factor present at the beginning, nevertheless, any mutant factor that might arise would be kept in heterozygous condition and, if recessive, it could not be selected against. For this reason it is to be expected that lethal factors and all sorts of "undesirable" recessive factors would gradually "creep into" the chromosomes of species which constantly out-crossed, so that if ever inbreeding were practiced upon them various abnormal types would appear and a state of balanced lethal factors in respect to one or more of the pairs of chromosomes would emerge. It might also very well happen that in such a contingency a lethal might "find itself" balanced by a non-lethal but disadvantageous recessive factor, such as one for sterility. Such a stock, if it persisted at all, would tend to change over into the condition of balanced lethals, for it would fulfill the condition of containing in one chromosome a dominant "beneficial" factor (the normal allelomorph of

the disadvantageous one) which nevertheless could not exist in pure stock (here owing to its linkage with a lethal).

All the above outlined circumstances, that tend to lead to the production of balanced lethals can in fact be regarded as cases fulfilling the condition first mentioned as the only one necessary for producing such a result. For, in cases where heterozygotes only are bred from, this must be because each chromosome contains a factor or factors, that produce some effect in heterozygous condition which leads to their selection as parents (i.e., some "beneficial" effect), but which nevertheless for some reason cannot exist in pure stock (otherwise the homozygotes too would be chosen as parents). And, in cases where out-crossing is always practiced, each chromosome of course contains "beneficial" normal factors potentially dominant to any recessive lethal mutant that may arise, yet, on account of the out-crossing, not one of these chromosomes can be obtained in stock pure for its particular self (that is, in stock having the homologous chromosomes so closely related genealogically that in cases where a mutation has occurred, both of them are likely to contain the same mutant factor.) So we see that the two modes of production of balanced lethals just considered are merely special cases of the general type first enunciated.

(6) *Effect of the continued heterozygosis on the chromosomes and on the evolution of the race*

The causes leading up to the state of balanced lethals having been examined, it now remains to consider what conditions the heterozygosis thus enforced would itself induce. The way for a treatment of this matter has already been paved by the consideration of the effects of out-crossing and of continually selecting heterozygotes as parents. In the balanced stocks, just as under the latter circumstances, any recessive mutant factors appearing in the chromosome regions involved cannot be selected against, for they would produce tangible effects only on homozygotes, which never appear (or at least never function as parents). Recessive mutants of all kinds would therefore gradually accumulate in the affected chromosomes, so that finally, even if it were possible to get rid of either of the original members of the pair of balanced lethal factors, it would still be impossible to obtain homozygotes, on account of the new lethals which had arisen near the old ones. In other words, the condition of balanced lethals itself gives rise to more balanced lethals, linked with the previous ones. If there were no crossing over, lethals anywhere within the affected chromosomes could persist, for no portion

of them would ever become homozygous and subject to selection with respect to recessive factors. In chromosomes where crossing over occurred, only regions near the lethals could become homozygous infrequently enough for selection with respect to recessives in these loci to be negligible; lethals would come to exist here, however, and these secondarily formed lethals would then constitute new foci, protecting the existence of still other lethals in the regions next adjoining: thus the "degenerative" process would gradually spread throughout the chromosome. Besides the lethals, other abnormal factors also might arise, and these too would be protected by the balanced lethal mechanism from being weeded out; nevertheless they might occasionally become homozygous, by some rare crossing over from their enfettering lethal, and then they might be hailed as new mutations.

Knowing the frequency of mutations, it would be possible to calculate just how quickly these degenerative changes would take place. Thus, if recessive lethals in a particular chromosome arise with such frequency that (on the average) one out of 500 gametes of each normal individual contain one such mutant factor, then, after 500 generations of enforced heterozygosis in regard to this type of chromosome, each individual would (on the average) contain one lethal in each of its chromosomes of this type, for the lethal should have been present as a mutant in one of the 500 ancestral gametes that transmitted one of these chromosomes, and, once having arisen, the lethal would of course have persisted.<sup>13</sup> On the same supposition of lethal frequency, after 1000 generations (about 40 years in *Drosophila*), each of the chromosomes which has been kept heterozygous would tend to have two lethals in addition to what it may have had at the beginning. In this calculation it is assumed that the whole chromosome has been involved in the enforced heterozygosis; if part of it was beyond the influence of the latter, owing to crossing over, correspondingly fewer lethals would have become established—i.e., only those of the above which happened to lie in the re-

<sup>13</sup> It might be urged that the lethal could mutate back to normal or to some non-lethal factor. But even if this tendency were as strong, on the average, as the tendency for the original normal factors to mutate to lethals, a condition of equilibrium would not be established, wherein mutations in either direction were equally frequent, until half of the normal factors had been transformed into lethals. Until that time, then, lethals would gradually accumulate, and in the early stages of the "degeneration," reverse mutations would be so infrequent as to be negligible. It should be noted moreover that if the same tendency which made lethals revert also caused other factors to revert to an ancestral form, the occurrence of lethals would really be favored, for a normal factor on returning to an ancestral form would usually not be well adapted to the life complex of the existing race, and so would tend to be lethal.

stricted region that was under the "protective" influence of the enforced heterozygosis.

There is now evidence in *Drosophila* (BRIDGES 1917) that besides the "point-mutations," in single loci, changes occasionally occur that involve a whole region of the chromosome, perhaps destroying, or, more probably, "inactivating" all the contained factors in some way; for it is found that the latter no longer give any evidence of their existence, inasmuch as any recessive mutant allelomorphs that may be introduced into the opposite chromosome will not be dominated over by factors in such a region of the once normal chromosome. (Such a region, moreover, even if relatively short, is always found to be lethal when homozygous, corroborating our conclusion that not many changes, or losses, can occur among factors without undoing some mechanism that is necessary for life.) Now, these recessive regional mutations, or "deficiencies," like the point-mutations, could of course persist in the chromosomes protected by heterozygosis from the action of natural selection, and although these changes must be very rare,<sup>14</sup> they might make up for their infrequency by the quantity of change produced by them when they did come. By this sort of change too, then, the chromosomes originally containing one pair of balanced lethals may gradually degenerate.

It should be noted, however, that degenerative alterations of any kind (whether deficiencies or ordinary mutations) could not become established in one member of the pair of chromosomes, if they involved loci allelomorphic to any that had already become changed in the opposite member of the chromosome pair, for individuals so affected would die. Thus not more than half of the loci of each chromosome could, on the average, become changed. These loci would, of course, be scattered anywhere along the chromosome.

Since there would be no process of selection either for or against any of these mutants, corresponding chromosomes in different individuals might, in the course of time, come to have very different compositions. The accidental decline and spread of certain lines would however, tend to keep the number of different sorts of chromosomes from becoming very large, except in extremely populous groups, for although this process of "extinction of families" is slow, that of mutation is slow also.

Since the synopsis of homologous chromosomes must depend in some way on a specific attraction of (certain?) like loci (allelomorphs) for

<sup>14</sup> 15,000  $F_1$  flies were examined by the writer, in a cross of  $s_a s_s k e^s r_o$  (all in chromosome III) by  $p_r c_v s_p$  (in II) but not one case of deficiency was found among them. Yet these crosses were so arranged that all deficiencies involving any one of the eight rather widely separated loci would be detected.

each other, the synaptic attraction of the members of the balanced pair would tend to diminish as they diverged more and more from one another through their unlike mutative processes.<sup>15</sup> This might lead to diminution in crossing over, which would be an advantage so far as productivity was concerned, for crossover chromosomes, containing lethals of both members, would cause death unless they met in fertilization with crossovers of just the opposite type. The weakening of the synaptic attraction might also lead to visible peculiarities of behavior in the maturation period, such as failure of homologous chromosomes to become finely spun out during maturation, and their late or imperfect (end-to-end?) conjugation. Non-disjunction too would then occur more often, for when chromosomes do not conjugate, it is a matter of chance whether or not they enter the same cell at the segregation divisions.

“Degeneration” of the general sort above described is not a process quite peculiar to pairs of chromosomes containing balanced lethals, but it must take place in every chromosome that is for any reason kept in company with homologous chromosomes of remote genealogical relationship to it, for the latter chromosomes, not having mutations in the same loci as the former, would by their dominant normal factors prevent the recessive mutants in the former chromosome from manifesting themselves, and so from being eliminated by selection. Thus, in hybrid Andalusian fowl or yellow mice, if the animals were for centuries bred from heterozygotes, and also in any of the chromosomes of species which propagate by continual out-crossing (as corn tends to do), lethals would

<sup>15</sup> The reasoning in detail is as follows: Chromosomes and regions of chromosomes having a certain genetic composition usually conjugate in a certain distinctive way (i.e., with each other only), regardless of what their derivation or previous history may have been. Now since the genetic composition of these chromosomes or regions has been, *ipso facto*, the only persistent feature which caused them to retain their individuality, it follows that it must be their own genetic composition which determines these distinctive features of their synapsis—i.e., how and with what they shall conjugate. That is to say, the genetic factors situated in each particular chromosome-segment cause a specific attraction between it and another chromosome-segment containing like factors. This means that the factors themselves or *local* products of them, attract each other, like for like. Of course there is no experimental evidence to prove that these genetic factors for synapsis are the same as the factors for the visible characters, but certainly there must be such “synapsis factors” present in every region of the chromonema, as shown by the exactness of the apposition which occurs. Moreover, these factors must, like the factors for visible characters, be subject to mutation, else they could never have become differentiated. This being true, the factors determining synapsis which are present in a balanced pair of chromosomes must gradually diverge more and more from one another in character, and so the synaptic attraction between the chromosomes of that pair will be weakened.

tend to arise, as we have already seen, and this whole degenerative process would in fact gradually take place.

The best examples of this sort of change are to be found in the Y chromosome of species of the *Drosophila* (XY) type of sex determination, and in the W chromosome of species of the *Abraxas* (WZ) type, for these chromosomes (or at least the region of them opposite the sex factor, in cases where crossing over was possible), have been continuously in company with remotely related homologues ever since the respective types of sex determination have been established. Moreover, the "degenerative" changes in these cases could be much more extensive than in cases of balanced lethals, because the Y and W chromosomes were more completely protected by their homologues from the action of selection. This is because their homologues (X and Z respectively) themselves remained quite normal, owing to the fact that they were subjected to selection when in the homozygous sex (XX or ZZ). Recessive changes could consequently be established in any locus of Y and W, whereas in cases of balanced lethals, where both homologues might become involved, only half of either chromosome might, on the average, degenerate. This consideration would apply to any case where the back cross was continually performed, for one chromosome would be kept normal while the other would be completely protected from selection, so far as recessive mutations were concerned. It is probably needless to point out that the W and especially the Y chromosome (which is perhaps of more ancient origin), do show the expected evidences of this degeneration and differentiation from their homologues, both genetically and cytologically. The evidences are now as follows: (1) Recessiveness of W and Y in respect to any mutant factors arising in the homologue, even though these mutants are usually themselves recessive to the original normal allelomorph; (2) similar non-dominance over normal factors in the homologue, as shown in cases where the normals are themselves recessive to mutants; (3) failure of occurrence, or relative infrequency, of dominant mutations in W and Y (recessives would not be detected); (4) visible differences in size and shape between them and their homologues; (5) great variations in their own size and shape even in closely related species; (6) weak synaptic attraction between them and their homologues, as indicated by the tendency for the sex chromosomes in the heterozygous sex to remain condensed during the growth period, while the autosomes are spinning out for intimate conjugation, (7) and as shown by their frequently delayed synapsis, (8) and by the lack of crossing over between them and their homologues

when they conjugate, even in the sex where other chromosomes are undergoing crossing over (shown in BRIDGES'S (1916) non-disjunctional females of type XXY), (9) and by the tendency, in these XXY individuals, for X and X to conjugate more often than X and Y. All of these peculiarities were to have been expected in the light of the previous considerations. The fate of the Y chromosome, then, illustrates what will eventually happen to the chromosomes in cases of balanced lethals, although in the latter cases each member of the pair can change only half as much.

Non-disjunction leads to the production of individuals with three, and eventually four, members of the chromosome pair originally involved. When this happened to chromosomes containing balanced lethals (as it probably would at some time), the members,  $A$  and  $a$ , of the originally balanced pair, would now tend to become two pairs,  $AA$  and  $aa$ , like pairing with like, provided the differentiation between  $A$  and  $a$  had become marked. Each of the members of these two pairs, now having identical mates, would (if this tendency was completely realized) behave like normal chromosomes, and every gamete would receive one of each pair, namely  $A$  and  $a$ . Every gamete and zygote would consequently contain all the dominant normal factors necessary for life; the lethal effect would thus be nullified and productivity would again rise to 100 percent.<sup>16</sup> If, however, the two pairs were not well differentiated and occasionally conjugated in a criss-cross fashion ( $Aa$  and  $aA$ ), intermediate values for productivity would result. These individuals of higher productivity would obviously be selected in the struggle for existence (unless the alteration which had occurred in the proportions of the chromatin materials had disturbed the mechanism of development, as it does in *Oenothera lata*). Thus the race containing balanced lethals might eventually return to a condition of normal genetic behavior, through the occurrence of non-disjunction, which would itself have been favored by the weakening of synaptic attraction between the degenerating chromosomes.

A similar situation would develop if, instead of non-disjunction merely of the balanced chromosomes, the whole nucleus became tetraploid. Here similarly the balanced pair would tend to form two independent pairs, the lethal effect would then disappear, and productivity would rise, provided the tetraploid condition was not harmful in other ways.

<sup>16</sup> Exception must be made of the special case in which the lethals are gamete lethals and dominant to their normal allelomorphs. In such a case, as all gametes would now receive the lethal, every gamete of the sex affected by that lethal would be killed.

Other things being equal, however, a tetraploid condition of all the chromosomes would not be as advantageous as the tetraploid condition of only the balanced pair (produced by non-disjunction), for evolution is hindered in tetraploid individuals by the fact that recessive mutants can manifest themselves here only in the rare coincidence that more than two of the mutant allelomorphs enter the same zygote.

Another important consequence of enforced heterozygosis, besides its allowing the degeneration of chromosomes, is that it hinders the establishment of progressive changes in them. That is to say, not only are (recessive) mutant factors which ordinarily would be disadvantageous not selected against, but the much rarer beneficial ones that would, if homozygous, produce advantageous effects, cannot be selected for, since none of them has an opportunity to manifest itself except in case of the (initially) very remarkable accident that the same mutation has occurred independently in both members of the chromosome pair. The evolution of the race is therefore hindered almost exactly in proportion to the fraction which the affected chromosomes form of the whole bulk of chromatin—e.g., if they form one fifth of the mass, selection has only four-fifths of the ordinary amount of genetic material to work upon, at least so far as recessive changes are concerned, and so evolution will (other things being equal) proceed at about four-fifths of the speed which it otherwise would have. For this reason, as well as on account of the disadvantage of their lowered productivity, species containing balanced lethals would in the long run have a poorer chance of survival than ordinary species. Nevertheless, in the long course of evolution by which any present-day form has been produced, this condition may have arisen more than once, especially since, as will now be explained, the retardation of evolution would not be absolutely permanent.

Although progressive change might for a long time be hindered in the way described, yet, after an extensive degeneration has taken place in either member of a pair of balanced chromosomes, many recessive changes in the other member will be able to "show," just as recessive mutants in the X chromosome can now manifest themselves in the XY male, owing to the "degenerate" condition of the Y. (In producing changes extensive enough to show these effects, point mutations would be vastly less effective than deficiencies). Thus, after a long time, selection and progress can start in again, rendered possible, paradoxically enough, by the degeneration which preceded it. After this degeneration, in fact, recessive mutants will be able to manifest themselves, and will be selected, even more readily than under ordinary circumstances, for



they do not need to be received from both parents in order to become evident, any more than sex-linked mutants do in the male *Drosophila*. A doubling in number of the balanced chromosomes through non-disjunction or "tetraploidy" would now make of them two ordinary pairs, the factors in one of which do not interfere with, or dominate over, the factors in the other. If the doubling had come before the differentiation, the same stage would nevertheless be reached in the end.<sup>17</sup>

Although the series of events described are entirely theoretical, they are the quite inevitable consequence of the random mutations and mitotic accidents that are continually though very infrequently occurring. Whether the course of processes outlined could actually come to pass therefore depends upon how long a race with balanced lethals would be able to maintain itself in competition with its less handicapped neighbors, and upon the actual frequency with which point-mutations, deficiencies, and mitotic abnormalities happen.

#### (7) *Special results obtainable with lethal factors*

Various peculiar sorts of results besides those seen in the crosses already reported may be obtained with lethal factors arranged in special combinations. For example, recessive factors present in heterozygous condition in both parents may be prevented from "showing themselves" in any of the offspring (i.e., appearing in homozygous condition), not only by having them completely linked with a recessive lethal factor, as before explained, but also, in case there is crossing over in one sex, by having a partially linked lethal on each side of them in the chromosome. For then the crossovers are killed too, inasmuch as the factor in question cannot cross over from both lethals at once (except in the rare case of double crossing over).<sup>18</sup> From the crossover fly containing  $l_{III\ 1}$  without  $C'$ , described on p. 444, flies were made up containing  $s_e D' p s_s k e^s l_{III\ 1}$  in one third chromosome, and their normal allelomorphs in the other.  $C'$  was not present. As  $D'$  is lethal when homozygous, the factors

<sup>17</sup> It is interesting to note that in tetraploid forms there would be a tendency for a series of changes to occur in the ordinary chromosomes similar to those occurring in balanced pairs. Groups of four would tend to differentiate into separate pairs, which would "protect" each other from selection; originally similar pairs would thus tend to undergo unlike degenerative changes, until they no longer protected one another, when degeneration would gradually cease, and selection of progressive mutants would at the same time gradually set in again.

<sup>18</sup> This of course does not apply to species in which crossing over occurs in both sexes; for a crossover egg containing the visible recessive factors without the lethal to the right, fertilized by a crossover sperm containing the visible factors without the lethal to the left, would produce viable individuals homozygous for the visible factors.

$p, s_s, k$  and  $e^s$  are penned in by a lethal on either side. Hence even though there is abundant crossing over these factors will nearly always be accompanied in the eggs by either  $D'$  or  $l_{III1}$  and as the sperm containing  $p, s_s, k$  or  $e^s$  will always carry both  $D'$  and  $l_{III1}$ , owing to the absence of crossing over in the male, no homozygous  $p, s_s, k$  or  $e^s$  flies can appear. The count was: completely normal 27, dichaete but otherwise normal 64, dichaete with sepia eyes 3. Pink-eyed, spineless, kidney-eyed, and sooty flies were completely absent. Since sepia has a lethal on only one side, but is linked to it, a few crossovers homozygous for  $s_e$  appeared, as expected.

If each of the above two lethals were balanced by another recessive lethal in a nearly opposite locus of the homologous chromosome, this stock would automatically maintain its original composition generation after generation without the production of  $p, s_s, k$  or  $e^s$  individuals, just like the stock  $\frac{s_s k e^s B'_a}{C' l_{III1}}$  described on p. 469. In that stock crossovers do not occur; in this one they would be killed.

In lieu of the two extra lethal factors, the  $D' p s_s k e^s l_{III1}$  chromosome was automatically maintained by crossing it to a chromosome containing  $p e C'$ . The flies were therefore of composition  $\frac{D' p s_s k e^s l_{III1}}{p e C'}$ .

Homozygous  $e$  flies are not killed but as they are somewhat less viable and much less fertile than most varieties, there is little danger of their crowding out the offspring of the above composition. This stock maintained itself fairly well, all flies appearing pink-eyed and either sooty-ebony compound or ebony, nearly all of the former being dichaete in addition. Dichaete crossed over occasionally, however. Spineless and kidney did not appear, so that the stock was seemingly pure for normal spines and normal eye shape. This race was then crossed to the self-maintaining  $\frac{s_s k e^s B'_a}{C' l_{III1}}$  stock, which, like it, was apparently pure for

normal spines and eyes, and unlike it, seemed pure for normal body color as well. It is evident that zygotes of the following four types, should be formed in equal numbers:  $\frac{p e C'}{s_s k e^s B'_a}$  (appearing  $e^s-e B'_a$ );  $\frac{p e C'}{C' l_{III1}}$  (appearing normal);  $\frac{D' p s_s k e^s l_{III1}}{s_s k e^s B'_a}$  (appearing  $D' s_s k e^s$ );

$B'_d$ );  $\frac{D' p s_s k e^s l_{III 1}}{C' l_{III 1}}$  (dies). The count was  $e^s-e B'_d$  34, normal 35,

$D' s_s k e^s B'_d$  26. Here, then, we have the spectacle of two races, both apparently agreeing in the complete "absence" of certain characters (spineless and kidney), which nevertheless, when crossed to one another, produce individuals (about one-third of the count) pure for those characters in the very first generation. It will be observed moreover, that "triple hybrids" were produced this time. If the cross had been

$\frac{e^s l_{III 1}}{D' C' B'_d}$  (appearing dichæte beaded) by  $\frac{e^s B'_d}{D' C' l_{III 1}}$  (likewise dichæte beaded), all the offspring would have been of the new type (not

dichæte, sooty, beaded) for only the  $\frac{e_s l_{III 1}}{e^s B'_d}$  individuals could live.

By crosses designed for the purpose, flies were made up of composition  $\frac{D' l_{III 1} B'_d}{e C'}$ . They appeared dichæte and somewhat beaded. These

flies maintained themselves in the same manner as the  $\frac{D' p s_s k e^s l_{III 1}}{p e C'}$

stock already described, there being always a mixture of dichæte beaded flies of the desired composition and weaker homozygous ebonies in the bottles. A dichæte male of the sort in question was then crossed to

beaded females of "pure" stock, and therefore of composition  $\frac{C' l_{III 1} B'_d}{l_{III 1}}$ .

In this cross sperm having the dichæte-bearing chromosome,  $D' l_{III 1} B'_d$ , should be checkmated, for it contains two lethals, and both kinds of eggs carry either one of these lethals or the other. Only offspring derived from the  $e C'$  sperm should therefore come to light, and these would consist of ordinary-looking beaded (composition  $\frac{B'_d}{e C'}$ ) and

normals ( $\frac{C' l_{III 1}}{e C'}$ ). On making the cross it was found that in truth,

among a large number of offspring examined, not a single dichæte fly appeared, and on breeding together the  $F_1$ , which consisted of beaded and normals as expected, dichæte failed completely to appear. Hence, this was a case in which a certain obvious character, dichæte, that is

clearly genetic and present in a majority of the individuals of a certain race, disappears entirely on crossing a fly exhibiting it to another stock, and never reappears in subsequent generations derived from the cross; it is no longer present even genetically. Yet ordinarily this character is a dominant. The result nevertheless presents no mystery; it was "made to order." Various other unusual results could be secured by carefully arranging lethal factors. For example, it would have been possible to "checkmate" both of the chromosomes of a parent in a properly chosen cross, in the same way as the  $D'$ -bearing chromosome was checkmated above. In that case, none of the offspring would be able to live, and we should have two otherwise fertile flies quite unproductive when crossed to each other. The results described in the sub-section dealing with the effects of crossing races with balanced lethals to ordinary races, or *inter se*, are comparatively simple and unexceptional compared with the results of crossing lethal-bearing races to other races also containing lethals, but other lethals, or lethals differently arranged. Triple, quadruple, and still more multiple hybrids may be produced, hybrids that breed true or those that segregate in all sorts of ways; impure stocks may be rendered "pure" on crossing, and *vice versa*, and bizarre ratios *ad nauseam* will appear.

It would be idle to continue the enumeration further. The factors may be put together like bricks, and the reader may work out for himself what the consequences of a cross will be. Enough crosses have been made to demonstrate that the results with these lethal factors obey the precise Mendelian regulations, and to piece together experimentally more unusual cases is an occupation no more requisite for determining the outcome of the various possible crosses than it is necessary for a civilized man to count on an abacus in order to be certain of the correctness of an addition. The study of the effects on crosses of lethal factors that have already been located, thus becomes merely a mental exercise.

(8) *The variability of the character beaded*

Practically all the peculiarities of inheritance in the beaded case have been shown to have their origin in just two circumstances: (1) that the mutant  $B_d'$  is a dominant, and (2) that the reaction by which the character is produced is readily modifiable. The first circumstance has been shown to be causally connected with the lethal effect of  $B_d'$  itself, and this lethal effect in turn explained the establishment of a balancing lethal. The second circumstance must be responsible for the fact that the character is so variable somatically, and also for the fact that so many (5 or

more) known genetic factors act as modifiers of it.<sup>19</sup> The question may now be asked, is there any connection between these two circumstances themselves.

There seems no *a priori* reason why dominant mutants should be more modifiable than recessives, although all the dominants so far found in *Drosophila*, without exception, are especially noteworthy for the relatively high variability of the characters they produce, as compared with the average of characters due to recessive mutants. Possibly the fact that the dominants are usually judged when in heterozygous condition has something to do with this result, although, again, there appears to be no theoretical reason why heterozygotes should be more variable than homozygotes. But whether or not individuals heterozygous for dominant mutant factors really are more variable than homozygous recessive mutants, as the facts certainly seem to show, there is a cogent reason for believing that mutant characters are more apt to be variable than normals are. The reason is that it is usually disadvantageous for an individual to have its characters depend on reactions that are easily modified, because then the normal, optimum, type would not so often be produced; factors will therefore have been perpetuated, by natural selection, which are especially undeviating in their effects. (Of course, in special cases, normal characters may be modified by ordinary agencies, but then they are only modified "adaptively," and under particular conditions; this is in fact physiology). Thus normal factors produce reactions that are less changeable than those of the average factor, or conversely, the characters produced by mutants would be, on the whole, more modifiable. The modifiability of the beaded character is thus correlated with the fact that it is a mutant.

All this character variation has of course nothing to do with *factor* variability, and, so far as this question is concerned, it should now be evident that the known peculiarities of beaded inheritance are entirely explained by the findings above reported. *The argument for factor variability in this case therefore falls*, and in similar cases the burden of proof must hence be on the proponents of that hypothesis, until the genetic facts are thoroughly analyzed. But, besides these experimental findings, some considerations have been raised in the preceding discussion that lead to certain theoretical objections to factor variability. These and other general arguments bearing on the problem here at issue will now be examined.

<sup>19</sup> The occurrence of *C'* in the chromosome with  $l_{III\ 1}$  does, however, seem to be an exceptional coincidence, not referable to either of the two circumstances named.

(9) *General considerations concerning factor variability*

If the conception of the organism sketched in the section dealing with the frequency of lethal mutations is correct, alterations in factors are very apt to have harmful, if not fatal, results on the individual. It is probable, moreover, that at least thousands of factors cooperate to make the individual. In view of these two circumstances, it is certainly venturesome to assume that the genetic factors are all continually varying from generation to generation. The individual formed of these factors would, to put it bluntly, have a very hard time holding together. Among thousands of fluctuating factors it would, on any such assumption, seem likely that a few at least in every individual would have varied so much as to be lethal or semi-lethal. In other words, as soon as it is postulated that the larger visible variations are merely the extremes of a fluctuation curve, and that innumerable factors in the individual are each producing curves of similar sort, it becomes difficult to see how an appreciable proportion of these visible, non-lethal changes can be taking place, without every individual also receiving many lethal, or at least very disadvantageous, variations at the same time. Under such circumstances, natural selection would be scarcely sufficient even for the work of conserving the factors as they are, and maintaining the race at its normal standard, let alone for the task of race improvement, or the alteration of particular characters in desirable directions, in addition to all this. On the other hand, if factor changes are rare and definite, these difficulties are not encountered.

Of course if the individual factor fluctuations were made indefinitely small, the total variation could be held within harmless bounds, but in that case it is very doubtful whether any one factor would vary enough for the changes in it to be appreciable, and it is difficult to see how, under such circumstances, natural selection could obtain a grip. Besides, it cannot be taken for granted that the variations may be indefinitely minute, for the factor itself is so tiny that changes in the number of its molecules, or in its radicles, might well produce quite discrete effects.

Factor changes, however, probably do not consist in mere variations in the number of molecules at all; they are probably qualitative, rather than quantitative in this sense, and so they should not be expected to follow a "normal curve." One reason against regarding factor changes as quantitative is that in FEDERLEY'S (1913) crosses of butterflies, where zygotes are formed in which some of the chromosomes are half the size of others, due to an extra division of the former, the size differences are

evened up again during development. The factors must necessarily have been halved when the chromosomes were; presumably they also recovered their form or size when the chromosome did, but if there is any doubt about this point it could be easily tested genetically. Another reason for believing that there is a definite size which each gene tends to approach as its equilibrium amount<sup>20</sup> is that, if this were not so, the sizes of corresponding genes in different cells would, by the accumulation of chance differences, surely come to vary enormously from each other after the long series of multiplications occurring during development (and generation after generation). For the genes, after all this time, to present just "tractable" differences from each other, of the order of magnitude postulated by the believers in fluctuation, would require a delimitation of variation adjusted with the utmost nicety, considering how many times the genes must reproduce themselves in all the cell generations from gamete to gamete. In doubling themselves in any one cell generation, then, their amount of growth would have to be regulated with infinite exactitude, and their course of variation would need to be steered along a knife edge.

The hypothesis of a continual fluctuation of factors, though regarded by many as such a natural assumption, really fails to account for the fact of continuous character variability which it was invented to explain. For if the factors are varying slightly all the time, they should vary in

<sup>20</sup> Although it is likely that the size of any particular gene is determined by a certain equilibrium point towards which its growth reactions tend, nevertheless we cannot, as in simple cases of mass action, consider all the genotypic material of a certain kind present in the cell as a whole, and say that this will tend to approach a certain amount, for the *total* amount is also dependent on the number of "units" of this gene that are present in the cell. Thus, if two units, or "doses," are present, there will be a different amount of genotypic material produced in the cell than if there is only one, as indicated by the different results produced by the sex factor, according to its dosage, and by the factors in a certain chromosome of *Oenothera* which in three doses cause the *lata* form and in two the *Lamarckiana*. Observations on the relative sizes of homologous chromosomes when they may be present in cells in varying numbers proves that the same principle holds here (thus each of the two sex chromosomes in a female is just as large, relatively, as the single one in an XO male), but of course one cannot surely argue from the size of the visible chromosome structure to that of the factors contained in it. We may therefore conclude that the growth of each individual gene and chromosome is carried on until a certain equilibrium point is attained, but that this equilibrium amount for any one gene or portion of a chromosome is more or less independent of how much material of this same sort is already present elsewhere in the cell. These principles appear to hold for the *visible structure* of chromosomes in FEDERLEY'S crosses of butterflies, where different numbers of chromosomes may be present, some of which may have been reduced in size by a double division during maturation, but where all chromosomes nevertheless eventually attain the normal size.

the embryo as well as in the adult, and in early gonidia as well as late, but a variation in a factor at that early stage would give a bias to the size or nature of that factor in all descendant cells in the direction of this original variation. Thus the offspring of an individual should often have their factors varying about a mean different from the value of that factor in the parent, and the curve of offspring should not be a normal curve at all, but polymodal, with more individuals grouped about some of the modes, representing variations that occurred in earlier gonidia, than about others, which would have been caused by variations in later cells that did not have so many descendants. Within the groups about each mode, moreover, there would be subsidiary centres of aggregation. This would seem true especially when we remember that the cells do not grow and divide synchronously but develop in more or less discontinuous groups.

To escape this dilemma it would seem to be necessary for the proponents of fluctuation to postulate that variation is confined to the maturation period of the germ cells, or at least to their later stages. But to do this is to dig the ground from under the very foundations of the hypothesis, for its chief claim to recognition lay in the inherent plausibility of the idea that factors, like so many other things, must be subject to continual slight variation, and could not be kept constant. To now admit that the factors may nevertheless be practically constant through most of the life cycle is to admit the existence of the whole mechanism necessary for factor constancy. To suppose that, in addition to this fixity, there is a variability at just about the time of maturation thus becomes a very special and unnecessary assumption; one not to be tolerated without the most cogent experimental proof.

In addition, then, to the experimental findings showing that there is another explanation for the cases used as arguments for factor fluctuation, we must bear in mind that the *a priori* basis for this hypothesis is very uncertain also. Now, when the definitely ascertained mutations are examined, still further evidence against this assumption appears, for *in factors in which large changes are found, small changes are not more frequent*; at any rate, there is evidence against the existence of a probability curve of variations, one with the minuter plus and minus changes more and more numerous. Perhaps the best example of what is meant here may be seen in the case of the mutations in the locus *W* of *Drosophila*, a case which has at times been cited in support of the variability contention. Seven distinct allelomorphs (all affecting eye color) are



already known at that locus, yet all six mutants are (in intensity of the color they produce) minus variants from the normal red, and furthermore, they show no tendency at all to group themselves in its vicinity. Instead of finding most of the allelomorphs very nearly like red in the character they produce, with the wider deviations most exceptional, there is only one factor ("blood") in the long interval between red and cherry, although cherry is, in intensity of color, about half way between red and white, the most extreme variant. The other four mutants are more extreme deviants than cherry. This is not because less extreme variants than cherry would be likely to escape observation, for hardly any of the mutations affecting eye color in other loci of *Drosophila* are even as extreme as blood, and very much smaller deviants than cherry or blood would be detected unfaillingly. It is therefore quite gratuitous to assume here that there are a very large number of small variations; certainly the number does not increase as we approach the parent type within the limits of observation, but, in fact, decreases conspicuously.

Of course, it would be scholastic to deny that some mutant genes may occasionally occur that are genetically unstable in some way or other. EMERSON'S (1911, 1914) variegated corn in fact proves this possibility, but it is noteworthy that even in this case there is no evidence of continuous fluctuation and a normal curve. Although such instability may be expected as an occasional abnormality, it is to the advantage of the organism that most genes shall be very stable, and present-day races are doubtless the products of a long process of selection in that respect as well as in regard to the constancy of the reactions whereby the factors produce the characters. In view of all the experimental data, and theoretical considerations, practically absolute factor fixedness seems the established rule, with the exception of occasional definite mutations that in any one locus must usually be exceedingly rare, and geneticists may well pause before ascribing any unusual results to a fluctuating variability of a single factor. The positive evidence against such a process still remains unchallenged by relevant facts or plausible hypotheses.

#### (10) *Contamination of factors*

It should be noted that, although  $B_d'$  has been kept continually heterozygous, in company with its normal allelomorph, for years, without then being subjected to artificial selection, nevertheless it shows no evidence of having been contaminated by that association, or weakened in any way.

## SUMMARY

1.  $B_d'$ , the factor for beaded wings in *Drosophila*, is, like the factor for yellow in mice, a lethal which kills all individuals homozygous for it. Like yellow it is recessive for the lethal effect and behaves as dominant in regard to a visible character, but in the present case the dominance is rather irregular.  $B_d'$  is located at about 2 units to the right of the locus of rough eyes; thus it lies at the extreme right-hand end of the known factors in the third chromosome.

2. In the so-called pure stock of beaded, although of course none of the individuals can be homozygous for  $B_d'$ , nevertheless all show the character beaded. This is because they contain, in the third chromosome homologous to the one carrying  $B_d'$ , another lethal factor,  $l_{III1}$ , which prevents individuals homozygous for itself from appearing. This factor, unlike  $B_d'$ , produces no visible effect when in heterozygous condition. Its locus is situated about 12 units to the left of that of  $B_d'$ . Since here it lies in the same chromosome with the normal allelomorph of  $B_d'$  no flies of this stock that are homozygous for normal wings can live, except when  $l_{III1}$  crosses over from the factor for normal wings.

3. There is, however, another mutant factor besides  $l_{III1}$  in the chromosome containing the normal allelomorph of  $B_d'$ . This is the factor  $C'$ , which produces no visible effect, but which, when heterozygous, practically prevents crossing over in the region of the chromosome near to it.  $C'$  is located somewhat to the left of  $l_{III1}$ , and effectually prevents the latter from becoming separated from the factor for normal wings, except in very rare cases. As neither the homozygous normals nor the homozygous beaded can live, the condition is created of a heterozygous stock which nevertheless breeds true to its own type.

It is proposed to designate a condition of this sort, in which heterozygosis is enforced by two opposed lethal factors, each of which in some way prevents the appearance of an opposite type of homozygote, as one of "balanced lethal factors."

4.  $I_{B_d'}$ , the factor in chromosome II which intensifies the beaded character, is not a lethal, and it is probably not an allelomorph of  $v_p$  (the factor for vestigial wings). It is partially dominant.

The composition of all flies in the "pure" beaded stock may now be

$$\text{represented } \frac{I_{B_d'} \quad c' L_{III1} B_d'}{I_{B_d'} \quad C' l_{III1} b_d'}$$

5. Investigation of the other dominant mutant factors of *Drosophila*

showed that it is a phenomenon of general occurrence for dominant mutants to be lethal. There are nine dominant mutants known, excluding beaded and truncate intensifiers. The viability of the homozygote was known in the case of three (all sex-linked); the remaining six have been tested. The total enumeration shows that 3 of the 9 factors are non-lethal, 1 is semi-lethal, and 5 are lethal. It is probable that lethals are more often found among the dominant mutants than among the recessives mainly because lethals have a better chance of being detected in the case of dominants, but perhaps also the dominants may on the whole exert more effect than recessives when they are homozygous, and therefore more harmful effect.

6. The lethal effect of  $B_d'$  not only explains why it was at first impossible to obtain pure stock of beaded, but also why stock that did breed true was finally secured; for if lethals are fairly frequent in occurrence (as there is good reason to believe) the selection of stock containing as high a proportion as possible of one lethal automatically tends to the establishment of a race containing another lethal in the opposite chromosome. In general the condition of balanced lethals should tend to arise whenever a "beneficial" lethal factor (one that is selected for) already exists.

7. Although  $l_{III_1}$  is the first lethal recessive factor found in autosomes of *Drosophila*, the evidence from sex-linked lethals, lethal dominants, and theoretical considerations, makes it probable that such factors form a large proportion of the factors arising by mutation. Hence they should be found to have arisen in any chromosome region that has been protected for a long time from the action of natural selection. The chromosomes involved in a case of balanced lethals are so protected by each other, and the Y chromosome is so protected by the X; chromosomes of stocks continually out-crossed or kept heterozygous are also removed from selection. In all these cases, then, lethal factors, and undesirable mutant factors of other sorts, would gradually accumulate in the affected chromosomes. Besides this, evolution of the species would be hindered in these cases by the fact that recessive mutant factors of a beneficial nature could not be selected for. It has been shown in the text, however, that if the race containing balanced lethals persisted very long, the degenerative processes themselves might cause it finally to revert to a condition of normal genetic behavior, in which each of the originally balanced chromosomes would be represented by an independent pair.

8. In out-crosses of the balanced lethal stock of beaded with other

racés *twin hybrids* and *constant hybrids* are produced, according to the Mendelian expectations for these cases. By the introduction of other factors into the affected chromosomes these results were made more striking. Balanced lethal stocks were obtained containing in heterozygous condition recessive factors that never came to light except on crossing, or as a result of a rare crossing over from one of the lethals. In the latter case *mutation* was simulated. The parallel results that have been obtained with *Oenothera* indicate clearly that this, too, constitutes a complicated case of balanced lethals. In double-flowered stocks there is a very similar situation.

9. Unusual and apparently non-Mendelian results of various other sorts can be, and have been, made to order with beaded flies. For example, it was possible to cause the total disappearance, on crossing, of a dominant character present in one of the parents, and its complete absence from all subsequent generations. Unfortunately for the geneticist who is still "open-minded" about the strict genotype interpretation of heredity, the Mendelian machinery at work here is quite transparent, owing to previous analysis. But of course one may always have recourse to taking the end results purely at their face value, as it is sometimes urged that, after all, we are concerned with facts, and that factors are merely concepts.

10. The apparently conclusive and unusually elaborate evidence of factor variability which the case at first presented has resolved itself, upon factorial analysis, into a quite different set of phenomena. This makes it imperative not to accept similar evidence in other cases until as complete genetic analyses have been made.

Theoretical objections to factor variability have also been presented.

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## ADDENDUM

Since the above has gone to press, DE VRIES (1918) has adopted an explanation identical with that of "balanced lethal factors" in order to account for the twin hybrids of his species crosses. For he finds that *Oe. grandiflora* is a permanent heterozygote for the semi-lethal factor "*ochracea*," and, as he points out, this circumstance must evidently be due to the existence of another, completely lethal, factor, which segregates from it. He finds, moreover, that in crosses of *Oe. grandiflora* with other species, the gametes containing the factor for *ochracea* give rise to one type of hybrid—*laeta*,—while the other gametes (those that must have the balancing lethal) give rise to the twin hybrid—*velutina*. In *Oe. Lamarckiana*, whose genetic behavior on crossing is similar to that of *grandiflora*, the "*ochracea*" type of gamete probably contains a completely lethal factor, as homozygous *ochracea* never appears.

This important confirmation of the conception of balanced lethal factors in *Oenothera* now opens the way to an explanation of the other peculiar results of crosses in that genus (constant hybrids, etc.) on the same basis. But it should be remembered that, whereas the lethals dealt with in *Lamarckiana* and *grandiflora* are zygotic (since both reciprocal crosses often give the two types of hybrids), the corresponding chromosomes in some of the other species evidently have gamete-lethals instead, or in addition. This is to be expected on the view of "chromosome degeneration" previously outlined. At the same time, the new findings of DE VRIES must also make more probable the view advanced in the present paper that most of the "mutations" are really the appearing, in homozygous condition, of previously existing heterozygous recessive factors which chanced to cross over from the lethals to which they had been attached. These recessive factors themselves must, however, have come into existence originally by a process of true mutation, although this may have happened in the remote past. It seems unfortunate to confuse their origin with the question of species hybridity. Since the time when the real mutations occurred, or before then, in the ancestry of any species of *Oenothera*, there may or may not have been species crossing, and so the mutant factors may or may not have previously been evident in homozygous condition, as the "normal" factors of some parental species, but there seems little object, from the present point of view, in affirming or denying such crossing, since there is no reason to believe that the occurrence of true mutations has anything to do with hybridity.

A paper of DE VRIES (1916) on seed viability in *Oenothera*, which has also just come to hand, is likewise of importance in this connection. It appears from this that RENNER too, some years ago, proposed a scheme of balanced lethals to explain the twin and constant hybrids, although he believed that the cause of this condition lay in original species hybridity. Although all the complicated results do not seem explicable on RENNER's one simple scheme, yet many of DE VRIES's own findings, here reported, do favor the general idea of balanced lethal factors. We may note here particularly the fact that not more than 50 percent of the offspring of *Oe. Lamarckiana* are viable, together with the fact that the productivity of some of its mutants is much greater. Among mutants of high productivity is to be noted especially the tetraploid form *gigas* (see discussion on page 484).