THE GENETIC BASIS OF TRUNCATE WING,—AN INCONSTANT AND MODIFIABLE CHARACTER IN DROSOPHILA

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THE INITIAL SELECTION EXPERIMENTS AND CROSSES

One of the first mutants which MORGAN found in Drosophila was a fly with truncated wings. It appeared in 1910, in a stock having beaded wings, and the inheritance of truncate, like that of beaded, seemed from the first to be irreconcilable with Mendelian principles, or indeed with any theory of fixed and segregating factors.

The truncate and the beaded cases thus stood as a constant challenge to those workers who hoped for a completely rational explanation of inheritance in Drosophila. Beaded has been dealt with in another paper; the truncate case will be considered here.[1]

The original truncate male, when crossed by its normal sisters, gave, even in F_1, a motley collection of offspring, the great majority being normal, but truncates of various grades also appearing. In F_2 the results were varied, and some of the F_1 matings of truncate by truncate gave nearly half the F_2 flies having some degree of truncation. It was to be expected that in the next generation pure stocks could be established; nevertheless crosses of the F_2 failed to produce any higher proportion of truncate than before, and for a few generations it seemed as though selection might be ineffectual. Broods yielding a higher percentage were found after several months, however, and by carrying on the selection work with these lines for about a year, MORGAN finally was able to obtain a stock in which sometimes as much as 90 percent of the flies showed the mutant character. The intensity of the character had increased markedly at the same time, but was accompanied by a greatly diminished fertility of the flies.

At this point one of the authors took up the work, and in an effort to obtain pure stock the process of selection was continued for nearly three years longer. It seemed, however, as though the climax had already been reached, for it was found impossible entirely to eliminate the normal individuals from the stock, or to obtain races that consistently yielded more than about 90 percent of truncates of all grades. It would not be justifiable to publish the protocols of these experiments in detail as this sort of breeding work is necessarily of an uncritical nature, not adapted to give definite factorial solutions to genetic problems. Table 1, however, is given as a sample of this series of experiments; it shows the effects simply of 13 successive generations of the selection, carried on during the latter part of the period. It will be seen that the average proportion of truncates (all grades) shows no significant change, being 84 percent for the first four generations, 90 percent for the intermediate five, and 88 percent for the last four.

These results suggest that this final stock might really be pure genetically, and homozygous for truncate, and that the differences between the individuals were due only to external conditions which accentuated or inhibited the development of the somatic character. In that case the normals and the truncates of various grades should all give identical results

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[1] A preliminary account of the truncate case was given by the authors in the book of MORGAN, STURTEVANT, MULLER, and BRIDGES (1915, pp. 191-194). Most of the crosses there reported were made in 1913.

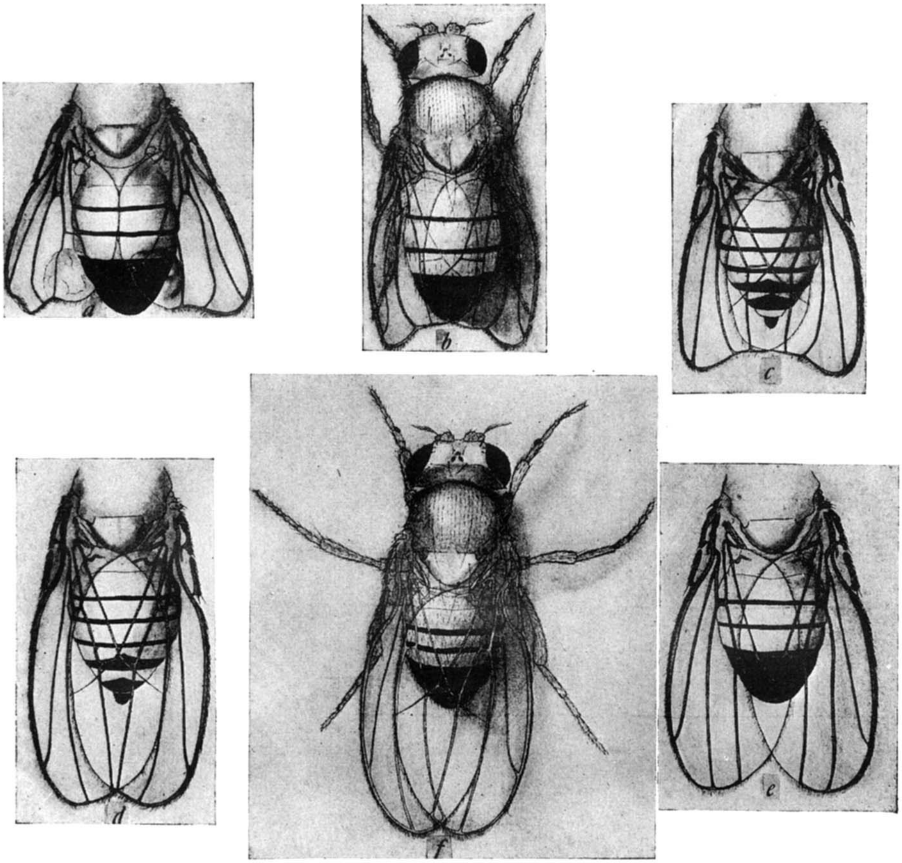

FIGURE 1.—*Types of truncation.*

a. Very short truncate male (the blistering is a frequent characteristic of this grade).
b. Typical short truncate male of the selected stock.
c. Long truncate female—the type of "truncate" usual in outcrosses.
d. Intermediate-winged female.
e. "Slight intermediate" male.
f. Normal-winged male from wild-type stock.

when bred. Table 2, however, demonstrates the wide genetic difference between the normals and the truncates thrown by this stock, for whereas the truncates on the average had given 91 percent of truncates in this experiment, the normals yielded only 5 percent. Even the selected stock therefore is undergoing a continual genetic variation, with which the variation in the character is correlated, and so selection back and forth within this stock still has immediate and marked effect, within the 90 percent limit.

TABLE I

"Main line" of truncates selected for 13 generations for homozygosis.

Number of generation	Truncates (all grades)	Normals	Average grade of truncation according to generation	Average grade according to groups of generations	Average percentage of truncates
1	*13	0	3.0	2.52	84
2	5 *19 16	0 4 8	2.3		
3	*11 15 12	0 1 3	2.7		
4	*36	8	2.5		
5	*18	2	2.7	2.70	90
6	23 8 *28	3 2 8	2.5		
7	*98 9 53	6 2 6	2.8		
8	*13	2	2.6		
9	*8	0	3.0		
10	30 *19	3 4	2.6	2.64	88
11	10 *29	1 7	2.5		
12	*79	10	2.7		
13	28	1	2.9		

* The asterisks denote those families from which the parents of the families recorded in the next generation were taken. It will be seen that, in some cases, as in generation 6, the family thus marked out is not the one that shows the highest percentage of truncates. Such a curcumstance is due to the infertility of the "best-truncate" families; attempts were made in each generation to breed from the "best" families, as well as from the others, but the "best" lines frequently died out after one or several genera-

tions, the race then being continued from the families of those poorer lines which had been carried on simultaneously as a safeguard. The above table shows none of the unsuccessful side lines which were in this manner always being carried on, both from better and from poorer stocks, but it gives a record only of those families that happened to lie in the line that was carried through continuously.

As a typical example of an unsuccessful side line, it may be stated that in generation 6, referred to above, three cultures were made of the truncate flies in the "best family" (that which consisted of 23 truncates, 3 normals). The "best" of these three cultures gave, in generation 7, 78 truncates and 1 normal; two families in turn were started from this culture; of these, one produced nothing at all, and the other yielded only three infertile flies in generation 8. The "main line" thus reverted to the descendants of the third bottle of generation 6, which has therefore been marked with the asterisk.

In determining the average grade of truncation of the above counts, the class of truncate is assigned an average grade of 3 and the class of normals a grade of 0, each grade is then multiplied by the number of flies in the class having that grade, and the sum of these products is divided by the total number of flies.

TABLE 2

Genetic behavior of normals thrown by truncates of the selected stock.

Number of generation	Truncates	Normals
1	38	3
2	21	1
3	17	1
4	62	9
5	0	27
6	1	19
7	5	67

Lines 1 to 4 show the counts given by the truncate stock for the four generations preceding that in which normals were chosen; only those families are here recorded which are in direct line with the one (4) from which the normals were derived. Lines 5 to 7 show the ratios thrown by the normals during three successive generations in which they were selected as parents. Individual matings (separate pairs of flies) were not used in this experiment.

The results of crossing truncate to wild-type flies were extraordinarily indefinite and inconclusive. A few examples of the numerous crosses of this sort which were carried out are given in table 3. Truncate behaves nearly as a recessive in F_1 (see table 3(A)), although a small percentage of flies appeared with the character more or less perfectly developed. In F_2 a good 3:1 proportion was never secured, but, as may be seen from table 3(B), ratios occurred varying from 168 normals: 1 truncate, to less than 2 normals: 1 truncate, the "truncates" being of various grades. In other crosses of the same type F_2 families have also occurred in which no truncates were produced at all. When an attempt was made to recover truncate stock by breeding together the "extracted" truncates

TABLE 3

Crosses of truncate by wild stock.

(A.) F_1 (from wild female by truncate male)

Bottle No.	Character of parents used	Truncate		Intermediate		Normal		Percentage of truncates (all grades)
		♀	♂	♀	♂	♀	♂	
1	Wild ♀, T♂ from selected stock	5	1	4	2	162	182	3.36
2	Wild ♀, T♂ from selected stock	5	0	1	3	129	127	3.4
3	Wild ♀, T♂ from selected stock	0	1	1	0	89	83	1.15
4	Wild ♀, T♂ from selected stock	4	1	1	3	214 (♀&♂)		4.03
5	Wild ♀, T ♂ from early unselected stock	0	0	0	0	672	676	0.0

(B.) F_2 (from $F_1 \times F_1$)

Bottle No.	Character of parents used	Truncate		Intermediate		Normal		Percentage of truncates (all grades)
		♀	♂	♀	♂	♀	♂	
6	T♀, normal ♂ from 3	22	9	3	4	103	68	18.2
7	" " " " " 4	15	11	7	1	32	21	39.2
8	I " " " " 3	28	15	20	14	173	120	20.8
9	N♀, N♂ both from 3	17	6	3	3	184	174	7.5
10	N♀, N♂ " " 5	0	2	0	0	208	128	.6
11	" " " " " " 5	12	20	3	5	168	125	12.0

(C.) F_3 (from $F_2 \times F_2$)

Bottle No.	Character of parents used	Truncate		Intermediate		Normal		Percentage of truncates (all grades)
		♀	♂	♀	♂	♀	♂	
12	T♀, T♂ both from 6	85	49	14	13	54	42	62.6
13	" " " " " " 8	101	43	13	10	77	111	47.04
14	N♀, N♂ " " 8	37	22	23	22	158	172	23.0

(D.) F_4 (from $F_3 \times F_3$)

Bottle No.	Character of parents used	Truncate		Intermediate		Normal		Percentage of truncates (all grades)
		♀	♂	♀	♂	♀	♂	
15	T♀, T♂ both from 12	132 (♀&♂)		11 (♀&♂)		44 (♀&♂)		76.5
16	" " " " " " "	79	58	43	42	1	8	63.0
17	" " " " " " "	32	17	1	0	112 (♀&♂)		30.9
18	N♀, N♂ " " "	35	31	4	5	50	73	37.8
19	" " " " " " "	0	0	0	0	464 (♀&♂)		0.0

of the F_2 families, it was found (table 3(C)) that truncate had been considerably "weakened," as only about 50 to 60 percent of truncates were now produced. Further selection, however, brought the extracted stock in later generations nearer to the value of the original selected stock. (An indication of this may be seen in table 3(D).) It was found that the percentage of truncates in F_2 and later generations of the cross tended to be higher if the P_1 truncate fly had been a female, than if the reciprocal P_1 cross had been made, and yet fair numbers of truncates of each sex were produced in both cases. All these results could be "explained" as due to varying "potencies," but this did not put the matter on a factorial basis.

Besides being crossed to wild-type, truncate was also crossed to various mutant stocks, and counts were made of the F_1, and of the F_2 derived from interbred F_1. Here the same indefinite results were obtained, in respect to the truncate wing character, as in the crosses with wild, but in addition there was also observable an apparently indefinite sort of linkage between truncate and the other character, which appeared, curiously enough, regardless of the linkage group to which the other character belonged. As examples of such experiments, the results of some of the crosses with jaunty (group II), and with pink (group III), are given in tables 4 and 5 respectively. It will be seen that in the F_2 of the cross with pink, although some degree of linkage was clearly indicated, yet a surprisingly large proportion of pink truncates were produced in certain bottles,—more than could be accounted for by weakness of linkage and by the amount of dominance shown by truncate in F_1.

ANALYSIS OF THE EFFECTS OF THE DIFFERENT CHROMOSOMES IN TRUNCATE STOCK ON THE TRUNCATE CHARACTER

It was thus necessary to discover, first, whether truncate depended on definite factors, lying in the chromosomes, and, secondly, if this proved to be true, whether the continual genetic variation occurring in the stock was due to fluctuation in these factors, or to heterozygosis that for some reason was forced to persist in the stock. As it was evident that at any rate the results did not represent simple monohybrid ratios, a method was required whereby the different factors, or sets of factors, that might be concerned in producing truncate could be dissociated from each other and studied separately. This object was achieved by making use, in a special way, of the linkage relations which had been worked out in Drosophila.

TABLE 4

Crosses of truncate by jaunty.

Generation of count	Bottle number	Character of parents		Straight-winged						Jaunty-winged (all normal*)		Percentage T and I among straight
				Truncate		Inter.		Normal*				
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
F_1	1	jaunty	T	1	0	16	2	120	141			6.8
F_1	2	T	jaunty	0	4	1	1	17	19			14.3
F_1	3	T	jaunty	0	4	9	6	66	38			15.4
F_2	4	N from 1	N from 1	1	2	1	1	58	63	8	9	4.0
F_2	5	N from 1	N from 1	5	9	2	0	91	79	15	17	8.6
F_2	6	I from 1	I from 1	21	10	8	3	19	43	14	7	40.4
F_2	7	N from 2	N from 2	4	0	2	0	29	30	11	4	9.2
F_2	8	N from 3	N from 3	13	4	9	1	72	113	7	7	12.7
Sum of F_2				44	25	22	5	269	328	55	44	13.8

* The word "normal," or "N," in this and the following tables, means normal in respect to truncation.

It may be recalled that in Drosophila all the hereditary factors are divisible into four groups, corresponding to, or rather contained in, the four pairs of chromosomes, and that in the male all the factors within any one particular chromosome are inseparable from one another during segregation (no crossing over), so that every offspring which inherits a single factor of that chromosome necessarily inherits also all the other factors which were in that particular chromosome. That is, all the factors lying within a given chromosome of a male have an identical distribution among his offspring. Thus it is only necessary to be able to observe the distribution, among the offspring, of a single easily recognizable pair of characters from the male parent, in order to learn the

TABLE 5

Crosses of truncate by pink.

Generation of count	Bottle No.	Character of parents		Red-eyed							Pink-eyed						
				Truncate		Intermediate		Normal		Percent T+I among reds	Truncate		Intermediate		Normal		Percent T+I among pinks
		♀	♂	♀	♂	♀	♂	♀	♂		♂	♀	♀	♂	♀	♂	
F_1	1	pink	T	4	2	9	2	184	227	4.0							
"	2	pink	T	16	10	9	2	66	86	19.6							
"	3	T	pink	1	3	3	6	57	66	9.6							
"	4	T	pink	2	6	10	9	88	101	12.6							
F_2	5	N from 1	N from 1	7	5	2	6	77	86	10.9	7	1	3	0	28	29	16.2
"	6	T from 1	N from 1	12	3	7	4	54	48	20.3	0	0	0	0	18	12	0
"	7	I from 1	N from 1	6	8	1	4	56	47	15.6	0	0	0	0	11	13	0
"	8	N from 2	N from 2	6	2	0	2	101	105	4.6	0	0	0	0	14	16	0
"	9	N from 3	N from 3	23	10	3	3	128	108	14.2	0	0	1	2	33	40	4.0
"	10	N from 4	N from 4	7	6	7	6	75	87	13.8	1	0	2	0	22	25	6.0
"	11	I from 4	I from 4	23	13	10	11	38	41	41.9	0	0	0	0	13	15	0
"	12	I from 4	I from 4	28	23	11	12	39	57	43.5	5	3	5	5	20	32	25.7
"	13	I from 4	I from 4	40	27	12	11	61	82	38.5	1	0	4	2	11	13	22.6
"	14	I from 4	I from 4	14	12	7	4	18	23	47.5	1	0	0	1	5	6	15.4
	Sum	of F_2		166	109	60	63	627	684	23.2	15	4	15	10	175	201	10.4

distribution of all the factors lying in the corresponding pair of chromosomes. This will tell nothing, however, about the distribution of the factors in the other three pairs of chromosomes, since the different pairs of chromosomes are distributed independently of one another to the offspring, and so there is opportunity for factors in different pairs of chromosomes, which may in previous generations have accompanied each other, to become dissociated from one another—i.e., transmitted to different offspring. Hence in order to know what was the distribution of all four pairs of chromosomes of a male parent among his offspring, it is necessary to have under observation the inheritance of four pairs of characters—one for each of the chromosome-pairs; each of these characters, then, will serve for the identification of an entire chromosome or group of factors in the offspring, and may be called an "identifying character." So, by this method, it is possible to determine the distribution, among the offspring, of the entire constellation of factors which a male parent contained. The offspring will contain different combinations of his factors (assuming he was heterozygous), but in each case it will be known just what combination is present. The somatic effects of these different known combinations of factors on the offspring may then be studied, and so it may be determined just what rôle each of the groups of factors plays in the production of any particular character that is under investigation. In this manner it was possible to ascertain what effect on the development of truncate wing each chromosome of the truncate fly produced.

It should be noted, however, that the simplicity of such an experiment is impaired when the character under investigation (e.g. truncate) is recessive, for then the factors for it must be introduced from the female parent as well as from the male, in order that the character may be observable in the offspring. The disadvantage of thus introducing the factors from the female parent is that in the female the factors for the character under investigation are liable to cross over from the identifying factors of the groups to which they belong, and it may therefore be impossible to tell, by means of one identifying factor in each chromosome, exactly what combination of factors for the character the offspring studied have received.[2] It would accordingly be desirable to have the character dominant, in order that in the investigation the factors for it

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[2] If, however, an identifying factor at each end of the chromosomes is used, it may be assumed with a fair degree of probability that the offspring receiving both identifying factors have also received those factors for the character studied which lay between them.

may be introduced from the male parent alone. In the case of truncate, this mode of procedure was rendered possible by a special circumstance.

Ordinarily, truncate behaves most nearly like a recessive character, when truncates are crossed to wild-type flies, although a very small percentage of F_1 showing some degree of truncation are almost always produced. Examples of such crosses have been shown in table 3. Here the flies which have wings at all truncated have been classified, for greater accuracy, into two divisions—typical "truncates," and "intermediates," and it will be seen that the number in the two classes is approximately equal. The total proportion of truncates—including both grades—is 3.1 percent (in the crosses of truncate of the selected stock). When truncate was crossed to various other types of flies somewhat similar results were usually obtained, but when the cross was tried of truncate from the selected stock by flies with black body color, the outcome proved to be conspicuously different, as shown in table 6. Here it will be seen that there are distinctly more typical truncates than intermediates, and the proportion of truncates of all grades appearing in this cross is also very much higher, being in fact 24.9 percent (in the cross of truncate males by black females), as compared with the previous 3.1 percent (when truncate males were crossed to wild-type females). The truncation of the two series of F_1 flies may be compared also by obtaining a figure which may be called the "average truncation" of each group of individuals. The typical truncates may be assigned a

TABLE 6

Crosses of truncates by black.

(A) F_1 from black ♀♀ × truncate ♂♂

Bottle No.	Truncate		Intermediate		Normal		Percentage of T and I
	♀	♂	♀	♂	♀	♂	
1	35	0	11	13	90	116	22.3
2	57	0	25	18	113	162	26.6
3	55	1	22	12	121	149	25.0
Sum	147	1	58	43	324	427	24.9

Average truncation among ♀♀ 1.33, among ♂♂ .19, among all .79.

(B) F_1 from truncate ♀♀ by black ♂♂

Truncate		Intermediate		Normal		Percentage of T and I
14	11	7	0	25	12	46.4

Average truncation among ♀♀, 1.52; among ♂♂, 1.91; among all, 1.65.

grade or value of truncation of 4, in this calculation, the intermediates a value of 2, and the normals 0. Multiplying each of these values by the number of individuals having the value, adding, and then dividing by the total number of individuals, it is found that the average grade of truncation of the F_1 from black females by truncate males is .79, whereas the average grade of the F_1 in the cross of selected truncate with normal is .10. This relatively high grade of truncation for the F_1 flies has been obtained consistently, in numerous and varied crosses of truncate with flies of black body color. Moreover, although there is still a considerable proportion of normals present among the F_1 even in these crosses, it will be shown later that "high grade" truncate is really almost always dominant in individuals heterozygous for black. These results with black were in themselves noteworthy, for here there seemed to be a type of interaction of factors hitherto not described, wherein two non-allelomorphic factors, both ordinarily recessive, re-enforced each other so as to produce a visible effect in the individuals heterozygous for both, just as happens in crosses of multiple allelomorphs. It should be added that the black character, unlike the truncate character, was not affected by this interaction, and remained recessive. The facts were more important, however, in their bearing on the truncate case, for they disclosed a means whereby truncate might be used as a dominant character, in the desired analysis.

In order to simplify the account of the analysis, crosses are here reported in which only the three large groups of factors were followed,—the fourth chromosome, which was too small to be likely to contain factors for truncate, was followed, however, in other experiments, and proved not to be concerned in the case. The sexes were used as the pair of "identifying characters" for the first chromosome; black body color, *b*, versus *B*, the normal gray, for the second chromosome; and pink eye-color, *p*, versus *P*, the normal red, for the third chromosome. The procedure was as follows: A truncate female with normal body and eye colors was mated to a normal-winged male with black body and pink eyes. The F_1 males, the distribution of whose factors among the F_2 flies was to be followed, had the following chromosome and factor composition, indicating the chromosomes by numerals and representing those derived from the truncate parent with asterisks, and the homologous chromosomes, derived from the black pink parent, without asterisks:

$$\frac{\mathrm{I}^{*}X}{\mathrm{I}\ Y}\ \frac{\mathrm{II}^{*}B}{\mathrm{II}\ b}\ \frac{\mathrm{III}^{*}P}{\mathrm{III}\ p}$$

Inasmuch as these flies are heterozygous for black, it was to be expected that truncate would behave as a dominant, at least partially, and the count designated as "cross 1" in table 7 confirms this expectation, for 33.3 percent of the flies had somewhat truncated wings. It is not in this generation, however, but in the next, that the desired analytical results appear.

One of these F_1 flies (a male) was back-crossed to homozygous black pink females of the composition

$$\frac{\mathrm{I}X}{\mathrm{I}X}\ \frac{\mathrm{II}b}{\mathrm{II}b}\ \frac{\mathrm{III}p}{\mathrm{III}p}$$

in order to obtain offspring (F_2) having various known combinations of the chromosomes derived from truncate stock. As all these offspring (F_2) alike received the non-dominant combination I*X* II*b* III*p* from their homozygous mother, the circumstance of receiving I*X or I*Y*, II*B or II*b*, and III*P or III*p* from their heterozygous father determined whether their characters were female or male, gray or black, and red or pink, respectively. The visible characters accordingly revealed just what chromosomes of the truncate stock these flies had or had not received. Thus, the female gray red flies must have received from their father all of his truncate-derived chromosomes,—I*X, II*B, and III*P,—while the male black pink flies could not have received any of these chromosomes, but must have gotten the non-truncate combination I*Y* II*b* III*p* instead. Corresponding with these facts, we find that the former flies (gray red females) are very largely truncate in wing shape, whereas all the latter (black pink males) have normal wings. These are the two extreme combinations; all the others received from the heterozygous father certain chromosomes derived from the truncate stock and others derived from the normal-winged (black pink) stock; a study of the amount of truncation shown by each of the different combinations may then reveal the rôle played by each chromosome in the production of the truncate character.

The results of the cross are designated as "cross 3" in table 7. There are eight possible combinations of the three pairs of identifying characters, and it will be seen that all of these combinations, or classes, appeared with about equal frequency, just as expected. In the case of each class the flies were further subdivided into four different grades, according to their wing shape. These grades (normal, "slight" intermediate, intermediate, and "truncate") may be given average values of 0, 1, 2.5, and 4, respectively, to bring them into proper relationship with the three grades previously used. The fact most obvious on examining the records of the different classes, with regard to their amount of truncation, is that all of the black flies have normal wings; i.e., none of the flies in any of the last four combinations, which failed to receive II*, shows any indication of the truncate character; on the other hand, some at least of the flies in each of the first four, gray classes—the classes that did receive II*—had wings with some degree of truncation. This proves that there is a factor (or factors) for truncate in II*, i.e., in the second chromosome. This factor (factors?) in heterozygous condition is sufficient "by itself" to cause truncation, even if none of the other chromosomes in the fly are derived from truncate stock, but if this factor is not present, truncate cannot appear (at least, not as a dominant), even if one of the first and also one of the third chromosomes in the fly are derived from truncate stock. The factor for truncate in chromosome II is therefore the "chief" factor for truncate—being both necessary, and self-sufficient,[3] for the appearance of truncate (as a dominant). It may be designated as T_2'; its normal recessive allelomorph would then be t_2', and in our notation t_2' may ordinarily be understood to exist in any formula of the second chromosome in which T_2' is not represented.

T_2' is not the only factor for truncate whose existence is disclosed by this table. Comparison of the four classes which received T_2' (the gray classes) shows that truncate appeared in them with very different frequencies and intensities. It is evident that the red-eyed gray classes—those which contain III**P* (the first two classes of the table)—contain more and better truncates than the corresponding pink-eyed gray classes—those having III*p* (the third and fourth classes of the table). The chromosome III* that came originally from truncate stock therefore contains a factor (or factors) for truncate also. This may be called T_3'; like T_2' it is dominant, inasmuch as it may produce an effect when in heterozygous condition. Unlike T_2' however, it may be called a "modifier" or "intensifier" rather than a chief factor, for it can produce an effect on the wing only in the gray classes, which already contain II**B* (T_2').

It was then shown that even when an opportunity is given for it to exist in homozygous condition, T_3', without T_2', is unable to cause truncation. This was done by crossing the males and females from the black-bodied, red-eyed classes, to each other; the result is given in table 7, cross 5. The parent flies must all lack T_2' but are heterozygous for T_3'.

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[3] Within the limits of the experiment.

Among the offspring, therefore, one-fourth should be homozygous for T_3', yet none of the flies had truncate wings. Three had a very slight unevenness of margin that approached the condition seen in slight intermediates, but this did not seem to be transmitted to their offspring. T_3', then, has practically no effect on the wing except in the presence of T_2'. Moreover, T_3' is not really necessary for the production of truncation, since some of the pink flies of the main experiment—flies that carried T_2' but could not have received T_3'—are truncate, although of course truncation in them is not so frequent nor so intense as when T_3' is present. T_3' thus fulfills the definition of an intensifier.

In a manner similar to that in which T_3' was deduced, we may also infer the existence of at least one intensifier, T_1', in the first chromosome (I*X) derived from the truncate stock. For the tables show that the female classes—i.e., the flies with I*X—contain a much larger propor-

TABLE 7

Crosses to determine the effects of the different chromosomes of selected truncate stock.

(A) Initial crosses of truncate by black pink

Generation of count	Number of cross	Nature of parents ♀	Nature of parents ♂	Gray red T	Gray red I	Gray red S	Gray red N	Gray pink T	Gray pink I	Gray pink S	Gray pink N	Black red T	Black red I	Black red S	Black red N	Black pink T	Black pink I	Black pink S	Black pink N
F₁	1	1 T from stock	Several bp from stock	3	8		18												
				4	7		26												
"	2	Several bp from stock	3 T from stock	13	10		39												
				1	12		56												

(B) Back-crosses to separate the effects of the chromosomes

Generation of count	Number of cross	Nature of parents ♀	Nature of parents ♂	Gray red T	Gray red I	Gray red S	Gray red N	Gray pink T	Gray pink I	Gray pink S	Gray pink N	Black red T	Black red I	Black red S	Black red N	Black pink T	Black pink I	Black pink S	Black pink N
F₂	3	Several bp from stock	1 T from 1	57	23	0	7	2	8	6	64	0	0	0	74	0	0	0	82
				2	17	12	47	1	4	4	62	0	0	0	67	0	0	0	73
"	4	1 T from 1	Several bp from stock	2	1	1	6	1	3	1	4	0	2	0	10	0	0	0	9
				0	1	1	2	0	5	1	4	0	1	0	4	0	0	2	2

TABLE 7 (continued)

(C) Crosses of derivatives of the separation cross

Generation of count	Number of cross	Nature of parents ♀	♂	Gray red T	I	S	N	Gray pink T	I	S	N	Black red T	I	S	N	Black pink T	I	S	N
F_3	5	Several black red N from 3	Several black red N from 3									0	0	0	121	0	0	0	36
												0	0	0	127	0	0	0	38
"	6	4 gray pink (2S&2N) from 3	1 black red N from 3	6	5	11	43	0	0	0	42	1	1	1	55	0	0	0	45
				4	2	3	60	0	0	1	59	1	1	1	73	0	0	1	44
"	7	4 black red N from 3	1 gray pink S from 3	5	11	14	22	0	3	1	39	0	0	0	49	0	0	0	47
				2	8	11	31	1	2	8	47	0	0	0	46	0	0	0	49
"	8	4 black pink N from 3	1 gray red S from 3	8	14	11	9	0	1	0	37	0	0	0	54	0	0	0	29
				5	6	8	14	1	3	1	30	0	0	0	17	0	0	0	24
"	9	2 gray red I from 3	Several bp from stock	3	5	11	48	0	4	9	45	4	1	2	52	0	3	5	55
				5	12	9	43	0	3	14	55	3	4	2	76	0	1	4	44

Females are recorded on the upper line, males on the lower, in the case of each cross. T = truncate, I = intermediate, S = "slight-intermediate," N = normal-winged; in section (A), both I and S have been grouped under I.

tion of truncates, and are more intensely truncated, than the corresponding male classes, provided of course that II*B (T_2') is present.

It might be objected here, however, that in the case of the sex chromosome the difference between the truncation of the males and females may not be due to an intensifier present in I*X and absent in IX, but that it may be due rather to sex difference, depending on the XX condition of the female and the XY condition of the male. This possibility was tested out by making reciprocal crosses of truncate to non-truncate flies. As will be seen from tables 4, 5, 6, and 7, the male offspring are distinctly more truncated (in comparison with their heterozygous sisters), when they have truncate mothers than when they have truncate fathers. In table 6, for instance, there was an average grade of truncation of .19 among the male offspring of truncate males, whereas the male offspring of the truncate female had a grade of 1.91—ten times as high. The daughters were not very different in the two crosses, being 1.33 in the first, and 1.52 in the second. The sons must therefore be able to receive a factor, or factors, for truncate from their mother, which they cannot receive from their father,—that is, there are one or more dominant factors for truncate that obey the scheme of sex-linked inheritance. There must consequently be one or more intensifiers, T_1', in chromosome I*; the symbol of the normal allelomorph, t_1', will ordinarily not be represented, but its presence may be taken for granted in all X chromosomes in which T_1' is not shown. T_1', like T_3', cannot cause truncation, even in homozygous condition, unless T_2' is present.

In the crosses in which the female parent was truncate the male offspring were nearly always somewhat more truncated than the female, because the males are haploid for sex-linked factors and therefore appear like homozygotes, whereas the females contain factors from both parents and show some effect of the normal allelomorph received from the father. T_1', in other words, is not a completely dominant factor.

Among flies which are homozygous in regard to the sex-linked factors, on the other hand, no matter whether they are pure for the intensifier, or for its normal allelomorph, the males are on the average less truncated than the females. This effect is especially evident where the normal allelomorph of T_1' is present and is clearly shown in tables 9 and 10 (to be discussed later in another connection), wherein the males and females are both lacking in T_1' throughout, and are like each other so far as all non-sex-linked factors are concerned. In these crosses the female parents were non-truncated and the male parents were heterozygous for T_2' and T_3', but lacked T_1' (having resulted from a cross of truncate male by non-truncate female). In table 9 (A) and (B), for example, it is easily observable that there is a great preponderance of females among the truncate and "good-intermediate" offspring; the intermediate class has about equal numbers of females and males, and the slight-intermediate and normal classes contain almost exclusively males. The grade of truncation among the 914 females in these two tables averages 3.2, whereas that among the 864 males averages 1.6. In table 10, the average grade of truncation in the 535 females is 3.1, that in the 517 males is 1.3.

We may say, then, that the character truncate is partially sex-limited, or rather, *sex-influenced,* its development being favored more in the female sex than in the male. Or, to put the matter in factorial terms, the sex-factor itself, or some factor normally lying in the sex chromosome, intensifies the development of truncate, to a degree depending upon whether this factor is present in one "dose" or two, just as is the case with sex itself. This fact has nothing to do with the existence of a mutant intensifier, T_1', in the same chromosome.

After truncate had thus been analyzed into its chromosomal components, it was demonstrated that it could also be resynthesized by breeding together individuals of non-truncated or imperfectly truncated classes (gray pink females, black red males, etc.),—classes which, according to the analysis, should contain "complementary" components. Thus, the gray pink females should contain T_1' and T_2', and the black red males just T_3'. By breeding them together, then, as good truncates should be obtained as were found among the gray reds, in fact, the count should be rather similar to that obtained from the heterozygous gray red females by black pink males, except that here there is a chance for crossing over between black and truncate. The count, designated as cross 6, table 7, is quite according to this expectation. It will be noted here that the male parent had been normal-winged, and the females were only slight intermediates or normal (not possessing T_3'); the knowledge of what results would be produced with respect to truncate was thus entirely derived from the identifying factors. Another, somewhat similar cross, was of black red (long) females by a gray pink (slight-intermediate) male. Here the male has T_1' and T_2', and the female T_3'. The count is shown on line 7 of table 7, and again shows the high-grade gray red truncates. Black pink females derived from the main experiment, and therefore containing T_1', were likewise crossed to one of their complementary brothers, a gray red male ($T_2'T_3'$). The count, on line 8 of the same table, shows typical truncates, as before.

These results are all in striking contrast to those of other crosses, in which the parental classes (derived from the same cross as those above) did not supply complementary elements; compare, for example, the case where black reds were crossed to each other (table 7, cross 5), with the crosses above. Crosses involving still other combinations of these classes will be presented later. In each case, the results conformed to the expectation based on the analytical findings.

Let us now return to the three crosses of "complementaries," for we shall find that closer scrutiny of them will give us some further information regarding the nature of the accessory factors for truncate. Crosses number six and eight, besides illustrating the reconstruction of truncate, provide an indication of whether the component designated as T_2' consists of a single factor, or a group of factors for truncate lying in the second chromosome. For, if T_2' is of multiple composition, a distinctly lower grade of truncate would be produced in cross six than in cross eight, since in the former cross T_2' is supplied to the offspring by their heterozygous mother, in which the process of crossing over would allow these supposed constituent factors of T_2' to become separated and scattered among different offspring, wheras in the latter cross T_3' comes from the father, and so its various factors would have to stay together (no crossing over), and re-enforce each other in the offspring, thus producing truncates of a higher grade than the average of the first cross. On the other hand, if T_2' is just a single factor, the truncates in the two crosses will of course be of the same grade. A test for T_2' alone is involved here, as the two crosses were identical (not reciprocal) so far as the chromosomes other than II were concerned, the cross six being $\frac{T_1'X}{X}\ \frac{T_2'B}{b}\ \frac{p}{p}$ female by $\frac{X}{Y}\ \frac{b}{b}\ \frac{T_3'P}{p}$ male, and the cross eight being $\frac{T_1'X}{X}\ \frac{b}{b}\ \frac{p}{p}$ female by $\frac{X}{Y}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$ male. When the averages were calculated, it was found that the average truncation of the red truncated offspring (including all grades showing truncation) in cross six was 2.34 and that the average in cross eight was almost exactly the same, 2.33. Blacks and grays are both included in the average, since, on account of crossing over between black and T_2' in the third cross, the truncates are distributed among both black and gray classes. Taking all the truncated offspring,—black and gray, red and pink,—it is 2.26 in cross six and 2.34 in cross eight, a difference that is scarcely significant. These results then, indicate that T_2' is a single factor.

In a similar manner, comparison of crosses seven and eight give data regarding the composition of T_3', for in cross seven there is a chance of crossing over between the possible constituents of T_3', since T_3' is supplied by the female, whereas in cross eight T_3' comes from the male. while the crosses are identical so far as the other chromosomes are concerned. The grade of truncation of the red flies in cross seven was 1.97, and of all flies in that cross, 1.91. These rather low figures incomparison with values 2.33 and 2.34, that we have seen were obtained in cross eight, thus indicate that T_3' consists of more than one intensifying factor in the third chromosome. Other evidence for this was obtained in later crosses, when it was found possible to obtain, by crossing over, stocks of pink truncate which there was reason to believe contained an intensifier in the third chromosome, that was not as marked in its effect as the original T_3'.

From the various experiments reported above, it is evident that truncate is a character that is particularly susceptible to modification by various factors, such as T_1', T_2', T_3', and "sex" (in the sense previously explained). Even the symbols T_1', T_2', T_3', moreover, probably do not represent separately all the mutant factors for truncate that exist in truncate stock, since, as has just been shown, it is likely that T_3' at least is composite. And, in addition to such specific factors for truncate, and sex, it should be remembered that there are mutant factors concerned with the development of other characters, which influence truncate at the same time. Thus, we have seen that the factor for black body-color must be regarded as an intensifier of truncate, as truly as T_1' or T_3'. There is evidence that a number of other previously known factors behave somewhat similarly. For instance, when a truncate male was placed in a bottle containing at the same time both a virgin bar-eyed female and two virgin round-eyed females (all non-truncate), the bar-eyed offspring that hatched in this bottle (bar is a dominant) were distinctly more truncated than the round-eyed offspring. As the environments of the two sets of offspring must have been exactly the same, the factor for bar, or some factor in the bar stock, must be a dominant intensifier of truncate. This result is shown in table 8. The average truncation of the bar offspring was 1.44, that of the rounds was 0.[4]

TABLE 8

Cross of a single truncate male (selected stock) by various females in a single bottle. One female parent bar (dominant, sex-linked), one vermilion miniature (recessive, sex-linked), one wild type; all three normal-winged. Count of F_1.

Females								Males											
Normal-eyed				Bar-eyed				Normal-eyed				Bar-eyed				Vermilion miniature			
T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
0	0	0	73	24	3	2	28	0	0	0	65	15	4	2	45	0	0	0	20

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[4] It may be added that in this experiment one of the round-eyed females had been a vermilion-eyed miniature-winged fly (v m), and this female left offspring as well as the other female, as shown by the presence of some v m sons (v and m are sex-linked), yet these two mutant factors, unlike bar, failed to intensify the truncate character.

A somewhat similar experiment was performed with the dominant factor for star eyes (star is in chromosome II). Heterozygous star females were crossed to truncate males (of a rather low-grade pink truncate stock containing that part of T_3' which had crossed over with pink). The count of non-star offspring was 100 normal males, 80 normal females, no truncates of any grade; the count of stars was: males, 69 normal-winged, 27 slight intermediate, 12 intermediate, 1 truncate; females, 103 normals, 36 slight intermediate, 25 intermediate, 16 truncate.

TESTS OF THE CONSTANCY OF THE FACTORS FOR TRUNCATE

a. *Tests of sibs*

Although it had thus been established that truncate is certainly a complex case of multiple factors, nevertheless this alone did not seem to explain satisfactorily the final failure of the persistent effort that had been made to secure homozygous stock. For, in an animal in which all the factors are linked into only four groups, it should not have taken so very many generations to secure complete homozygosis, no matter how many factors had been in heterozygous condition at the start. It therefore seemed important next to obtain a method of investigation in which the effects on truncate due to the segregation of the multiple heterozygous factors that had been found should be recognizable, and could be eliminated from consideration, so that it could be determined whether or not there was any residuum of genetic variability in this character, due to actual fluctuations in the genes themselves. It seemed possible to carry out this mode of attack by a continuation of the previous main experiment, reported on pages 11 to 15 (table 7(B)), wherein the various factors had been followed out according to their chromosomes. The "residuum of variation," if there were any, should appear among the different males or females contained within any one of the separate eye-color body-color classes shown in table 7, cross 3.

The gray red males, for example, should, according to the accepted scheme of inheritance in Drosophila, all have contained identical factors for truncate, for all alike had received, without crossing over, corresponding truncate-bearing second and third chromosomes from the same heterozygous male parent. Their composition was $\frac{Y}{X}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$ as already noted. Yet, as we have seen, some of these males appeared truncate, others intermediate, and others normal. If, then, these differences between them were really genetic, the factors for truncate, having been transmitted similarly to all of them, must have been subject to intrinsic variation. In order to determine whether the differences were genetic, the gray red males of various wing shapes needed merely to be bred to black pink females from stock; counts of the number of truncates of different grades among the offspring of the separate males would then reveal whether their character differences had a genetic basis.

The advantages of using the gray red males rather than the gray red females for the testing are threefold:—(1) a larger number of offspring can be obtained from individual males than from individual females; (2) when males are tested, since there is no crossing over between the factors for truncate and the "identifying factors," gray and red, the offspring which are gray and red like their parents furnish an ideal test of the truncate which was present in the original gray red parents, and only this class of offspring need be examined for this purpose; if female flies are tested, on the other hand, their factors for truncate become scattered more or less indiscriminately amongst all the different classes of offspring, because of crossing over. (Compare the distribution of truncate among the offspring recorded in table 7, cross 9, representing the tests of heterozygous gray red females, with the distribution among the offspring of heterozygous gray red males, shown in table 7, cross 8); (3) when males are tested, these gray red offspring, being descended substantially without recombination from their gray red fathers, may be used again for similar tests in the next generation.

Tests were therefore made of gray red males derived from crosses similar to that shown in table 7, cross 3. The results of these tests are shown in table 9, (A) and (B), the count from each male being shown on a separate line. Only those offspring of the tested males which, like the latter, are gray red, are recorded, for the reason explained in (2) of the paragraph above; the gray pinks, etc., appeared as expected, however. Table 9 (A) represents tests of males which we may call group A; these all derived their truncate-bearing chromosomes from the same heterozygous $\frac{Y}{X}\,\frac{T'_2B}{b}\,\frac{T'_3P}{p}$ father; they should therefore be genetically identical in truncation, if factors are constant. Table 9 (B)—group B—represents tests of males derived from a different father from that of group A; they may therefore be different from group A genetically. They should, however, be like one another, as they too are brothers to each other, and were derived from a single $\frac{Y}{X}\,\frac{T_2'B}{b}\,\frac{T_3'P}{p}$ father.

It will be seen, on comparing the results of the tests of the different brothers, either in group A or group B, that although there was a certain amount of "chance" variation in the proportion of truncates produced by the different males, nevertheless this variation was entirely uncorrelated with the grades of truncation in the wings of the male parents themselves. Thus, in group A, the four truncate-appearing males produced families with the following average amounts of truncation: 2.0, 2.2, 2.4, 2.6; the final average for the offspring of the truncate males therefore is *2.3+*. In the same group (A), the 3 "good intermediates" produced averages of 1.8, 2.5, and 2.8, respectively, which gives a final average of *2.5+*; the intermediates produced 2.15, 2.25, 2.4, 2.4, final average *2.3*; the 2 very slight intermediates and one normal gave 2.15, 2.47, 2.5, final average *2.4—*. In group B a similar situation exists, the families of the 2 truncates and 1 good intermediate together averaging 2.5—, those of the 3 intermediates also averaging 2.5—, and those of the 2 slight intermediates and 2 normals together averaging *2.7*. It is here evident that the variation between the families is entirely an individual matter, not connected with the class of wing shape to which the parent belongs. For there is much greater variation between the families within each parental class than between the average values for the different classes. In no case do the latter values (within either group A or B) differ by as much as 0.3 grade, in spite of the fact that the parents themselves, of these various classes, present average differences ranging from 1.0 grade (in the minimum cases) to 4.0 grades (in the maximum). Moreover, those slight differences which do exist between the offspring averages have absolutely no tendency to be of the same sign as the parental differences. It must therefore be concluded that, so far as these tests can show, the gray red brothers which were tested had been alike genetically; the relatively slight deviations in truncation, between their families of offspring, were not of genetic origin, since they were in no way connected with the variations in truncation between the brothers themselves.

Besides the above tests of males, similar but less extensive tests of sister gray red females were also carried on, which had a composition similar to that of the males, but contained in addition heterozygous T_1'. These tests yielded results identical in kind with those of the males, except that the counts were smaller, and less critical in nature, as explained on p. 21. In these tests too there was a conspicuous lack of correlation

Table 9

Tests of brother gray red males $\left(\frac{Y}{X}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}\right)$ *by black pink females.*

All tested males within a single group (A or B) are derived from a single father of same type as themselves by black pink mother. Only their gray red offspring are recorded; the numbers not in parenthesis give the total males and females, those in parenthesis give the females.

(A) (Group A)

Grade of truncation of tested male parent	Average grade of truncation of offspring	Truncate	Good intermediate	Intermediate	Slight intermediate	Normal
4 (truncate)...	2.0	20(20)	9(9)	32(13)	41(0)	2(0)
4 (truncate)...	2.2	22(21)	11(9)	22(9)	32(1)	1(0)
4 (truncate)...	2.4	12(12)	8(8)	6(4)	15(0)	0(0)
4 (truncate)...	2.6	51(49)	24(16)	56(12)	33(0)	0(0)
Total of truncates (4)....	2.34	105(102)	52(42)	116(38)	121(1)	3(0)
3 (good intermediate	1.8	5(5)	6(6)	17(11)	20(1)	2(0)
3 (good intermediate	2.5	17(15)	10(8)	18(4)	16(0)	0(0)
3 (good intermediate	2.8	52(45)	26(17)	59(9)	11(0)	0(0)
Total of good intermediate(3)	2.53	74(65)	42(31)	94(23)	47(1)	2(0)
2(intermediate)	2.15	14(14)	6(5)	24(14)	22(0)	1(0)
2(intermediate)	2.25	20(15)	5(4)	16(12)	28(0)	0(0)
2(intermediate)	2.4	20(20)	11(10)	34(17)	17(1)	0(0)
2(intermediate)	2.4	25(24)	11(8)	17(9)	28(0)	2(0)
Total intermediates (2)....	2.30	79(73)	33(27)	91(52)	95(1)	3(0)
0.5 (very slight intermediate)	2.15	14(14)	7(7)	25(11)	19(1)	3(0)
0.5 (*very* slight intermediate)	2.5	29(22)	18(15)	50(25)	20(0)	0(0)
0.0 (normal)...	2.5	15(15)	9(7)	15(3)	9(0)	3(0)
Total of slight intermediate and normal (0.3).......	2.38	58(51)	34(29)	90(39)	48(1)	6(0)

TABLE 9 (continued)

(B) (Group B)

Grade of truncation of tested male parent	Average grade of truncation offspring	Truncate	Good intermediate	Intermediate	Slight intermediate	Normal
4 (truncate)...	2.5	26(22)	12(10)	31(15)	21(0)	0(0)
4 (truncate)...	2.5	6(6)	2(2)	11(9)	3(0)	0(0)
3 (good intermediate).....	2.4	29(23)	12(5)	32(17)	23(0)	3(0)
Total truncate and good intermediate (3.7)	2.45	61(51)	26(17)	74(41)	47(0)	3(0)
2(intermediate)	2.3	4(4)	1(1)	7(4)	4(0)	0(0)
2(intermediate)	2.4	24(24)	14(12)	28(15)	19(0)	3(0)
2(intermediate)	2.5	23(23)	13(11)	36(6)	14(1)	0(0)
Total (intermediate (2.)...	2.46	51(51)	28(24)	71(25)	37(1)	3(0)
1 (slight intermediate)	2.1	4(4)	2(2)	8(8)	8(3)	0(0)
1 (slight intermediate)	2.55	12(12)	8(7)	10(5)	10(0)	0(0)
0 (normal)....	2.9	18(18)	8(7)	13(3)	4(0)	0(0)
0 (normal)....	3.0	21(14)	3(2)	8(2)	7(1)	0(0)
Total slight and normal (0.5).	2.71	55(48)	21(18)	39(18)	29(4)	0(0)

between the grades of the parents and those of the offspring,—the truncate females (grade 4) yielded an average offspring grade of 2.3—, among their 147 gray red offspring; their normal sisters (grade 0) yielded a grade of 2.24, among their 107 gray red offspring.

On the basis of the various tests of sibs, then, the conclusion seemed practically inevitable that the variation in the truncate character, aside from that due to segregation of multiple heterozygous factors, is entirely "somatic," and not due to fluctuations in the genes.

b. *The "pure-line" experiment*

It was thought desirable to carry the tests of factor constancy further, however. For it might have been contended that the genetic variations in any one generation may be rather small, and as such inappreciable, but that they would have a chance to become evident if allowed to accumulate through several generations of selection. A scheme of selection was therefore undertaken, with this question in view. The selection had to be carried on in such a way, however, as to exclude the possibility of effects due to recombination and the sorting out of those combinations that tended in the direction of selection. This is usually the insuperable difficulty in the way of significant selection experiments in sexually reproducing, cross-breeding organisms; it is the flaw which invalidated the early conclusions on hooded rats, for example, and other similar experiments, in so far as their bearing on the question of factor variability is concerned. What is needed in such an experiment is the prevention of effective recombination (recombination which might have an effect on the progress of the selection experiment). This is the essential feature in the pure-line idea of Johannsen. But it is not really necessary for this purpose that the organisms be homozygous and self-fertilizing, as in Johannsen's definition of the pure line, or—what amounts to the same thing—that they be inbred until, according to the probability ratios worked out by JENNINGS, they have become homozygous. Neither is the only other alternative that they reproduce asexually, as in the work of JENNINGS and of a number of other investigators. There is a third method of pure-line work, which we wish to present here, that may be used in cross-breeding organisms like Drosophila, in which all the linkage groups are known. This method consists in exposing to view completely, in one parent at least, the process of recombination, by making use of "identifying factors," and then picking out, generation after generation, the same combination for selection.[5]

This process is really, in essential principles, the one which has been

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[5] A useful, but not so efficient, modification of this is to carry on the plus and minus selection lines without reference to recombination, and then, at the end of the experiment, to "purify" the lines, or bring them to a common basis so far as modifying factors may be concerned, by means of crosses with a common stock containing the identifying factors. If there is any effect of selection still noticeable after the "purification," it must have been in the factor studied. This method is not so effective as that above, however, for the reason that it reduces the rigor of the selection with respect to possible variations in the factor under consideration, to have other variations, indistinguishable from these, occurring at the same time, which are due to recombinations of modifying factors.

already employed in the experiments just reported, where gray red flies having the same chromosome combinations were produced, and those with different grades of truncation were "selected" and bred, to find out whether they were genetically different. Here, however, the "selection" was carried on through only one generation. But in the cases where the gray red parents were males, it was possible to continue the selection further. For in these cases the gray red male offspring received (without crossing over) precisely the same paternal combination as their fathers had contained, and so they could be used for similar test crosses with black pink females from the stock bottle. As the latter crosses likewise produce offspring of the same type, these may again be used, and so, generation after generation, the selection might be continued indefinitely, by mating the gray red males to black pink females from stock. Such an experiment was accordingly undertaken with these flies.

It will be desirable, before examining the results, first to analyze the conditions of this selection experiment a little more closely. The original gray red males, that were to be tested, contained the chromosome combination $\frac{Y}{X}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$. Of this combination, they transmit their Y, $T_2'B$, and $T_3'P$ chromosomes, without crossing over, to their gray red male offspring. The entire paternally derived equipment of the offspring is therefore a direct and unalloyed continuation of the paternal side of their father, at least so far as the first three chromosomes are concerned. The maternally derived chromosomes of the offspring, on the other hand—the X-, b-, and p-containing chromosomes—are not descended from the similar chromosomes of the father, but are received directly from the black pink stock, which was used as female parent. Thus these maternal chromosomes in the offspring have a chance of being slightly different from those which the gray red father contained, although they are really derived from the same black pink stock as those in the father. In the same way, when this cross is repeated in each generation the same paternal Y, $T_2'\ B$, and $T_3'\ P$ chromosomes are continuously handed down, but the X-, b-, and p-containing chromosomes of the father are always lost in the subsequent generation and a new lot of them is received from the black pink stock. It might at first sight appear as though this polygenetic origin of the maternal chromosomes might invalidate the pure-line experiment. However, all tests have indicated that the black pink stock is sensibly homozygous, so it is improbable that differences in these maternal chromosomes would appreciably influence the characters of the offspring and thus interfere with the selection work. And much more important in this connection is the fact that since these chromosomes are discarded and renewed *at random* from the stock in each generation, any selection of factors in them (supposing factor differences do exist) can have no constant or cumulative effect on the progress of the selection. Any final and sustained differences in the effects of selection, on lines differently selected, would hence have to be due to changes which took place in that part of the germ plasm that has been handed down continuously in the selected lines—namely, the Y, $T_2{}'B$, $T_3{}'P$ chromosomes.

The above conclusion, it should be noted, implies the assumption that there are no factor-differences for truncate, existing outside the three large chromosomes. For only the first three chromosomes are under watch in the present experiment, and while these are being handed down without recombination, as in a pure line, it must be remembered that any heterozygous factors in the fourth chromosome or elsewhere (i.e., in the cytoplasm) would have a chance of being sorted out and so influencing the final result of selection. The fourth chromosome could have been watched in the same way as the first three by crossing the flies to bpb_e stock instead of to bp, and always choosing the gray red straight-winged male offspring as parents. If this had been done (as it was, in fact, for one generation) the transmission of the entire chromosomal genetic complex would then have been exposed to view and under control continuously. It was thought unnecessary to keep watch on the fourth chromosome, however, both because it is extremely small and also because, as has been previously mentioned, other tests had indicated there were no modifiers for truncate located there. It should, besides, be noted that such disregard of the fourth chromosome in the experiments could not invalidate the significance of a negative result. For, in case of a negative result (i.e., finding that there was no effect from selection), it would be proved, not only, (1) that all factors for truncate that had been included in the pure-line system (i.e., those located in the first three chromosomes, that had not been permitted to undergo recombination) had remained sensibly constant, but also, (2) that no factor-differences affecting truncate had *existed* outside this system,—in loci that had been allowed to undergo recombination. For if the latter factor-differences had existed, selection would have been effective by sorting them out. The same conclusion would apply both to factors in the fourth chromosome and to any supposititious factors (factor-differences) affecting truncate, that might be imagined to exist outside any of the four accepted chromosomes or linkage groups of Drosophila—that is, a negative result of the selection experiment would prove them non-existent.

The selection experiment was carried out in two lines—a "minus" or "low" line, and a "plus" or "high" line. The two lines were of course both derived from the same gray red ancestral male of composition $\frac{T_2'B}{b}\ \frac{T_3'P}{p}$. The count from this male (crossed by black pink female) is shown in table 10 (A), which gives as usual, only the gray red offspring of the cross. The average grade of truncation of these flies was 2.3+. One of the slight-intermediate males of this family was then chosen as the start of the low line, and one of the truncates for the high line, each being crossed to black pink females, according to the scheme explained above. The low line was then carried through twelve generations of selection, in each of which several gray red males showing the nearest approach to normal wings were chosen for parentage, and bred in separate bottles with black pink females from stock. In cases where there were conspicuous differences in truncation, between the families produced by these several males, that family giving the lowest grade of gray red male offspring was used to furnish the male parents of the next generation. The condition of this line after twelve generations of "minus selection" is shown in table 10 (B), which gives the distribution of truncation among the gray red offspring of the seven least-truncated males of this generation. It will be seen that the average grade of truncation for all these families together is 2.2+. The extremely slight difference from the original value, 2.3, is in the direction of selection, but in view of the much larger differences between families of the same line and generation, this figure must be considered as in extremely close agreement with the first finding.

The high line was carried on for thirteen generations. The method used was the same as in the other line, but of course the gray red males selected in this case were always of the highest grade of truncation available, instead of the lowest. It was found, as was to be expected, that in many cases no really high grade males were produced which could be used as parents in this line, owing to the sex-limitation of truncate and the fact that T_1' was not present; nevertheless the males chosen represented the highest grade of truncate that was being produced, and averaged about a grade higher than the males used as parents in the low

TABLE 10

Results of selection in the absence of recombination.
Only gray red offspring are recorded; total males and females not in parentheses, females in parentheses.

(A) Count from first cross of single gray red truncate male $(\frac{Y}{X}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p})$ by black pink females, from which the two selection lines were derived.

Truncate	Good intermediate	Intermediate	Slight intermediate	Normal	Total	Average truncation
11(9)	5(5)	25(12)	10(0)	0(0)	51	2.33

(B) Counts from similar crosses of males of the "low line" after twelve generations of "minus selection."

Grade of tested male **parent**	Truncate	Good intermediate	Intermediate	Slight intermediate	Normal	Total	Average truncation
0 (normal)	28(28)	37(37)	30(28)	43(3)	27(0)	165	2.0
1 (slight intermediate)	29(29)	27(26)	32(15)	52(0)	16(0)	156	2.0
1 (slight intermediate)	23(23)	5(5)	20(6)	36(1)	0(0)	84	2.2
1 (slight intermediate)	26(25)	22(21)	32(10)	25(0)	0(0)	105	2.5
1 (slight intermediate)	12(12)	5(4)	11(0)	7(0)	0(0)	35	2.6
1 (slight intermediate)	17(16)	5(5)	4(1)	10(0)	0(0)	36	2.8
1 (slight intermediate)	10(9)	3(3)	8(3)	3(0)	0(0)	24	2.8
Total	145(142)	104(101)	137(63)	176(4)	43(0)	605	2.22

(C) Counts from similar crosses of males of the "high line" after thirteen generations of "plus selection."

Grade of tested male parents	Truncate	Good intermediate	Intermediate	Slight intermediate	Normal	Total	Average truncation
2 (intermediate)	9(9)	7(6)	30(18)	21(1)	1(0)	68	2.0
2 (intermediate)	8(7)	28(26)	49(24)	23(1)	0(0)	108	2.2
2 (intermediate)	19(15)	22(17)	45(19)	40(2)	0(0)	126	2.2
2 (intermediate)	14(14)	27(25)	25(13)	28(2)	0(0)	94	2.3
Total	50(45)	84(74)	149(74)	112(6)	1(0)	396	2.18

line. The condition of this line in the last generation, after thirteen generations of "plus selection," is shown in table 10 (C). Here there is close agreement between the grades of truncation in the different families, and the average of all turns out to be 2.2—. There is absolutely no genetic difference, then, between this line and the low line, in spite of a dozen generations of divergent selection. Neither of them, moreover, departs significantly from the original value. This same agreement in genetic behavior was shown moreover not only in the final generations of the two lines, but throughout their entire history. We must accordingly draw the conclusion that the truncate character, usually so plastic and so very responsive to the touch of selection both in crosses and in inbred stock, and capable as it is of existing in all degrees of "potency," nevertheless remains absolutely unaltered by selection when all recombinations of its multiple heterozygous factors is rigidly prevented, in a form of pure-line experiment which enables the distribution of practically the entire chromosomal germ splasm to be placed under observation. In other words, the actual factors or genes for truncate, lying in the chromosomes, each had a definite value, and remained constant. The experiment at the same time demonstrates that no inheritance other than chromosomal is taking place, even in the case of a character genetically so complex and so elusive as truncate. That its inheritance was entirely confined to the chromosomal linkage groups—in this case, indeed, to the first three chromosomes—is proved by the fact that there were no hereditary modifications of the character possible, so long as only these chromosomal contributions remained the same.

Thirdly, the experiment disposes of the supposition, which certain geneticists might have advanced, that the factors for truncate may in heterozygous individuals be weakened, or undergo contamination by their normal allelomorphs. For here the truncate factors of both lines were kept heterozygous for twelve generations without their value or "potency" being affected.

THE CAUSE OF THE INCONSTANCY OF TRUNCATE STOCK

a. *Proof of the viability and non-sterility of flies homozygous for the intensifiers*

The question still remained unanswered, then, why had all the attempts to obtain genetically constant truncate stock ended in failure. Since the factors themselves were constant, and the stocks were inconstant, genetically, it followed that all the stocks must be, for some reason, heterozygous in one or more of the factors for truncate. This means that the homozygote, of one sex at least, must be either sterile, or non-viable, or else incapable of being formed at all. It was evident, however that this condition did not apply to all the factors for truncate, otherwise it would be possible, by out-crossing to normal, to obtain heterozygous truncates that were somatically and genetically of as high a grade of truncation as the best truncate flies of the stock bottle. Nearly all the truncates resulting from out-crosses were, on the contrary, of conspicuously lower grade than their truncate parents. And it could be shown, more specifically, that the factor T_1' was not subject to this limitation. For, in the first place, T_1' could exist in fertile truncate males. This had been proved by the reciprocal crosses considered on p. 15 and 16. And as males which carry a given sex-linked factor in their single X chromosome are usually of a similar type, somatically, to the females homozygous for that factor, it was fairly certain that the females homozygous for T_1' were viable also. It was less certain that they were fertile, on account of the difference between the reproductive systems of male and female. The viability and fertility of females homozygous for T_1' were, however, independently proved by the fact that the daughters of the out-crossed truncate females were, on the average, just as frequently truncate, and of just as high a grade of truncate as the daughters out of the out-crossed truncate males (see page 16, and table 6). The males necessarily transmitted T_1' to all of their daughters; the females therefore must have done the same, and so must have been homozygous for T_1'.

It was also clear that not only T_1' but in addition either T_2' or T_3' (or, if they be compound, then one or more of the factors contained in them) could exist with some degree of fertility in homozygous condition. For the truncate or intermediate T_1' males derived from outcrosses of truncate females were usually of much lower grade than the truncate males of truncate stock. Since both alike might possess T_1' in their single X chromosome these differences in truncation were evidently due to the heterozygosis of the F_1 males in respect to some non-sex-linked factors (T_2' or T_3' or some component of them), for which the stock males were homozygous.

Crosses were then made to secure evidence, in the case of the two latter factors, as to whether or not they could exist in homozygous condition. For this purpose recourse was again had to crosses of single heterozygous gray red males $\frac{T_1'X}{Y}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$ by stock black pink females. One of these crosses is shown in table 11 (A); it is identical in nature with that of table 7, cross 3. From the offspring of this cross, flies were chosen for five parallel crosses, the results of which were to be compared.

The first three of these crosses were concerned with the question of homozygosis in respect to T_3'. The first cross was of a slight-inter-

TABLE II

Crosses to test the possibility of securing homozygous T_3' and T_2'.

(A) Preliminary back-cross of heterozygous truncate male to black pink stock

Nature of parents		Gray red					Gray pink				Black red				Black pink			
♀	♂	sh T	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
$\frac{\quad}{}\ \frac{b}{b}\ \frac{p}{p}$ Black pink long	$\frac{T_1'}{}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$ Gray red truncate		18 (0)	9 (4)	16 (16)	3 (3)			18 (3)	38 (17)				47 (21)				48 (23)

(B) Crosses of flies derived from (A)

♀	♂	sh T	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
$\frac{T_1'}{}\ \frac{b}{b}\ \frac{p}{p}$ Black pink long	$\frac{T_2'B}{b}\ \frac{T_3'P}{p}$ Gray red slight		10 (6)	18 (11)	11 (0)	3 (0)		8 (4)	14 (10)	19 (6)				40 (22)				41 (23)
$\frac{T_1'}{}\ \frac{b}{b}\ \frac{T_3'P}{p}$ Black red long	$\frac{T_2'B}{b}\ \frac{p}{p}$ Gray pink long		5 (2)	20 (11)	14 (6)	5 (1)		8 (7)	20 (13)	23 (11)				34 (14)				43 (20)
$\frac{T_1'}{}\ \frac{b}{b}\ \frac{T_3'P}{p}$ Black red long	$\frac{T_2'B}{b}\ \frac{T_3'P}{p}$ Gray red slight	11 (8)	7 (4)	4 (2)	7 (3)	1 (0)		3 (2)	3 (1)	5 (2)				24 (15)				7 (3)
$\frac{T_1'}{}\ \frac{T_2'B}{b}\ \frac{p}{p}$ Gray pink slight	$\frac{b}{b}\ \frac{T_3'P}{p}$ Black red long			5 (3)	2 (1)	5 (2)			4 (4)	4 (3)		2 (1)	1 (1)	7 (5)				17 (8)
$\frac{T_1'}{}\ \frac{T_2'B}{b}\ \frac{p}{p}$ Gray pink long	$\frac{T_2'B}{b}\ \frac{T_3'P}{p}$ Gray red slight	1 (1)	5 (3)	8 (4)	4 (2)	7 (3)		1 (0)	9 (4)	13 (7)		5 (2)		16 (8)			2 (1)	11 (3)

(The number of males is given in parenthesis under the number of flies of both sexes.)

mediate gray red male (composition $\frac{X}{Y}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$) by one of its black pink sisters ($\frac{T_1'X}{X}\ \frac{b}{b}\ \frac{p}{p}$) derived from the same bottle. This cross is of the same type as the parental one of table 11 (A) except that T_1' is distributed to half of the daughters and to half of the sons, instead of to all of the daughters and none of the sons. The count, given on line 1, table 11 (B), shows the results to be expected on the basis of the parental compositions, there being the usual four classes, gray reds, gray pinks, black reds, and black pinks, in remarkably equal numbers. Only the grays are truncated, and the gray reds especially, showing a count of 10 truncate: 18 intermediate: 11 slight-intermediate: 3 normal. ("Good intermediates" and "intermediates" are classed together in this and the following records.)

The second cross was like the first, except that the female was heterozygous for $T_3'P$, instead of the male. This cross therefore had the formula $\frac{X}{Y}\ \frac{T_2'B}{b}\ \frac{p}{p}$ (long-winged gray pink male) by $\frac{T'X}{X}\ \frac{b}{b}\ \frac{T_3'P}{p}$ (long black red female). It will be seen that the results, shown on line 2 of table 11 (B) are very similar in character to those of the first cross, except that there may be a slight decrease in the proportion of high-grade truncates among the gray reds, as compared with the first cross. This change, if not a chance deviation, is due to the crossing over between T_3' and P, which was possible in the female.

These first two crosses really served as controls for the third cross, which was concerned with the possibility of securing homozygous T_3'. The third cross was really a combination of the first and second, in that both the male and female parents were heterozygous for $T_3'P$, instead of the male alone, as in cross 1, or the female alone, as in cross 2. The parents, then, were a slight-intermediate gray red male which was a brother of the similar one used in the first cross and one of its black red sisters which was also a sister of the similar female that was used as a parent in the second cross. The formula for the cross is $\frac{X}{Y}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$ by $\frac{T_1'X}{X}\ \frac{b}{b}\ \frac{T_3'P}{p}$. Here, since both parents are heterozygous for $T_3'P$, the reds should bear the ratio of 3 : 1 to the pinks, instead of 1 : 1, as before, and some of the reds,—one-third or fewer of them, depending on the exact amount of crossing over between T_3' and P,—will be homozygous for T_3', provided T_3' can exist in homozygous condition. This homozygosis in T_3', if it can exist, might then be reflected in a more strongly truncated condition of the flies of the gray red classes; if, on the other hand, homozygous T_3' cannot exist, the gray red flies will not, on the average, be more markedly truncated than those of the previous cross. They would, if anything, average less truncate than in cross 1, because of the possibility here of crossing over between T_3' and P (red). Moreover, the ratio of reds to pink would be 2 : 1, or at least less than 3 : 1, if the reds homozygous for T_3' failed to appear. The count is shown on line 3 of table 11 (B). The ratio of reds to pinks is exactly 3 : 1 (54 : 18), as it should be if the homozygous T_3' live. Examining the gray reds, it is clear that there is among them a new class of individuals, with respect to truncate, that is never found in conspicuous numbers in out-crosses. This has been called "short-truncate"; it is of the same grade as the highly-truncated individuals of selected truncate stock. Almost exactly one-third of the gray red individuals belong to this grade. (It will be noted that the gray pinks, on the other hand, are no more truncated than before, there being no special environic conditions here favoring truncation.) It is therefore evident that the whole chromosome containing T_3' is capable of existing in homozygous condition, and that, provided T_1' and T_2' are present as heterozygous factors, then homozygosis in T_3' alone is necessary in order to produce an average grade of truncation equal to that of the best selected inbred stock. It was found, on attempting to breed the short-truncates produced in this experiment, that although the flies were not sterile, yet the infertility (of the females especially) was so great as to make it extremely difficult to perpetuate the line, just as in the case of the "best" inbred truncate stock. Crosses of the same type as that of table 11 (B), cross 3, have been repeated several times, and exactly similar results, including the production of a conspicuous class of short-truncates, have always been obtained.

The first, fourth and fifth crosses were concerned with homozygosis in respect to T_2'. The fourth cross was similar to the first except in that the female was heterozygous for T_2' instead of the male, so that here there could be crossing over between T_2' and B. The formula is $\frac{X}{Y}\ \frac{b}{b}\ \frac{T_3'P}{p}$ by $\frac{T_1'X}{X}\ \frac{T_2'B}{b}\ \frac{p}{p}$ (long black red male by slight-intermediate gray pink female). The parents were sibs of the parents used in the other crosses. This cross accomplishes the same purpose for T_2' that the second cross did for T_3'. The very small count, given on line 4 of table 11 (B) is nevertheless sufficient to show that the general character of the cross is like that of the first, except in that the grays here include fewer truncates, and that some of the blacks are truncated, owing to the crossing over between T_2' and B in the female. The first and fourth cross served as controls for the fifth, in which opportunity was given for T_2' to become homozygous. In the fifth cross, both male and female (sibs of the previous parents) were heterozygous for $T_2'B$, instead of just the male, as in cross one, or just the female, as in cross four. This time, then, a slight-intermediate gray red male like that in the first cross was mated to a gray pink sister like that in the fourth cross $(\frac{X}{Y}\ \frac{T_2'B}{b}\ \frac{T_3'P}{p}$ by $\frac{T_1'X}{X}\ \frac{T_2'B}{b}\ \frac{p}{p})$. In this case, as both parents contain heterozygous $T_2'B$, the expected ratio of grays to blacks would be 3:1, instead of 1:1, as in the other crosses, and one-third or fewer of these grays (both reds and pinks) should be homozygous for T_2' and might therefore be more truncated than the corresponding classes of grays (red or pink) in the first cross. But if the grays homozygous for T_2' fail to appear, this 3:1 ratio should be reduced towards 2:1, and the grays in this cross should not be more truncated than those in the first cross. The count, on line 3, table 14, shows 48 grays to 34 blacks. This is nearer 2:1 than any other simple ratio possible here and certainly it could scarcely be taken for a 3:1 ratio. The gray reds, although including one short-truncate, such as appear in isolated instances in outcrosses to black, are not on the whole significantly more truncated than those in the first cross; the gray pinks, which are just as useful for the present purpose, are, if anything, less truncated than in the first cross, and do not include any flies that have a higher grade of truncation than that found among the gray pinks there. The two most truncated gray red males, moreover, were tested by crossing to black pink, and both proved to be heterozygous for T_2', giving both gray truncate and black long-winged offspring, and no black truncates. The evidence of these experiments hence indicates that T_2' cannot become homozygous. Yet it is not conclusive, for there is a possibility that homozygous T_2' is no more truncate in appearance than heterozygous T_2'. The deficiency in the number of grays, also, might be explained away, by supposing that special circumstances made all the grays here relatively less viable than the blacks. It should be mentioned, however, that crosses one and five were later repeated, with results similar to those of the present experiments: the ratio of grays to blacks in the repetition of experiment five was 61:32, and the truncate averaged of no better grade there than in the control cross corresponding to cross one (no short-truncates at all were produced this time).

b. *Experiments to test the possibility of securing homozygous T_2'*

Other crosses were then undertaken, which might determine more definitely whether the T_2' homozygote could occur. It would not have been feasible here to test flies for homozygosis in the ordinary simple manner,—that is, merely by finding the total proportion of truncates thrown, because the proportion of truncates is subject to modification in so many ways. A way was therefore found of "marking" the T_2' factors, by binding them to identifying factors (B and b), so that it could be determined in the tests whether a fly had only one T_2' factor, or two T_2' factors differently "marked", as will be explained presently. For this purpose, black truncate flies were secured that had arisen as crossovers in a cross similar to number four above (see line 4, table 11 (B). From these a stock of black truncate (inconstant for truncate, of course) was obtained. Black truncates (presumably heterozygotes containing $\frac{T_2'\,b}{b}$) were then mated to heterozygous gray truncates having the second chromosome composition $\frac{T_2'\,B}{b}$. Both reciprocal crosses were made. From these crosses gray truncate offspring,—usually the male offspring,—were chosen, and tested by crossing to black long-winged stock.

In the cross of $\frac{T_2'\,b}{b}$ males by $\frac{T_2'\,B}{b}$ females, the gray truncate male offspring that were tested may have received either a crossover gray chromosome,—B,—or a non-crossover gray chromosome,—$T_2'\,B$,—from their mother. In the former case, which would be less frequent, they must have received $T_2'\,b$ from their father, since they showed the truncate character; their composition, then, would be $\frac{B}{T_2'\,b}$. They would reveal this composition, in the test crosses with black long, by producing gray offspring $(\frac{B}{b})$ all long-winged, and black offspring $(\frac{T_2'\,b}{b})$ some of which were more or less truncated. (Enough intensifiers were present to insure the visibility of the truncate in most of the flies carrying T_2'.) Of the fifteen gray truncate males tested, four proved to have this composition, as they threw black truncates, but no gray truncates. Here there was no chance for homozygosis of T_2'. The other eleven gray truncate males proved to have received $T_2' B$ from their mother, for they all gave rise to some gray truncates on crossing to black long. It was to be expected, if T_2' can be homozygous, that half of these eleven males would have received the $T_2' b$ chromosome from their father, the rest merely the b. The former, then, would throw black truncates, the latter only black longs, in addition to the gray truncates. As a matter of fact none of these flies which threw gray truncates threw black truncates, only longs appeared within the black class of offspring. The composition of all eleven must therefore have been $\frac{T_2' B}{b}$, although, if T_2' can occur in homozygous condition, half of them should have been $\frac{T_2' B}{T_2' b}$.

From the reciprocal cross, of $\frac{T_2' B}{b}$ male by $\frac{T_2' b}{b}$ female, 10 gray truncate male offspring were tested. In this cross, there will be no $\frac{B}{T_2' b}$ class, for all the gray offspring must receive $T_2' B$ from their father, since there is no crossing over between T_2' and B in the male. Correspondingly, it was found that all the (gray) males tested threw gray truncates. Half of these males, as before, may have received b from their other parent, and the rest $T_2' b$. But, as before, it was found that all the black offspring were longs; here again, then, all the flies had been heterozygous, $\frac{T_2' B}{b}$, although there had been a chance for the half of them to have been homozygous, $\frac{T_2' B}{T_2' b}$. Five of the gray truncate female offspring of this cross were also tested, by crossing with black long males; similar considerations apply to them, except that a heterozygous $\frac{T_2' B}{b}$ female would throw some black truncate offspring by crossing over; it would not throw nearly as many black truncates as gray truncates, however, and so it could be distinguished from the homozygous $\frac{T_2' B}{T_2' b}$ female. It was found that all five females were of the heterozygous type, $\frac{T_2' B}{b}$, just as in the case of the males.

Another cross, similar in principle, involved the dominant identifying factor star, in chromosome II, as well as black. A black pink truncate fly (obtained by crossing and subsequent selection) was crossed to star pink. A son showing the characters star, truncate and pink,—composition $\frac{T_2'\ b}{S'\ B}\ \frac{p}{p}$, —was then crossed by a short-truncate female from the inbred truncate stock. Forty-six male offspring which did not have star eyes, and which had therefore received $T_2'\ b$ instead of $S'\ B$ from their father, were then tested by back-crossing them separately to non-truncate black pink females. The composition of these males must have been $\frac{Y}{T_1'\ X}\ \frac{T_2'\ \ b}{T_2'(?)B}\ \frac{p}{T_3'\ P}$; the question was whether any of them had received $T_2'\ B$ from their truncate mother, in addition to the $T_2'\ b$ that they must have gotten from their father. If T_2' can be homozygous, at least half should have received $T_2'\ B$, and should therefore give rise to gray truncate offspring as well as black truncates. All forty-six, however, failed to produce any gray truncates, although they all threw black truncates in very high percentage, particularly among the red female offspring, which contained T_3' and T_1'. The original short-truncate female from selected stock must therefore have been heterozygous for T_2', and when her eggs were fertilized by the T_2' b-bearing sperm, only those which had failed to receive the maternal T_2' developed. Adding these 46 to the previous 26 tests, there is a total of 72 flies, at least one-half of which should have been homozygous for T_2', had this been possible, but all of which were found to be heterozygous.

We may therefore conclude with certainty that homozygous T_2' cannot exist. This explains not only why there was such difficulty met with in the attempt to get pure truncate stock, but also why truncate, even when it acted as a dominant in crosses with black, never gave more than half of the F_1 showing truncate (see tables 6 and 7). Twin hybrids, truncate and long, were produced, as in out-crosses of Oenothera, Matthiola, and beaded Drosophila (see page 41), half of them in the present case receiving the T_2' chromosome and half the t_2'. Tests of such F_1 longs, moreover, showed many of them to be genetically long, and not merely T_2'-carrying flies that lacked intensifiers or happened to vary somatically in the direction of normal (although of course, some of the longs were of this type, in cases where more than half of the F_1 appeared long). Table 12, lines 1 and 2, shows the result of such a test of an F_1 long male which had arisen from an out-cross of a short-truncate female of inbred stock by a black pink male. When this long was back-crossed to black pink, many of the gray red offspring should have appeared truncate, had the F_1 carried T_2', but, as the table shows, not one truncate appeared. One of the slight intermediate F_1 males, on the other hand, which was a brother of the long, gave the count on the second line, when tested similarly. Thus, even the short-truncate of inbred stock is heterozygous for T_2', and gives the results expected of a heterozygote, on out-crossing. It may be noted that the tests of these normal-appearing F_1 males are in striking contrast to the results obtained by crossing normal-appearing males from a back-cross like that in table 7, cross 3, where the identifying factors show that the flies, though normal, must really carry the factors for truncate.

TABLE 12

Tests of F_1 males from a cross of short truncate female by black pink male. F_1 males tested by back-crossing to black pink.

Character of F_1 male tested	Gray red				Gray pink				Black red				Black pink			
	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
Normal	0	0	0	21	0	0	0	25	0	0	0	23	0	0	0	26
Slight intermediate	0	3	3	2	0	0	2	14	0	0	0	17	0	0	0	11

c. *The reason for the non-appearance of homozygous T_2'*

The failure of T_2' to appear in homozygous combination must be due either to the fact that it acts, when homozygous, as a lethal factor, or that there is a selective fertilization of T_2' eggs by t_2' sperm exclusively. In the latter case, when heterozygous $\frac{T_2'\,B}{b}$ flies were crossed with each other, a ratio of 3 grays $(\frac{T_2'\,B}{b})$: 1 black $(\frac{b}{b})$ should be produced, since all $T_2'\,B$ eggs would be fertilized by b sperm, and half the b eggs would be fertilized by $T_2'\,B$ sperm. In the case of a lethal factor, on the other hand, only 2 grays : 1 black should appear, for one-third of the grays would consist of the homozygous $\frac{T_2'\,B}{T_2'\,B}$ combination, that would die. We have seen (page 35) that the 2 : 1 ratio is actually obtained; thus T_2' is a lethal, like yellow in mice, and like most dominant mutant factors, in Drosophila at any rate.

d. *The cause of the infrequency of normals in the selected stock*

This "yellow-mouse" hypothesis had of course often been considered, as a possible explanation of why stocks of truncate were always genetically inconstant, but it had not previously been thought probable, because the highly selected, inbred truncate threw only 10 percent of normals (and many of these, even, carried T_2') whereas yellow mice throw as much as 33⅓ percent of normals (or non-yellows). A cross of $\frac{T_2'}{t_2'}$ by $\frac{T_2'}{t_2'}$ likewise should give 2 $\frac{T_2'}{t_2'}$ (more or less truncated) : 1 $\frac{t_2'}{t_2'}$ (long), provided the $\frac{T_2'}{T_2'}$ die. Either, then, in the case of the selected truncate, most of the $\frac{t_2'}{t_2'}$ which are formed die also, owing to the existence of another lethal or semi-lethal factor in the chromosome with t_2', or, if they live, many of them must appear truncate, like the $\frac{T_2'}{t_2'}$ flies, owing to some other chief factor for truncate that they may contain. This other factor would have to lie in the second chromosome, for we have seen that flies containing other chromosomes of truncate stock, but not having the second so derived (the black red flies of table 7, cross 3), even when inbred, cannot give rise to truncates (table 7, cross 5). It would, moreover, have to be a recessive, for we have seen that heterozygous flies which fail to receive T_2' are never truncate, even if one of their second chromosomes is derived from truncate stock (p. 39).

A test was therefore undertaken, to determine whether there existed, in the t_2'-bearing second chromosome of the selected stock, a recessive factor for truncate, which, when homozygous, was able to cause the truncate character to appear, even in the absence of T_2' itself. This test was performed by inbreeding the gray red flies ($\frac{T_1' X \text{ or } Y}{X} \frac{t_2' B}{b} \frac{T_3' P}{p}$) obtained in the last tests for homozygous T_2', reported on p. 38. All these flies had received, it will be remembered, one gray-bearing chromosome derived from a short-truncate female of the selected stock; this chromosome had been found not to contain T_2'. If, however, it contained a recessive "chief" factor for truncate, in any of the families (the chromosome was not necessarily the same in the different families, on account of the possibility of crossing over in the stock truncate fly from which it was derived), then, when the gray flies of such a family were crossed together, some flies homozygous for the recessive truncate would be produced, and many of these should show the truncate character (since the intensifiers also are present). Nineteen of these tests were made in all, the gray red offspring of each of nineteen of the males that had been tested for homozygosis being inbred to each other in mass culture, family by family; thus, if the original truncate female had been heterozygous, so that only certain families contained the factor, it would have a chance to become homozygous in these lines. None of the 19 families produced any truncates, however, and the hypothesis of a recessive "chief" factor to explain the low proportion of normals in the selected truncate stock therefore had to be discarded.

The only remaining possibility was that the truncate stock contained a lethal (or semi-lethal) factor in the second chromosome that carried t_2', for since it was shown that a $\frac{t_2'}{t_2'}$ combination in the selected stock would not give rise to truncate-winged flies, the excess of truncates (or deficiency of longs) proved that there was a real deficiency, i.e. a dying off, of the $\frac{t_2'}{t_2'}$ flies that were formed at fertilization.

A similar condition of affairs had been found by MULLER (1917) to exist in the case of the beaded-winged stock of Drosophila, and was called by him a state of "balanced lethal factors." In such a case, there are present, in homologous chromosomes, two different lethal factors, each of which kills off an opposite type of homozygote, and which, together, thus cause a heterozygous stock apparently to breed true, or nearly true. The balanced lethals in the case of the truncate stock are T_2', which kills the homozygous truncates, and the recessive lethal factor in the opposite chromosome, which kills the homozygous $\frac{t_2'}{t_2'}$ flies.

As shown in the report on the beaded case, the occurrence of such recessive lethals by mutation must be comparatively frequent. Bearing this in mind, the existence of this factor in the truncate race is easily explained, as follows: A stock having a character such as truncate, which is itself due to a lethal (T_2'), must continually throw normals. When such a character is selected, therefore, an endeavor will be made to select a line throwing as few normals as possible. In the course of time, various lethal factors will arise, in different loci, most of which will be eliminated by natural selection, but when a lethal arises in the normal homologue of the chromosome containing the factor that is being selected for (T_2'), it will cause the killing off of a large number of the undesired normals; consequently the line in which this factor has arisen will be perpetuated in the selection. The nearer the locus of this factor lies to the locus of the desired factor (T_2'), the fewer will be the normals that can escape, by crossing over, from the lethal action, and so the better will be the chance of the perpetuation of this race in the experiment. After a long course of selection, then, it is to be expected that the stock would show the presence of a lethal factor nearly opposite, in location, to the lethal factor that is being selected for, and a condition of "balanced lethals" would have arisen. For the further consequences of this condition the reader must be referred to the account of the beaded case.

e. *The source of the normals which did occur in the selected stock*

In the case of the truncate stock, it is likely that there was a certain amount of crossing over between T_2' and the recessive lethal. This would partly explain the presence of the 10 percent of normals in the selected stock, for such normals could arise from a $\frac{T_2'\,L}{t_2'\,l}$ female, from a crossover gamete $t_2'\,L$ (l here indicates the recessive lethal; L its normal allelomorph). Such normals would never give truncates if crossed together, as they do not contain T_2'.

Table 2 showed, however, that some of the normals thrown by the stock could give rise to truncates. Some of these normals, then, may have been merely somatic variants towards normal, which nevertheless had the full complement of truncate factors. In that case it would have to be assumed that in these experiments they had been mated to the crossover normals, which lacked T_2' and which would be indistinguishable from them somatically, for these matings gave fewer truncates than ordinary matings of somatic truncates. But it seems unlikely that this special sort of mating should be made frequently, and so it is more probable that most of these normals, which were able to throw some truncates, were T_2'-bearing flies that lacked one or more of the intensifiers. It has been explained that females homozygous for the intensifier, T_3', are extremely infertile, and since in the selected stock even those eggs which are laid are killed off in large numbers by the lethals of chromosome II, it will be readily understood that truncate stock homozygous for T_3' could not be long maintained. It was, in fact, found continually in the original selection experiment that the very high lines died out through infertility, and that the stock had to be replenished from mass cultures of somewhat lower grade. The stock consequently was probably impure for T_3' as well as T_2', even though many of the individuals in it must have been homozygous for the former factor.

THE LOCUS OF TRUNCATE

The above experiments were sufficient to demonstrate the nature of the mechanism of inheritance of the truncate character. They showed that it conformed regularly to the principles of chromosome heredity, factor constancy, etc., and disclosed the causes of the inconstancy of the inbred stock, and of the indefiniteness and variability of the ratios thrown in crosses.

Further determination of the precise number of modifying factors for truncate present in each chromosome, and the location and exact effect of each, in the various possible combinations, would have required, for the most part, elaborate tests—much more elaborate than the preceding ones—and yet these details would have been of little general significance, once it had been found out what was really "the trouble" in the truncate case. Some more exact tests were at first undertaken, with these objects in view, but many identifying factors had to be used for this purpose, in order to follow the crossing over, and it was found that some of these factors also influenced the truncate character. This circumstance, taken together with the fact that the classes containing the intensifiers far overlapped those lacking them, on account of the natural somatic variation of truncate, made a direct estimation of the percentage of crossovers impossible. An example of the confusion caused in these ways is seen in table 13, which represents a count of 196 offspring of a back-cross designed to find the locus of T_3'. A female heterozygous for truncate and for three third-chromosome identifying factors—pink eye (*p*), kidney eye (*k*), and sooty body color (e^s), was back-crossed to a pure *p k* e^s male. It will be seen that the truncate shows rather close linkage to *P*, and yet the crossover classes all show a fair amount of truncate, which is distributed among them nearly proportionally. Thus the truncate intensifier shows no closer linkage to *P* than to either of the other factors, and its locus is indeterminable. Such a result can be explained only by supposing that several modifiers of truncate are here concerned.

Difficulties of the above sort would not have been insuperable, as the flies from such a count could each be tested out, separately, but a much

TABLE 13

Back-cross of truncate to pink kidney sooty.

$$\left(\frac{T_1'}{}\,\frac{T_2'}{}\,\frac{T_3'}{pke^s}\ ♀ \times \frac{p\ k\ e^s}{p\ k\ e^s}\ ♂\right)$$

	$P\ K\ E$				$p\ k\ e^s$				$P\ k\ e^s$				$p\ K\ E$				$P\ K\ e^s$				$p\ k\ E$			
	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
♀	3	1	0	34	1	4	2	36	1	1	0	1	0	0	0	8	0	0	0	6	0	0	0	4
♂	3	4	1	30	0	4	0	32	0	0	0	4	0	1	0	2	0	1	1	7	1	1	0	2

more elaborate system of experiments would have been necessary for this purpose than the value of such information justified. There would, moreover, have been no such thing as a "complete account" of the truncate case, unless all known and unknown mutant factors of Drosophila had been tested, in all possible combinations, with respect to their effect on truncation. It was therefore decided to confine the "intrachromosome" investigation simply to a determination of the locus of T_2', the chief factor for truncate.

Certain data regarding this may be obtained from the counts of table 7(B), crosses 4, 6, and 9, where females heterozygous for truncate and black $(\frac{T_2'\,B}{b})$ were back-crossed to black long males. Here the long-winged offspring must be disregarded, as they are genetically heterogeneous, including many flies that carry T_2 besides the really genetic longs. The percentage of crossing over may, however, be obtained from the count of those that show any degree of truncation. Among the latter, the grays are of course non-crossovers, the blacks crossovers. There are 173 flies in these three crosses showing some grade of truncation, and among them 41 are black (crossovers), the rest gray. This would make the crossing over between truncate and black $\frac{41}{173}$, or 24 percent. In various crosses of the same type 375 truncates have been counted in all, including 100 blacks; this makes the percentage of crossing over nearly 27.

Truncate was also crossed to a stock of black purple curved (all in chromosome II), and F_1 truncate females were back-crossed to $b\ p_r\ c$ males. The count of 530 individuals is shown in table 14; there is the usual percentage of crossing over between b, p_r and c (b-$p = 6$ percent, p_r-$c = 17$ percent). To find the linkage with truncate, only the 119 offspring showing truncation can be used, just as before. Among these there are 22 percent of blacks, indicating a "distance" of 22 units in this cross. This, averaged with the previous results, would place T_2' at 25.5 units from black. Double crossing over is so free in this cross, however, that it is not possible to tell with certainty whether T_2' is on the same side of black as curved is (i.e., to the "right") or on the opposite side (the "left"). Yet the fact that in crossing over between black and purple the truncate remained with the black, indicated that T_2' was probably on the opposite side of black from purple,—that is, to the "left".

A test was accordingly made to determine on which side of black T_2' lay. This was done by means of a back-cross involving truncate, black, and star. Star (S') is a dominant factor lying 46 units to the left of black, at locus 0. In the first place, a star truncate stock was secured by crossing truncate with star and selecting the crossovers in the second generation, and in the second place this star truncate was crossed to black, and one of the heterozygous female offspring, which appeared star truncate, was back-crossed to a black male (triple recessive). The count, given in table 15, shows clearly that T_2' is to the left of black, since twenty-three of the twenty-four flies known to be crossovers between truncate and black (i.e., those showing both truncate and black) are also crossovers between star and black (star, truncate, black). The exact percentages are not very significant in a count of this size (total count 130) but the order of the factors is determinable with certainty.

When all these tests of truncate with black are averaged together, a

TABLE 14

Back-cross of truncate to black purple curved.

$$\left(\frac{T_1'}{\quad}\,\frac{T_2'}{b\ pr\ c}\ \text{♀} \times \frac{b\ pr\ c}{b\ pr\ c}\ \text{♂}\right)$$

	BPr C				*bpr c*				*Bpr c*				*bPr C*			
	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
♀	26	13	6	56	7	5	0	69	0	1	0	3	0	0	0	10
♂	20	12	11	83	5	1	2	99	0	0	0	4	0	0	0	8

	BPr c				*bpr C*				*Bpr C*				*bPr c*			
	T	I	S	N	T	I	S	N	T	I	S	N	T	I	S	N
♀	0	0	0	10	3	2	0	25	1	1	0	1	0	0	0	2
♂	4	0	0	10	0	0	1	30	0	0	0	1	0	0	0	0

TABLE 15

Back-cross of star, truncate and black.

$$\left(\frac{T_1'}{\quad}\ \frac{S'\,T_2'}{b}\ \frac{T_3'}{\quad}\ \text{♀} \times \frac{b}{b}\ \frac{T_3'}{p}\ \text{♂}\right)$$

Non-crossovers		Crossover 1		Crossover 2		Double crossover 1, 2	
Star truncate	Black	Star black	Truncate	Star truncate black	Normal	Star	Truncate black
41	36	6	6	23	14	3	1

percentage of crossing over of about 27 is obtained. The distance between truncate and star, as given in the last test, is 12.3. As the percentage of separations between star and black is known to be about 39, the results of the tests of truncate with star and with black agree fairly well, and it may be concluded that truncate is between star and black, at a locus of about "12"—i.e. 12 units to the right of star, which is taken as being at 0.

ON THE MODE OF ORIGIN OF THE TRUNCATE COMPLEX

On the basis of the information that has been gained concerning the genetic basis of the truncate complex, its mode of origin may in a general way be reconstructed. It is probable that either the factor T_2' or one of the intensifiers had arisen by mutation and persisted unnoticed in the ancestral beaded line for some time before the first truncate fly was discovered. For the original truncate, it will be remembered, gave rise to a moderate proportion of truncate F_1, and to a much higher proportion of truncates in some of the F_2 families. Now, the moderate proportion of truncates in F_1 would not have been possible in the first place, had not an intensifier of truncate been present,—it is only necessary to recall here the extremely small proportion of truncates produced in F_1, in the absence of black, even when the intensifiers are present in heterozygous condition (see table 3(A), representing crosses of selected stock to wild type). Secondly, had not an intensifier been present, it would not have been possible to raise so materially the proportion of truncates in F_2, since only intensifiers, and not T_2' itself, could have been made homozygous by this process.

One possibility, then, is that T_2' had existed for some time in the stock, and suddenly burst into visibility when T_1' or T_3' arose by mutation. Before this occurrence, it would have been detectable practically only as a lethal factor, by the disturbances it produced in ratios, or, if elaborate tests were made, as a factor giving the fly a very slight "instability" of wing shape, or "tendency" to have shortened wings, which manifested itself once perhaps in 100 cases ordinarily, but much more often in the presence of the factor for black (thus recalling the alleged mode of inheritance of certain nervous defects in man). On the other hand, it is more probable that one of the intensifiers had existed first, for these would have been better able to persist in the stock than a lethal factor. The intensifier would not have been detectable at all at this time, unless, in the case of T_3', tests of fertility had been made. But, whichever of the two suppositions is true, it is evident that one of the mutant factors had arisen and persisted unobserved until the second mutation took place.

This situation raises the question as to how many such mutations—producing no visible result on the organism, but changing only the composition of the chromosomes, and, through them, the ultimate potentialities of the race—may be continually occurring unseen. Certainly the occurrence of such mutations is by no means rare, judging by the comparative frequency with which modifying factors are found, in races which happen to have the "chief" factor for that character already present. Thus, when selection is made for bar eye, bar modifiers are found; on selection for beaded wings, beaded modifiers; on selection for dichaete bristles, dichaete modifiers; all of which modifiers have little or no effect on the fly lacking the chief factor for bar, beaded, or dichaete, respectively. Yet such mutations must be occurring with similar frequency (though not selected for and artificially perpetuated) in stock lacking the specific chief factor; so, in wild stock, modifiers must be arising for bar as in the bar stock, and at the same time for beaded as in the beaded stock, for truncate as in the truncate stock, and similarly for innumerable other potential characters not actually realized in the stock at that time. Hence it may well be that there are more mutations for which no means of influencing the somatic characters exists, than visible "character-mutations," and undoubtedly there are many on the edge of visibility. Many of these "invisible" or "barely visible" mutant factors should persist in particular lines, merely as a matter of chance, and they would affect not only chromosome composition and behavior, but also the possibilities of future character-evolution for that line. Such, at least, was the case with the truncate line which, by means of the prior possession of an "invisible" factor for truncate, was enabled to produce the truncate "evolution series." Here is a situation which may give some satisfaction to believers in orthogenesis.

The truncate which had thus appeared probably contained, as yet, only one intensifier, and no balancing lethal, for it could not be increased by selection for a considerable time. When, however, each of the other contributing factors finally appeared in the course of the breeding, the line containing it was purposely selected, since it threw more and better truncate. It has been previously explained that there is nothing remarkable about the fact that a balancing lethal did arise. The fact that it was also possible to find one or more additional intensifiers may however call for some comment. This responsiveness of truncate to selection,—the appearance of intensifiers when sought for,—may be shown to be an inherent characteristic of the truncate race, dependent on a particularly high modifiability of the embryological process whereby the truncate character is produced. The degree of modifiability of the developmental reaction that causes any given character may be estimated by at least two criteria: (1) by the somatic variability of the character among individuals of the same genotype (a test of responsiveness to environmental influences); (2) by the proportion of previously known factors for other characters which are able to influence the character under consideration (a test of responsiveness to genetic influences). To both of these tests truncate gives a positive result, for we have seen, (1) that, when flies carry T_2', the wing shape is unusually variable even among individuals of identical factor composition, and (2) that, among these flies with T_2', a number of factors, like that for black body color, star eyes, bar eyes, sex, etc., which ordinarily produce no noticeable effect upon the wing, now influence its shape decidedly. Hence the developmental reactions that determine the wing shape must be particularly unstable, or susceptible to modification, in the race containing T_2'. From this conclusion in turn it follows that relatively many modifiers of wing shape should be found to arise by mutation in the truncate race,—not because mutation itself happens oftener in this race, but because, of the mutations that do occur, more would be able to affect the wing character in this race than in non-truncate races, where wing shape was not so easily influenced. A race of this kind, accordingly, would be able to undergo an evolution or cumulative change, under the directive action of selection, which would be next to impossible in another race, that lacked the factor causing this embryological modifiability. Here again, then, we have an example of invisible peculiarities of the germ plasm of certain lines, influencing the possibilities of character-variation and evolution.

The question might here arise—if characters may differ so considerably in regard to the modifiability of their development reactions, is it not conceivable that certain characters may be so highly modifiable,—influenced by such a large proportion of the total number of mutations that occur,—and that this *total* number of mutations is at the same time so large, that, even though each separate single factor of the germ plasm is highly constant, nevertheless these characters would, in effect, really be undergoing a continual genetic fluctuation. This contingency cannot be categorically denied, as an academic possibility for exceptional cases, but we have seen that in the case of truncate, although it is a particularly unstable character, yet the number of mutations which affected it were not nearly so frequent as would be required for such an effect,—the pure line remaining constant through twelve generations. Of course, if selection had continued indefinitely, some distinct genetic changes affecting the character would have been bound to occur, but not of the order of frequency of "continuous variation" (fluctuation). The reason that the inbred stock, on the other hand, was so much more plastic genetically is simply because there was in it an opportunity for the formation of all sorts of genetic types by mere recombination of the various mutant factors that had already arisen at widely separated occasions. The inheritance of truncate therefore provides no evidence even for this modified type of genetic fluctuability which might appear as the resultant of innumerable mutations in many different factors, each of which itself was highly constant. Moreover, no other cases affording evidence of such a situation have been reported thus far.

We may now sum up the causes why the truncate character, when it was analyzed, was found to depend on so many factors. (1) Two successive mutations were practically necessary, in the first place, before truncate became visible at all. (2) Since truncate depended on a lethal, and was, in addition, inconstant in somatic expression, it was subjected to selection, which perpetuated any new factors (balancing lethals and intensifiers) that still further differentiated truncate from normal. (3) Since the truncate character depended on an unstable developmental reaction, the initial appearance of such intensifiers was made more likely.

The history of the truncate stock, after the appearance of these contributing factors, consisted merely of the attempt to get the line as pure as possible for T_3', and to keep the line in the balanced lethal condition by eliminating crossovers.

THE ORIGINATION OF TRUNCATE IN OTHER STOCKS

It is not very uncommon to find truncate appearing sporadically, like a mutant, in other stocks. Thus, in DUNCAN'S (1915) search for mutations, truncate was the second most common in occurrence, beaded arising most frequently. Now, if the above account of the origin of the truncate complex has been correct, such truncate should not be provided with the accessory factors present in the selected stock, or at least not with the same ones. That this is true is indicated by the fact that often, when these truncates are bred, they fail to reappear, except in trifling proportions, in subsequent generations. The latter flies, then, if they owe their truncation to the same factor as the T_2' of truncate stock, evidently lack all the intensifiers, just as we should expect to be usual in stock not subjected to selection. In this connection, it may be noted that since the intensifiers are missing it is by no means certain that all these appearances of truncate represent separate mutations, as the factor for truncate may have been present for a long time, under such circumstances, without manifesting itself. Hence the actual mutations may really be rather rare.

Truncate has also appeared several times in stock containing factors for other characters, that at the same time intensify truncate, and these cases may with more certainty be regarded as real mutations. The composition of such truncates is accordingly more worthy of examination. One such truncate was found by MORGAN in a stock with "cut" wings; special crosses proved that it contained T_2', identical with the T_2' of truncate stock. Cut, itself, intensified the truncate, but there was no evidence of the regular intensifiers in addition. Another truncate was found by the writers in a cross between black and a stock that had been continually tested by crossing to bar. The mutant, a "long-truncate" female, was crossed to black pink, and gave offspring half of which were intermediate and the rest normal-winged. Two of the heterozygous intermediate F_1 males were then back-crossed separately to black pink. As in the similar crosses of ordinary truncate, truncate appeared only among the gray offspring (F_2), but it appeared about as often among the gray pinks as among the gray reds, giving in both classes the percentage and grade ordinarily present among the gray pinks. Thus it was evident that the intensifier T_3' was not present. The T_2' was evidently the same as that in ordinary truncate stock, for when the gray pinks $\left(\frac{T_2' B}{b} \; \frac{p}{p}\right)$ were inbred they produced 81 grays and 51 blacks, instead of a ratio of 3 : 1, thus showing that this T_2' also was lethal. There was, moreover, a fairly large percentage of crossing over between black and truncate.

We may accordingly conclude that the origin, by mutation, of the intensifiers present in truncate stock, was quite independent of the origin of T_2' itself by mutation.

APPLICATIONS OF THE METHODS

It is believed by the authors that the general method of attack developed in the truncate case,—whereby, by the use of "identifying" genes, a refractory character may be taken apart, put together, or held in a desired combination—will become of more widespread applicability as the linkage groups of the organisms commonly used for genetic study become better known. Since a large part of the work on truncate was carried out nearly seven years ago this general method has already had a chance to be tried out rather extensively in Drosophila work, and it has been used with success by students of this organism in a considerable number of problems, some of which have been reported in the literature.

In the case of other organisms, however, it will be necessary to know and have the use of identifying factors in the various linkage groups concerned, before the present method can be used at all. Moreover, certain modifications are necessary in the case of organisms that have crossing over in both sexes, and also in case the character to be studied is recessive. In either of these circumstances, it will be desirable to have two identifying factors in each chromosome involved, one on each side of the factor under investigation,—in order to make sure that the factor has not crossed over from its identifier.

Remote as the possibilities of such work may seem to be in the case of such animals as mammals, it is nevertheless difficult to conceive how the genetic bases of the more elusive and complicated characters in them can be determined adequately by any other means. Even in the case of man, an attempt in such a direction would be justified, for here the most important characters,—such as the psychological ones,—are perhaps more plastic, obscure, and complicated in genetic basis, than any others in the entire animal kingdom, and it would seem next to impossible ever to give any real Mendelian analysis of most of them without studying them by the method of linked identifying factors. In place of controlled crosses, and large families, however, reliance would here have to be placed on finding, by means of wide examination of data, a sufficient number of crosses of similar type, and then seeking in them the requisite identifying factors.

It would accordingly be desirable, in the case of man, to make an extensive and thorough-going search for as many factors as possible that could be used in this way, as identifiers. They should, preferably, involve character differences that are (1) *of common occurrence,* that are (2) *identifiable with certainty,* and that are (3) *heritable in a simple Mendelian fashion.* It seems reasonable to suppose that in a species so heterozygous there must really be innumerable such factors present, if only an examination of the inheritance of *small, definite physical traits** were made on a large scale. As the study of such factors should naturally be accompanied by an examination of their inheritance with relation to each other, a knowledge of their grouping would at the same time gradually become available. All this would of course require very detailed and intensive work (rather, perhaps, than a superficial study of numerous individuals), and as yet little work has been done that is of this character. For, hitherto, the study of factors which are inconspicuous, or unimportant in actual life, has been largely avoided, in order to make an immediate and direct attack on the more important, more difficult characters. Now, as any two parents of a human family would probably differ nearly always in a very large number of factors, it would not be at all surprising if it were found that there were usually differences in one or more identifying factors in the case of any given one of the twenty-four (±) pairs of chromosomes. Thus, if a far-reaching investigation of definite physical traits were carried out first, then, when investigation of the more difficult characters was later undertaken, the requisite identifying factors, suitably arranged in the appropriate chromosomes, would probably be found, in many crosses, ready provided for the study of the more complicated and important trait.

The implication is not intended that no results of importance in human genetics can be attained by simpler methods; it would of course be desirable to carry on such studies at the same time as the more rigorous ones, but the inconclusiveness of the pedigrees of most important human characters, when studied directly, and their resemblance to the early pedigrees in the truncate case, indicates that nothing like an adequate understanding of the intricacies of inheritance in man can be reached without some such far-reaching and difficult plan as that just outlined. Meanwhile, too, partial knowledge of "identifiers" would be of partial help, in the larger problems.

However the situation may be for human genetics, it does seem clear that in the more tractable organisms, such as the domesticated and laboratory races of animals and plants, character analysis by means of linkage studies with identifying factors will come into more general use,

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* Or chemical, such as the blood agglutinins.

both for the investigation of general problems and for the dissection of particular characters.

RESUMÉ

The general hereditary behavior of truncate

1. Truncate is a variable character, appearing in all grades between short-truncate and normal wing. Some normals are almost always thrown, even by the "best" truncates, and it was found impossible, through four years of selection, to secure a permanent stock that threw no normals, although the proportion of the normals was reduced to about 10 percent, and the average grade of the truncates which did appear was increased markedly at the same time.

2. This variation is not only somatic, but also genetic, for in the selected truncate race the high-grade truncates that appear throw relatively more and "better" truncates than do the intermediates, and the latter in turn greatly surpass the normals in this respect. It is thus possible, in the final stock, to modify the average grade and percentage of truncates back and forth by selection, within the limits above stated.

3. When truncate is crossed to the wild-type fly it behaves as an incomplete recessive, the great majority of F_1 being normal, but a small percentage of flies that show some truncation usually appearing also. The ratios in F_2 vary greatly with individual pairs of F_1 flies, ranging from nearly four normals to one with truncation through various values down to over 100 normals to one truncate, while some of the F_2 families contain no truncates at all. The percentage of truncates in F_2 is higher if the P_1 truncate was a female, but in either case the truncates in F_2 consist of both males and females in comparable numbers. The extracted F_2 truncates throw about two normals to one truncate but can often be improved by selection until they reach the limit shown by the selected stock.

4. It was found that on crossing to flies with black body-color (recessive), truncate behaves as a dominant, and manifests itself in some degree in nearly all of the F_1 flies that carry it. Two heterozygous factors, ordinarily recessive, may thus reenforce each other so that one becomes a dominant. Similar results were obtained in crosses with bar-eyed flies, and with star-eyed flies, but bar and star are both dominants themselves.

The dissociation of the truncate genotype

1. Truncate was crossed to stock having black body-color (chromosome II) and pink eye-color (chromosome III). In this cross, truncate could be used as a dominant (since black is present), and F_1 truncate males were back-crossed, separately, to black pink. In this way flies having all possible combinations of the chromosomes present in the truncate stock were obtained, and could be recognized, according to their sex, body color, and eye color, these latter being used as "identifying factors" for the chromosomes containing them. By then studying the amount of truncation in the flies having the different combinations, the effect of each of the chromosomes of the truncate stock, on the truncate character, could be determined. The following results were arrived at:

(a) There is a chief factor for truncate (T_2'), lying in the second chromosome; flies having only T_2', in heterozygous condition, and no other mutant factor for truncate, may occasionally have truncated wings. T_2' is partially dominant.

(b) The third chromosome of truncate stock also contains a mutant factor or factors for truncate, which may be designated as T_3'. This acts as an intensifier of T_2', and when it is heterozygous at least it produces no visible effect on the wing unless T_2' is present.

(c) These crosses, when made in both reciprocal forms, showed that there is a similar intensifier, T_1', in the first (X) chromosome, which intensifies truncate to about the same extent as does T_3'. When both intensifiers T_1' and T_3' are present they have a summative action. T_1', like T_3', produces no visible effect unless T_2' is present. T_1' is partially dominant, under the conditions of these experiments (with black present).

2. Crosses of truncate flies lacking T_1', obtained in these experiments, showed that the truncate character is also influenced by sex ("sex-limited"), in that it manifests itself more readily in females than in males.

3. Flies from these experiments, that were known to contain T_1' and T_3', but lacked T_2', were crossed with each other. As no wing modifications were produced, it was evident that both T_1' and T_3' together, in the absence of T_2', cannot cause truncation, or any visible effect on the wing, even when opportunity is given for them to become homozygous.

4. It was found possible to resynthesize truncate, according to calculation, from the flies of the non-truncate or imperfectly truncated classes of the dissociation experiment. Flies were chosen for mating together

which were scheduled, by their identifying factors, each to contain factors for truncate that were missing in the other. High-grade truncate then appeared in the offspring in the classes expected to contain it. This result furnished a confirmation of the conclusions from the dissociation experiment, and showed that the flies of the non-truncate classes had received the factors for truncate which they were supposed to have, and transmitted them regularly to their descendants.

5. In the crosses just mentioned, T_3' was sometimes introduced through the mother, sometimes through the father. The slightly lower grade of truncation, when T_3' is derived from the mother, indicates that T_3' is multiple in composition, consisting of two or more factors that may cross over from one another in the female.

6. Similar tests with T_2' indicated that this includes only one factor (at any rate one dominant factor).

7. It was found in later crosses that this "chief factor," T_2', is located in the "left-hand" end of the second chromosome, about 12 units to the right of star.

8. Experiments involving, in addition to black and pink, a fourth-chromosome factor, bent, showed that the truncate stock does not contain an intensifier in chromosome IV. In these latter experiments the distribution of the entire germ plasm was under observation.

Tests of the constancy of the factors for truncate

1. Individual tests were made of brother and of sister flies from the F_2 count of the "dissociation experiment," which could be seen, by their identifying factors, to have received from their father a given chromosome combination,—T_1' T_2' T_3' in the case of the sisters, T_2' T_3' in the case of the brothers. The tests showed that the differences in truncation between the flies having a given combination were not genetic; that is, the flies which receive the same chromosome combinations from their parents contain identical factors for truncate. It must be concluded from this that the factors for truncate follow exactly the distribution of the chromosomes, and that they do not undergo fluctuating variation.

2. A "pure-line" experiment was then undertaken, by repeated back-crossing of males of the above sort $(\frac{T_2'B}{b}\ \frac{T_3'P}{p})$ to black pink females. In each generation, in such a cross, males with the same truncate combination as their father are again produced, and may be recognized by their identifying characters (gray body and red eye-color). These are

then mated, as before, to black pink females from stock, and so the process may be repeated indefinitely. The danger of recombination is here excluded as effectually as in the case of self-fertilizing or asexually reproducing organisms, and so the purpose of a pure-line experiment is fulfilled. Two lines of flies were carried on in this way,—a "high" or plus-selected line, for twelve generations, and a "low" or minus line, for thirteen generations. The selection was absolutely ineffective; this corroborates the conclusion derived from tests of brothers and sisters, and shows that the factors for truncate are constant, and entirely contained in the (three large) chromosomes. It proves at the same time that the factors for truncate are not contaminated by their allelomorphs in the heterozygote.

The continual genetic variation occurring in the truncate stock must therefore be due to recombinations of factors for which the stock is perpetually heterozygous.

The cause of the inconstancy of the selected stock

1. To determine the reason for this perpetual heterozygosis of the stock, tests were undertaken to discover whether T_2' could exist homozygously. As it was next to impossible to determine this by direct tests, T_2' was placed in a chromosome with *b* (black), and flies with this combination were crossed to others having T_2' bound to *B* (gray). 72 offspring which had received at least one T_2' were then tested, by backcrossing to black, to discover whether they contained both the T_2' with black and the T_2' with gray. None of them, however, had more than one of the T_2' factors, and the ratios showed that T_2' acts as a lethal when homozygous.

Thus T_2' resembles the majority of dominant mutant factors of Drosophila in being lethal when homozygous, and pure stock of it cannot be obtained.

2. It follows that crosses of truncates should yield 1 non-viable: 2 potential truncates : 1 normal. As in the selected stock much fewer than one third of the flies that hatched were normal, tests were made to see whether any of the normals that should have occurred had been caused to appear truncate by reason of some other, recessive, "chief factor" for truncate. It was found, however, that no such factor existed; consequently the deficiency of normals was due, not to their appearing truncate, but to their actual absence,—this means that they were prevented from hatching by a lethal factor, which must have lain in the chromo-

some homologous to that bearing T_2'. Occasional crossovers between T_2' and the lethal would give rise to a few normals, however.

It is thus evident that there is in truncate stock a condition of "balanced lethal factors," similar to that existing in beaded Drosophila, Matthiola, and Oenothera.

3. Reciprocal out-crosses of truncate stock showed that T_1' was present, homozygous, in viable, fertile flies of the selected stock.

4. Crosses of flies from the dissociation experiment were arranged in such a way that T_3' was given opportunity to become homozygous, while T_1' and T_2' remained heterozygous. Many of the flies thus synthesized were as high-grade truncate as the best inbred stock, and much higher than the truncates from out-crosses. This proved that T_3', when homozygous, is not lethal, but that its effect on truncation is much stronger than when heterozygous. Females of this composition are so infertile, however, that it would be next to impossible to maintain stock pure for T_3'. This explains the occurrence of low-grade truncates, and normals carrying truncate, in the selected stock.

The origin of the truncate complex

1. Truncate is one of the most frequent characters to appear sporadically, by apparent mutation, in various stocks of Drosophila. In most of these cases, however, intensifiers of truncate are absent, and so the possibility is not excluded that truncate was present previously in the stocks, and merely failed to manifest itself before. In accordance with these circumstances it is usually found that the truncate fails to reappear in the descendants of such flies, except in a minute percentage of individuals.

When, however, truncate appears in stocks containing bar, or other factors which themselves intensify truncate, it may be concluded that truncate has here arisen as a real mutation. Several such truncates have been tested, and found to be inherited, and evidence was obtained that their mutant factor for truncate was identical with T_2', the chief factor of ordinary truncate stock. None of the intensifiers of the ordinary stock were present, however.

2. The hereditary behavior of the original truncate shows that one of the factors for truncate had been present, undetected, in the parental long-winged stock, and that the appearance of truncate was due to the origin, by mutation, of a second factor. Thus the first truncate-winged fly contained T_2' and an intensifier. The other mutant factors of the

truncate stock appeared later, and they were perpetuated through the process of selection, because they influenced the truncation in the direction desired.

It is of significance for evolution, as well as for genetics, that particular races may thus have pre-existing in them factors which by themselves are invisible, but which favor the manifestation of a certain character.

3. The occurrence of more modifiers for truncate than for most characters is explained not only by the fact that the modifiers were specially picked out by selection in the case of truncate, but also by the marked instability of the developmental reaction whereby the truncate character is produced. This instability, or susceptibility of modification, is disclosed by (1) the somatic variability of the character, when the genotype is kept constant, and (2) by the number of mutant factors for other characters which act upon truncate in addition. On account of this modifiability it should happen that relatively many of the mutant genes which arise would be able to affect truncate, and it should therefore be easier to find either intensifiers or "inhibitors" for truncate than for most characters. The reason for the readier discovery of modifiers would hence be, not that the process of mutation is influenced in some way by truncate, but, on the contrary, that truncate is especially susceptible of being influenced by mutation.

The existence, in certain races, of factors like T_2', which make a certain character (here, the wing shape) more highly modifiable, may be of particular importance in evolution.

General applications

1. The results described in the first section of this resumé would undoubtedly have been regarded by certain writers as positive proof of the ineradicable fluctuability, miscibility, or, as it were, fluidity, of a unit character. The case was, in fact, more extreme than those upon which they rely as evidence for their doctrine. And yet it has been found that the inheritance of this character is entirely confined to the recognized chromosome system, and depends on definite chromosomal factors which are neither miscible nor inconstant.

Although mutations affecting this character may occur with somewhat greater frequency than those for many other characters, the mutations are not nearly of the order of frequency which would be necessary in order for them, by themselves, to give rise directly to a true genetic fluctuability. Practically all the genetic variability and modifiability of the stock is, on the contrary, due to the occurrence of recombinations among those mutant factors which have, on rare occasions, previously arisen, and variation due to a real new mutation, although possible, is highly exceptional. Even in such cases, moreover, the mutations do not all consist in variations of some one particular factor, but they may affect any one of many factors that are concerned with the character.

The use of cases resembling the truncate case, in order to support the doctrine of fluctuating variability of single unit-factors, is therefore entirely unjustifiable, in the absence of analyses comparable with those made in this case.

2. It is believed that the general methods developed in this case will become increasingly useful with the growth of knowledge of the linkage groups in organisms. It has been shown how, by the use of "identifying factors," any given genotype may be cut up for study, put together again in various ways, or held in a particular combination for the maintenance of stock or for observations on factor constancy. At the same time the distribution among the offspring, of the entire (chromosomal) germ plasm of a parent may be completely exposed to view.

The suggestion is made that such methods may also be necessary before much progress can be made in the study of the more important characters in human genetics.

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