THE EFFECT OF LONG-CONTINUED HETEROZYGOSIS
ON A VARIABLE CHARACTER IN DROSOPHILA

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The belief that a factor may sometimes be contaminated by its allelomorph when the two meet in the hybrid has received support from Castle and from some geneticists of the non-Mendelian camp. As far as can be seen there is no reason why this might not occur, but there is no evidence for arriving at such a conclusion. The bulk of Mendelian inheritance seems to show that factors are not affected by their allelomorphs.

Bateson supposes some cases of multiple factors to be due to fractionation and that the products of this quantitative disintegration segregate independently of one another. He explains these supposed fractional degradations as due to irregularities in the segregation of the factors in the germ cells, during cell divisions in which he imagines the qualities to be sorted out each to its place. In such a case a character might become weaker and weaker as a result of continued crossing to other stocks.

The aim of the experiment considered in this paper was to contribute evidence in regard to the constancy of factors in a state of heterozygosis; and, further, to show that the apparent fluctuation in factors, which is thought by certain workers to be contamination or some sort of quantitative disintegration, can well be accounted for on other and more satisfactory grounds.

The investigation which follows is concerned with a variable wing character in ¹ which is called balloon, and the factor for which lies in the second chromosome.

The balloon factor was kept heterozygous for at least sixty generations, covering a period of nearly three years. Heterozygous males that contained the factors streak*, dachs, jaunty, curved, and balloon in one chromosome, and the factors black, purple, vestigial, arc, and speck in the other, were repeatedly crossed to homozygous females containing black, purple, vestigial, arc, and speck. All the mutants involved were recessive except streak, so that the males containing streak were easily picked out. Since there is no crossing over in the male and the females were pure for b p v a sp, the factors of each chromosome were kept together, so there were only two kinds of males and females produced in each generation, of the same types as those above described. One kind contained streak, a character on the dorsal side of the thorax, and in the other kind this character was absent. The males containing streak thus always contained the factors for balloon wing and for its normal allelomorph. The close association of these allelomorphs over such an extended period furnished an excellent opportunity for contamination if such influences occur.

*The factor streak was introduced in the way described only during the later generations.

Before the balloon character could be studied it was necessary to separate it from the other mutants and obtain flies homozygous for balloon. This process of purification was a somewhat complicated one. The accompanying diagram will serve to illustrate the method of 'freeing' the balloon factor. Males of the heterozygous type just described were crossed with normal females. The F_1 offspring were heterozygous, some containing the $S d j c ba$ chromosome and the normal chromosome. Others, containing the $b p v a sp$ and normal chromosomes, were eliminated. Males and females of the former were crossed in order to get the recessive factors together in the same fly, otherwise their normal allelomorphs would obscure them and the desired combinations could not be identified. Now, certain recombinations produced flies in the F_2 which were homozygous for balloon but heterozygous for the other factors. These flies were identified by the phenotypic appearance of the balloon and streaked factors. The other combinations were discarded. Next, males of this type $\frac{S d j c ba}{ba}$ were crossed to wild (normal) females in order to eliminate the $S d j c$. Part of the offspring (F_3) from this cross received from their father balloon but none of the other mutant factors (the balloon having crossed over). And of course they received from their mother the normal chromosome. Such flies were identified by the absence of the character streak. Those flies containing streak were discarded. These F_3 males and females that were normal in appearance were bred together and

Female normal (normal)
normal

normal

Female S d j c ba (streak) b p v a sp (normal)
 $\swarrow \searrow$ normal normal

(non-cross-overs) (cross-overs) (Discarded)

(All other combina- (tions discarded.)	Female <u>normal</u> (from <u>normal</u> wild stock)
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normal normal

Female ba (normal) S d j c ba (streak)
 normal
 (Discarded)

ba_ normal ba' normal

~~ba~~ (balloon) (Discarded)
~~ba~~

their resulting offspring (F_4) that showed phenotypic balloon were also homozygous for this character.

Now that this balloon factor which has been in a state of continued heterozygosis is free, it remains to be compared to flies which have been kept homozygous for balloon for a considerable length of time. The flies used for this comparison had been kept homozygous for an even longer time than the others had been kept heterozygous, the latter having been derived from the homozygous stock.

The next problem that confronted was to arrange the variations of the character into a consistent series. After a large number of wings had been observed seven grades were established which approximately accounted for all the varieties that had appeared. The accompanying diagrams will serve to show the nature of these grades, which are based upon the wing venation. The sorts of variation used as standards for these classes were fairly typical, and although all intermediate conditions were found there was seldom doubt in deciding to which grade a particular wing belonged. The different grades may now be considered in detail.

1. Some few wings showed no modification whatever, these were classed as normal. In all others the balloon character made its appearance near the posterior margin of the wing by an abnormality in the region of the cross-vein which connects the fourth and fifth longitudinal veins.

2. If the modification was merely a bend in the cross-vein,

it was classed as 'slight'. Likewise if the disturbance took the form of a slight projection, either proximal or distal, it was considered in the same class, or if the extra mark was apparently disconnected or removed from the cross-vein.

3. If short projections or markings appeared, both proximally and distally, they were classed as 'moderate'. Also if only one projection occurred and this was more extended than those of the class 'slight', even so much as to become branched, it was recorded in this grade.

4. If fairly extended projections in both directions from the cross-vein appeared, the wings were graded as 'marked'. Such projections were frequently branched, especially distally. Wings in which the venation in this region was confused or considerably disturbed also fell into this lot.

5. Now if a small watery blister appeared in this region of modification which occupied an area less than half that of the entire wing, it was placed in the class 'small blister'.

6. When the blister covered approximately half the size of the wing, it was classed as 'medium blister'.

7. The grade 'large blister' was established for a similar condition that occupied the entire or nearly all of the wing surface.

The type and the range of each class being fixed, the question next occurred as to what their order or relative degree of deviation from the normal might be. After a comparison of the amount of modification in each class, the order in which they have been described was adhered to, and experimental

1. Normal.

2. Slight.

3. Slight.

2. Slight.

3. Moderate.

3. Moderate.

4. Marked.

4. Marked.

4. Marked.

5. Small Blister.

6. Medium Blister.

7. Large Blister.

support for it was also found and will be described presently.

The method of observing and recording the variations was carefully considered so as to avoid bias. The flies of either the stock (of homozygous ancestry) or outcrossed (of heterozygous ancestry) were inconspicuously marked by amputating a small portion of one member of the front pair of legs. The front pair was chosen because these legs usually project backwards beneath the body rendering them less obvious, and secondly because these were most easily accessible. The 'branded' flies were thoroughly mixed with those to be compared before the record was taken. Each wing was first graded and then identified as stock or out-crossed type. In this process of classification a diagram of the grade types was constantly referred to so as to make the judgment as uniform as possible. Each wing was recorded separately, there being no satisfactory way in which to determine the variation of the fly as a whole.

As far as possible the crosses for comparison were started on the same day, using food from the same batch of the same age and consistency, and placed in the same conditions of environment. Approximately the same amount of food was used in each case; and with the exception of a few instances the same number of parents were allowed to breed in each bottle and have access to this food.

The food and rearing problem was without doubt the most difficult question encountered. It being necessary to start many crosses in order to insure a sufficient number of offspring. Unfortunately, owing to the infertility of the flies, it was

often impossible to obtain counts on the same date from stock and outcrossed bottles that had been started simultaneously and with similar food. But the results include observations on a number of bottles and are consistent enough to show that variations in the character due to environmental differences between the bottles were not responsible for the main outcome of the comparison.

The flies for each day were recorded in the correlation table, one mark representing both wings, the modification in the right wing being placed in its proper column within the row labeled for the modification of the left. An arrangement of the counts in this way gave a correlation between the right and left wings which justified the order in which they were previously classified. For example that 'small blister' is a greater deviation from normal than is 'marked' is proved by the fact that 'small blister' (in one wing) shows a greater tendency to be associated with 'marked' (in the other wing) than with 'moderate'. This may be seen by consulting the table. Similarly each of the other grades may be proved to have the order in the series which has been assigned to it. Exception must be made in the case of the last grade 'large blister'; but in this case the numbers are not large enough to be significant, and it seems fairly obvious that the 'large blister' represents a greater deviation from normal than medium blister.

The accompanying table contains a record of the stock and outcrossed types treated separately. The offspring were kept

Normal Slight Moderate Marked Small B. Med.B. Large B.

Normal	122	28	1	0	0	1	0
Slight	14	190	38	4	1	3	0
Moderate	2	53	130	45	8	4	3
Marked	0	5	23	204	36	20	20
Small B.	0	2	6	30	77	36	17
Med. B.	0	0	3	20	28	36	4
Large B.	0	1	1	12	21	3	3

one day after being taken from the bottles, therefore both types were of the same age when graded. The record shows a comparison of the two types. The grades are numbered and the averages refer to the mean grade of each lot counted. All wings produced by bottles that were started on a certain date (with similar food) are grouped together.

It will be seen from these figures that the outcrossed type which has been kept in a state of continued heterozygosis deviates further from normal than does the stock. The average grade of the former is 4.2, which places this type in 'marked', and the average grade of the stock is 3.0, or 'moderate'.

The figures show that in twenty-five cases the stock is weaker than the outcrossed type. In only five cases the outcrossed is weaker than the stock, and in one case both are of the same value. In four of these six cases the number of outcrossed flies (1, 2, 3, & 4) was too small to be of any significance at all. In the other two cases the number was somewhat larger than above, although still rather small, but here there is very little or no difference between outcrossed and stock (-.2 and 0).

By comparison of the lots that were started on different days in either stock or outcrossed, it is quite evident that there is variation in the character (presumably due to environment) quite apart from any difference in the stock and the outcrossed. The differences between the latter two types, however, are too consistent to be due to the same cause.

Neither is the difference due to contamination from the

A. STOCK

Date started	Date graded	Nor.	Sli.	Mod.	Mar.	S.Bl.	M.Bl.	L.Bl.	Tot.	Aver.
Apr. 30	May 15									
	" 16									
	" 17									
	" 18									
	" 19									
	" 20									
May 1	May 15	2	18	26	6	3	1	2	58	3.0
	" 16	2	5	1	1	4	1	0	14	3.3
	" 17	2	6	2	1	1	0	0	12	2.4
	" 18	0	4	2	5	2	0	1	14	3.6
	" 19	0	2	1	7	4	0	0	14	3.9
	" 20	2	2	0	0	0	0	0	4	1.5
		8	37	33	20	14	2	3	116	3.1
May 2	May 15	9	29	11	10	6	3	0	68	2.7
	" 16	19	63	23	19	2	3	3	132	2.5
	" 17	28	51	15	20	4	5	1	124	2.5
	" 18	19	19	11	6	0	1	1	58	2.1
	" 19	15	18	9	7	5	2	5	60	2.9
	" 20	12	13	8	5	4	3	1	46	2.9
		102	193	77	67	21	17	11	488	2.6
May 3	May 15	12	15	29	24	2	0	0	82	2.8
	" 16	34	43	39	19	4	3	0	142	2.4
	" 17	14	39	16	14	3	5	1	92	2.5
	" 18	6	29	9	17	2	0	1	64	2.7
	" 19	16	22	13	17	1	4	1	74	2.7
		82	148	106	91	12	12	3	454	2.6
May 5	May 18	0	6	17	15	11	12	5	66	3.4
	" 19	0	8	6	1	3	0	0	18	2.9
	" 20	0	4	4	3	4	2	1	18	3.9
	" 21	0	3	4	7	4	0	0	18	3.6
	" 22	0	2	1	6	1	0	0	10	3.6
	" 23	6	2	2	1	5	0	0	16	2.8
	" 24	0	0	0	1	7	2	0	10	5.1
		6	25	34	34	35	16	6	156	3.8
May 6	May 18	8	3	4	3	1	2	1	22	2.8
	" 19	7	9	7	2	2	2	3	32	3.0
	" 20	2	7	10	1	1	0	1	22	2.8
	" 21	3	3	3	4	0	0	1	14	2.9
	" 22	4	1	1	2	0	4	0	12	3.4
	" 23	1	2	1	13	4	4	1	26	4.2
	" 24	0	0	0	3	0	0	1	4	4.7
		25	25	26	28	8	12	8	132	3.2
May 13	May 24	12	0	10	13	31	14	4	84	4.2
	" 25	2	6	7	11	2	4	0	32	3.2
	" 26	4	5	13	12	5	6	1	46	3.4
	" 27	10	23	25	24	1	11	4	98	3.3
	" 28	9	7	16	16	4	4	4	60	3.4
	" 29	0	0	0	2	5	1	2	10	5.3
		37	41	71	78	48	40	15	330	3.7
		260	469	346	318	138	99	46	1676	3.0

B. OUTCROSSED

Nor.	Sli.	Mod.	Mar.	s.Bl.	M.Bl.	L.Bl.	Tot.	Aver.	Diff. (B-A)
0	4	17	31	4	1	1	58	3.7	
1	3	6	5	1	0	0	16	3.1	
3	13	14	27	2	1	0	60	3.2	
2	0	3	0	9	4	0	18	4.4	
0	0	6	2	5	3	2	18	4.6	
0	0	0	2	1	1	0	4	4.7	
6	20	46	67	22	10	3	174	3.6	
0	3	1	5	1	1	1	12	3.9	0.9
0	0	1	4	0	1	0	6	4.1	0.9
0	2	0	2	0	0	0	4	3.0	0.6
0	0	1	3	2	0	0	6	4.1	0.5
0	0	1	3	2	0	0	6	4.1	0.2
0	5	4	17	5	2	1	34	3.9	0.7
2	0	0	2	2	0	0	6	3.3	0.6
1	1	2	0	0	0	0	4	2.2	-0.6
0	0	1	1	0	0	0	2	3.5	1.0
0	2	0	0	0	2	0	4	4.0	1.9
3	3	3	3	2	2	0	16	3.2	0.6
0	0	0	1	0	0	1	2	5.5	2.7
0	0	0	0	2	0	0	2	5.0	2.3
0	0	0	1	2	0	1	4	5.2	2.6
2	5	1	16	7	5	2	38	4.1	0.7
1	8	5	12	6	4	0	36	3.7	0.8
0	1	2	4	4	2	3	16	4.8	0.9
1	1	0	5	1	0	0	8	3.5	-0.1
0	0	2	8	0	2	0	12	4.0	0.4
0	0	1	8	4	4	3	20	5.0	2.2
0	0	0	2	0	0	0	2	4.0	-1.1
4	15	11	55	22	17	8	132	4.2	0.4
3	1	2	30	29	13	4	82	4.6	1.8
0	2	4	17	12	5	2	42	4.4	1.4
0	0	4	2	13	4	7	30	5.2	2.4
1	1	0	15	17	8	4	46	4.8	1.9
0	0	0	1	7	4	0	12	4.9	1.5
0	0	0	4	8	7	1	20	5.2	1.0
0	0	0	2	2	2	0	6	5.0	0.3
4	4	10	71	88	43	18	238	4.8	1.6
0	1	3	7	4	3	0	18	4.2	0.0
6	5	6	11	4	0	0	32	3.0	-0.2
3	1	9	14	24	3	6	60	4.4	1.0
3	3	3	25	10	8	2	54	4.2	0.9
0	1	1	33	15	5	3	58	4.5	1.1
0	0	2	1	2	1	0	6	4.3	-1.0
12	11	24	91	59	20	11	228	4.2	0.5
29	58	98	305	200	94	42	826	4.2	1.2

normal allelomorph, otherwise the outcrossed would be nearer the normal than the stock. We find, on the contrary, that the outcrossed type is really better balloon than the stock. Moreover the standard deviation of the stock is 1.55 grades, and of the outcrossed is 1.37. Hence there has been no increase in the variability in the outcrossed. The variability of the stock is greater and therefore gives additional evidence that there has been no contamination or frationation.

The difference, however, must be due either to variation in the balloon factor itself (multiple allelomorphs) or to a difference in modifying factors (multiple factors). Assuming that variation of one type or the other could occur at all, natural selection would tend to cause the races finally to differ in the direction found. For the wings that made for stronger balloon, being inadequate for efficient flight, would tend to be eliminated. Also the watery blister, which usually bursts, causes the fly to adhere to the sides of the bottle and perish. This inbred stock has had an excellent opportunity to accomplish these results in a period of three years, but the outcrossed did not have a chance for such selection, since balloon is recessive and was kept hidden during the state of heterozygosis.

Tests could be made to determine definitely whether it is due to multiple allelomorphs by the use of linkage experiments, since the knowledge which has already been obtained of linkage groups in . is adequate for this purpose. It is important to know whether this difference is due to multiple

allelomorphs or multiple factors. For if it were found to be due to multiple allelomorphs the further question would be raised as to whether it is due to one or two mutations in this factor for balloon or whether it is due to natural selection working on continual fluctuations in this factor. There is no evidence at present to show whether the latter is true, but this question too could be determined by certain breeding tests.

Another experiment was made with the curved wing character in _____, which was treated in the same general way. The factor curved (c), like balloon, was in the chromosome S d j c b: that was subjected to the long-continued outcrossings. After it was purified by a series of crosses in a manner similar to those made in 'freeing' balloon, it was compared to stock which had been kept homozygous for an extended period. Owing to the infertility of the curved wing flies the number of resulting offspring was small. But the results corroborate those already given for the balloon character. This character appears as a convexity throughout the general surface of the wing and the grades are based on the degree of variation found. After careful observation these were determined as follows: (1) 'Slight curvature', (2) 'less moderate ~~ture~~ curvature', (3) 'more moderate curvature', (4) 'marked curvature', (5) 'spongy wing'. Of those started on the same date with similar food, there were only two strictly comparable lots (made up and counted on the same day). Owing to the small numbers of stock offspring, an extra lot of stock flies started on a different day was counted.

The following data were obtained:

	Date	1	2	3	4	5	Total	Average grade
	started	Slight	Less mod.	More mod.	Marked	Spongy		
Total stock	May 2	1	4	2	2	1	9	2.5
Total outcrossed	May 2	2	5	16	14	1	37	3.1
Total stock	May 5	4	31	28	10	3	73	2.6

Standard deviation of stock, .81; of outcrossed, .84 grades

In the totals and averages the number of spongy winged flies were not included, because the position of this grade in the series was uncertain, but the number of these flies was too small to be of any account. Here, too, the rather unexpected result was obtained, wherein it was found that not only was there no evidence of contamination but the outcrossed was, if anything, less normal than the stock.

A table is also given showing the correlation between the right and left wings:

Right.						Left.
	Slight	Less mod.	More mod.	Marked	Spongy	
Slight	4	2	0	0	0	
Less moderate	0	14	8	4	0	
More moderate	0	6	17	3	1	
Marked	0	3	1	13	1	
Spongy	2	0	2	0	1	

It should be mentioned that the same result had previously been obtained in the case of the character dachs legs, when comparisons were made between stock flies and outcrossed flies derived from the race $\frac{S d j c}{b p v a} ba^*$.

CONCLUSION

In conclusion it may be said that the evidence thus far shows that characters are variable and may change without any artificial selection, but in the case of the characters dealt with in this paper at least/^{the}variation that was observed was certainly not due to contamination or fractionation. This was true in spite of the fact that the factors had been kept in a state of heterozygosis for over fifty generations. If the balloon and the other characters observed had had a higher survival value instead of a lower, they might have varied in the opposite direction from that observed and the results might then have been mistakenly attributed to contamination.

It is important not to accept results apparently showing contamination or fractionation of factors at their face value without a thorough factorial analysis.

*Muller, H. J. The Mechanism of Crossing-Over. American Naturalist, July, 1916.