THE INHERITANCE OF THE MUTANT CHARACTER "VORTEX"

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INTRODUCTORY

There seems to be an impression that the great majority of mutants of Drosophila are comparatively simple in their genetic behavior. Because of their superior usefulness the mutants whose inheritance is clean-cut have been practically the only ones employed in the experiments for the analysis of genetic phenomena. Other mutants occur, and not infrequently, which must be made the objects rather than the tools of investigation. In the following paper is given an account of such a character, "vortex."

ORIGIN OF THE VORTEX CHARACTER

In looking over the "California wild" stock of *Drosophila melanogaster* in November 1913, an occasional fly was found which showed on the thorax a pair of "rosettes"; that is, in the areas lateral to the dorso-central bristles the microchaetae or small hairs were arranged in a pair of whorls. Specimens of this character were noticed on other occasions, but no breeding work was done until August 7, 1916, when a female was found that had in addition to the posterior rosettes an anterior pair. This female gave rise to the stock "vortex," with which the present work has been done.

DESCRIPTION OF THE VORTEX CHARACTER

In appearance and in degree of development the vortex character is quite variable. In the modal condition (figure 1*b*) two brown-pigmented spots are present, located lateral to and midway between the anterior and posterior dorso-central bristles. The pigment lies in the walls of an indentation or funnel that extends more or less deeply into the thorax. The microchaetae for a considerable area around this focus are arranged in a whorl. Also the dorso-central bristles, especially the anterior pair, are involved in the whorled formation.

In more pronounced specimens (figure 1*c*) these vortices are much more conspicuous and the central funnel is partly evaginated like the finger of a glove, or even wholly evaginated into a horn-like elevation. In these extreme specimens an anterior pair of whorls is also developed. These anterior vortices are never as pronounced as the posterior ones.

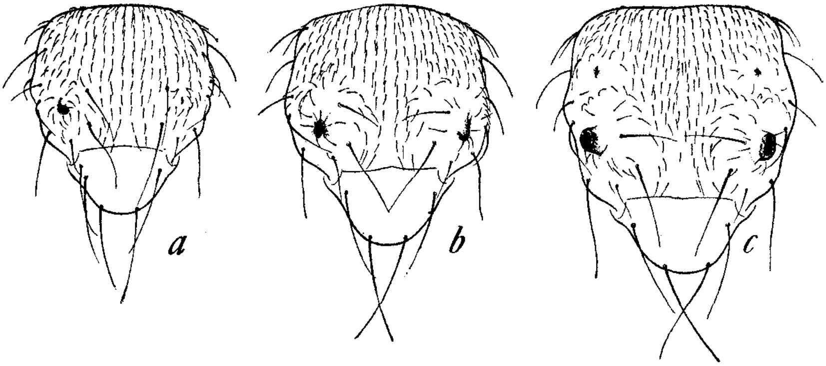

FIGURE 1.—The mutant character vortex. *a* represents a "slight" type (♂) with no anterior vortices; *b* represents the "modal" type (♀) with conspicuous funnel-like vortices; *c* represents an "extreme" type with everted funnels, and an anterior pair of vortices. *a* shows a symmetrical development that is fairly common.

In the "slight" cases (figure 1*a*) only the rosette-like arrangement of the hairs, or only a small brown pigmented depression indicates the character. A very small proportion of the individuals, genetically the same as the others, entirely fail to show the character, and are somatically normal.

All intergrades between this complete absence of the character and its fullest expression are met with in the same culture, although the bulk of the individuals are of the "modal" type.

In general the degree of development is approximately the same on the two sides. But within all grades there may be asymmetrical development of the character, so that the anterior vortex on one side may be absent giving a tri-vortex condition; also uni-vortex individuals are not uncommon.

The character is sex-limited to a considerable degree; i.e., females exhibit a higher grade of the character than do the males. The slight grades are preponderantly males and those few individuals that fail to show the character are practically always males.

The vortex flies do not differ from wild flies in any other respect that we have observed, and they are of good viability.

THE BIGENIC NATURE OF THE VORTEX CHARACTER

The first test usually made with a mutant character is the determination of the chromosome to which its gene belongs. The most approved method for doing this is to cross the flies in question to flies carrying the dominant characters star and dichaete, star being in the second chromosome (at 0.0) and dichaete in the third (at 11.7). By means of back-cross tests of the F_1 star dichaete males it is possible to ascertain whether the gene for the mutation lies in the second or the third chromosome or whether it is independent of both. The star dichaete back-cross method takes advantage of the fact of no crossing over in the male. Thus, if the gene for the mutant in question is in the second chromosome all the back-cross progeny will fall into two classes, namely, those showing star and those showing the mutant, with a total absence of individuals showing both star and the mutant, or, conversely, neither.

Accordingly, the original vortex female was at once out-crossed to a star dichaete male. Apparently this female was non-virgin and had already been fertilized by a brother heterozygous for vortex, since of the F_1 offspring, 17 showed the character vortex while the remaining 61 did not show vortex but did show the star and dichaete in all combinations but with some excess in the wild-type class(culture 4955, Aug. 17, 1916).

The fact that criss-cross inheritance was not shown—that among the F_1 males none of the stars or dichaetes showed vortex—proved that the character is not sex-linked.

The fortunate presence of the vortex flies in the F_1 culture bottle gave immediate materials for making the desired back-cross test. Four back-cross cultures were raised from matings between sister vortex females and F_1 males heterozygous for star, dichaete and vortex.

TABLE I

P_1 mating, star dichaete male by vortex female; back-cross mating, vortex female by F_1 star dichaete male.

1916 Aug. 31		Vortex	Vortex star	Vortex dichaete	Vortex star dichaete	Wild-type	Star	Dichaete	Star dichaete
5056	♀	16	—	—	—	2	11	12	16
	♂	6	—	—	—	11	12	12	22
5077	♀	12	—	1	—	—	18	10	21
	♂	6	—	—	—	1	17	5	18
5078	♀	18	—	—	—	27	11	13	26
	♂	15	—	—	—	18	22	10	20
6126	♀	13	—	1	—	13	2	9	9
	♂	16	—	—	—	7	9	14	20
Total	♀	59	—	2	—	42	42	44	72
	♂	43	—	—	—	37	58	41	80
Grand total		102	—	2	—	79	100	85	152

The first point observed was that none of the back-cross star flies showed the vortex character. This linkage between star and vortex indicated that a second-chromosome recessive was essential for the production of vortex. Aside from two female exceptions none of the dichaete flies was vortex; by the same reasoning it is then obvious that the production of vortex depends also upon the action of a third-chromosome recessive gene. That is, the vortex character is the product of the simultaneous action of two independent genes, one in the second and one in the third chromosome.

If the above hypothesis is correct the vortex class should appear as one quarter of the offspring of such a back-cross test. In fact, the vortex individuals totaled 104 out of 520 or 21.2 percent, which is a fairly close approximation to the 25 percent expected. Furthermore, taking account of the fact that there is no crossing over in the male the back-cross flies should be in the ratio 1 vortex : 1 star : 1 dichaete : 1 star dichaete, which is approximated in the observed ratio 102 vortex : 100 star

: 85 dichaete : 152 star dichaete, although the star dichaete class is too large.

There were two further points in which the results failed to agree with the simple explanation thus far suggested. Two dichaete vortex females occurred, which were explained by the assumption that occasionally an individual homozygous for vortex II and heterozygous for vortex III shows the character. Later work has confirmed this hypothesis, for it has shown that the vortex III gene is not strictly recessive in flies homozygous for vortex II. The second point is the occurrence of the large and unexpected class of wild-type flies. An explanation for some of these wild-type flies was obtained when vortex flies were bred together (table 2).

TABLE 2

Progeny from crosses of vortex female by vortex male.

1916, Sept. 20	Vortex ♀	Wild-type ♀	Vortex ♂	Wild-type ♂
5255	98	1	84	19
5271	99	1	95	6
5458	12	—	10	—
5466	2	—	5	—
5467	8	—	2	—
5468	10	—	2	—
5469	17	—	17	—
5676	55	1	45	13
5701	27	—	45	—
5702	37	—	44	5
5867	64	2	59	10
Total	429	5	391	53

In making the counts of table 2 it was apparent that especially in the males a considerable proportion (about 20 percent), showed the character to a very slight extent. It is probable that some of the so-called wild-type flies in the back-cross counts of table 1 would have shown the vortex in this slight form had they been classified with knowledge of this point. However, even with close examination certain flies of the "pure cultures" of table 2 failed to show the character. In this connection an interesting fact was noted; namely, that those few wild-type females which occurred were among the last to hatch. Thus, in cultures 5255, 5271, and 5676 the single wild-type females occurred in the last day's count. A similar phenomenon was noted with regard to the males. The first flies to hatch were all quite extreme vortexes. Somewhat later the males were less extreme on the average, and an occasional wild-type male occurred. In those cultures in which a large number of wild-type males appeared the majority were in the latest counts.

But that the above points do not entirely explain the back-cross results is apparent when it is realized that in two of the cultures (5078, 6129) there were many wild-type females. It seems probable from later work that in these two cultures the vortex mother was of the type heterozygous only for the vortex III gene, in which case the number of wild-type and of vortex offspring should be about equal.

THE LOCI OF THE VORTEX GENES

The same mating which gave material for the above back-cross tests of the male, gave an opportunity to test at once the question of the localization of the two genes concerned. By crossing the F_1 star dichaete females from the cross of vortex by star dichaete, to vortex males the amount of crossing over between star and the second-chromosome gene, and at the same time the amount of crossing over between dichaete and the third-chromosome gene, could be found. Out of the more than twenty such cultures that were started only three were successful (table 3).

The results of the female back-crosses parallel quite closely those of

TABLE 3

P_1 mating vortex female by star dichaete male; back-cross mating, F_1 star dichaete female by vortex male.[1]

1916 Aug. 28	v_o	S v_o	D v_o	S D v_o	+	S	D	S D
5047 ♀	16	—	8	1	2	15	7	11
5047 ♂	6	—	1	—	2	15	7	11
5254 ♀	3	—	2	—	1	10	—	9
5254 ♂	8	—	1	—	2	3	4	6
5866 ♀	11	—	2	—	2	5	17	13
5866 ♂	12	1	6	—	—	6	13	22
Total ♀	30	—	12	1	5	30	24	33
Total ♂	26	1	8	—	4	16	29	42
Grand total	56	1	20	1	9	46	53	75

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[1] The symbol for the dominant character star is the capital letter S; that for the designated v_o; the vortex gene situated in the second chromosome is $v_{o\text{II}}$, that in the third is $v_{o\text{III}}$. The + sign is read "wild-type."

the male back-cross of table 1, except that apparent crossovers occurred. The small number of wild-type flies, especially males, in these cultures indicates that there was very slight changing over of vortex into wild-type, and it is probable that these wild-type flies, certainly the females, represent crossing over between star and vortex II. A calculation of the location of the second-chromosome vortex gene gave a star vortex crossover value of about 10.

There was uncertainty with respect to the dichaete vortex class, since in the male tests of table 1, where there was no crossing over, there had appeared two such dichaete vortex females. A calculation made on the uncertain basis that the dichaete vortex flies were crossovers gave a total of 21 vortex dichaete among the 78 vortex or 27 percent of crossing over. It was accordingly decided as possible that the third-chromosome gene for vortex lies to the right of dichaete at a position near to the center of the third chromosome.

It had been hoped to get far more adequate data with respect to the location of the second- and third-chromosome genes. But an unexpected obstacle presented itself. It was only with the greatest difficulty that the cultures involving vortex could be reared. The back-cross tests of the female were started on a large scale but of the more than twenty cultures all but three proved sterile, and these three produced relatively few flies. The same low productivity had been apparent in some of the pair cultures of vortex by vortex of table 2.

From the preceding considerations it was apparent that several points were capable of further elucidation. The occurrence of the class of wild-type flies in the back-cross tests of the male (table 1) was not accounted for beyond question. The appearance of dichaete vortex flies, which simulated crossovers in the male back-cross test, had received an explanation requiring experimental tests. It seemed possible that sterility was in some way connected with the above aberrations. Furthermore, a case ("pale") had arisen the characteristics of which gave a suggestive parallelism with the vortex case, namely, simultaneous linkage to both the second and the third chromosome, the appearance of unexpected classes, and of lethal effect. The analysis of that case had led to the hypothesis that a piece of the second chromosome had been removed and had been attached to the middle of the third chromosome. The removal of the piece of the second chromosome (deficiency) gave the effect of a lethal located in the second chromosome. The attachment of that piece to the third chromosome (duplication) explained the linkage of the contained genes to the third chromosome genes. The place of attachment of this "transposed" piece was apparently at the middle of the third chromosome, which is the place of the spindle-fiber attachment. If the simultaneous linkage of vortex to the second and to the third chromosome was based on some such "transposition" then the transposed piece should likewise be attached to the spindle fibre and the linkage of vortex should correspond to a locus at the middle of the third chromosome. The preliminary calculation had suggested that this indeed was the case since there seemed about 27 percent of crossing over between dichaete and vortex, dichaete being known to be some 25 units from the center of the chromosome. A test of the above points demanded first of all a repetition of the original experiments on a larger scale and with close attention to the questionable features.

TESTS OF THE WILD-TYPE FLIES OF VORTEX STOCK

The first point tested was the assumption that the wild-type flies that occurred in the stock of vortex were simply fluctuants and were of the same genetic constitution as those flies which showed the character. A pair of such wild-type flies (M251) gave 62 vortex individuals and only two wild-type individuals, which were males. This was an entirely regular result comparable with the progeny given by vortex pairs. A second pair (M312) gave 71 females all of which were vortex and only four wild-type males among the 65 males of the culture. A pair of extreme vortex individuals mated at the same time gave 91 vortex and 13 wild-type individuals (M236). From these tests it is apparent that flies of the stock may give the same results irrespective of their grade, that is, of their somatic appearance.

REPETITION OF THE MALE BACK-CROSS TEST WITH STAR DICHAETE

The second test was a repetition of the male back-cross which had given the numerous wild-type flies. Ten such cultures were raised with no sterility (table 4).

During the classification of the flies of table 4 particular attention was paid to the classes which had caused confusion before, namely the wild-type and the dichaete vortex classes, in order that none of the flies classed as wild-type should show vortex even slightly, and likewise that all of the dichaete flies which showed the vortex might be separated out.

The first point which appeared in the new cultures was that the number of wild-type flies in no case exceeded the number which could read-

TABLE 4

P_1 mating, vortex female by star dichaete male; back-cross mating, vortex female by F_1 star dichaete male.

1918 July 9		v_o	S v_o	D v_o	S D v_o	+	S	D	S D
M337	♀	21	—	—	—	—	20	18	39
	♂	20	—	—	—	3	21	16	28
M338	♀	18	—	—	—	—	21	23	15
	♂	10	—	—	—	9	10	11	12
M339	♀	28	—	7	—	—	26	17	22
	♂	12	—	—	—	1	35	25	24
M340	♀	15	—	—	—	—	23	18	30
	♂	21	—	—	—	1	18	22	23
M341	♀	21	—	3	—	—	31	14	20
	♂	20	—	—	—	2	18	23	26
M347	♀	29	—	3	—	—	30	19	28
	♂	25	—	—	—	2	21	22	26
M348	♀	26	—	3	—	—	21	15	19
	♂	23	—	—	—	1	34	20	29
M350	♀	12	—	1	—	—	14	13	16
	♂	10	—	—	—	—	15	11	18
M354	♀	18	—	8	—	—	17	10	12
	♂	17	—	—	—	3	15	14	22
M356	♀	26	—	2	—	—	17	20	26
	♂	20	—	—	—	1	21	20	27
Total	♀	214	—	27	—	—	220	167	227
	♂	178	—	—	—	23	208	184	235
Grand total		392	—	27	—	23	428	351	462

ily be explained by the amount of overlap of vortex into normal. This wild-type class was further characterized by being entirely confined to the males, not a single wild-type female having occurred. As had been discovered in the case of the wild-type fluctuants in the vortex stock these wild-type males were in general restricted to the late counts.

The sums of the wild-type and the vortex flies gave a class of the same size as each of the other expected classes and not a large excess as had been the case in the two exceptional cultures of table 1. Definite proof in the case of two of the wild-type flies that they were genetically vortex was furnished by crossing them to vortex females from stock. One of the tested males gave 62 vortex females, 79 vortex males and only three wild-types which were males (M431). The other gave 32 vortex females and 21 vortex males with no wild-type flies (M440).

The apparent crossover class of dichaete vortex reappeared in the new experiment, but was entirely confined to the females. It had been suggested on the basis of the former experiments that these dichaete vortex flies were not homozygous for both vortex genes, but were homozygous for the second-chromosome gene and only heterozygous for the third-chromosome gene. If this were true such flies should give, when tested by homozygous vortex males, about half of the offspring vortex and half not vortex. Were they really homozygous for both genes, almost all of the offspring ought to be vortex as in a stock culture. One such vortex dichaete female (from M354) was accordingly tested by crossing to vortex males from stock. The offspring (M396) were: v_o♀ 29, +♀ 0, D♀ 41, Dv_o♀ 1, v_o♂ 26, +♂ 6, D♂ 39, Dv_o♂ 0. That is, the dichaete-bearing third chromosome did not carry the vortex III gene, and approximately half of the flies were vortex instead of nearly all being vortex. This result proved that it is possible for a female only heterozygous for the third-chromosome vortex gene to show the character when homozygous for the second-chromosome gene. Furthermore the single dichaete vortex female which occurred in the above test culture was of the same constitution as her mother as was proved by the progeny obtained by crossing her to a vortex male from stock. The progeny were: v_o♀ 76, +♀ 0, D♀ 70, Dv_o♀ 15,v_o♂ 83, +♂ 3, D♂ 83, Dv_o♂ 0, among which progeny the vortex constituted 174 out of 330 flies.

Since the suggested explanation has proved to be correct, an interesting comparison between this dichaete vortex and the wild-type class presents itself. All the wild-type flies were male, but the dichaete vortex flies were without exception female. While this is apparently an inverse relation it is in reality an expression of a single phenomenon—the partial sex-limitation of the character. This sex-limitation permits a readier and more marked expression of the character in the female than in the male, both in the double homozygous condition and also in the special case of the heterozygote just considered.

LOCATION OF THE VORTEX GENES THROUGH LINKAGE TESTS WITH STAR AND DICHAETE

The first experiment to locate the vortex genes more accurately was a repetition of the female back-cross test with star and dichaete (table 5).

The results to be expected from the female back-cross are much more complex than those that form the male tests; in addition to the changing over of vortex into wild-type (♂♂) and the presence of supernumerary vortexes of the heterozygous type (♀♀), in the case of the female test

TABLE 5

P_1, vortex ♀ × *star dichaete* ♂ ; *F_1 star dichaete* ♀ × *vortex* ♂.

1918 July 14		v_o	S v_o	D v_o	S D v_o	+	S	D	S D
M359	♀	34	10	7	1	2	30	26	35
	♂	36	3	—	—	5	32	32	27
M366	♀	34	1	1	—	5	38	47	44
	♂	28	4	—	—	8	37	48	43
M367	♀	36	5	13	2	7	28	39	38
	♂	40	2	—	—	3	33	48	59
M368	♀	40	3	8	1	3	41	29	52
	♂	38	2	—	—	8	44	60	37
M397	♂	27	2	10	2	7	26	40	35
	♀	37	3	—	—	5	27	39	32
M398	♀	16	1	7	5	2	11	15	24
	♂	16	2	—	—	2	17	16	13
M399	♀	44	—	10	2	2	36	29	41
	♂	31	7	—	—	8	37	42	35
M400	♀	32	5	11	—	5	39	26	35
	♂	35	2	—	—	9	36	36	39
M401	♀	22	3	6	1	4	17	26	27
	♂	27	1	—	—	3	13	27	20
M402	♀	31	2	1	—	2	26	25	33
	♂	38	7	—	—	5	27	27	31
M425	♀	22	2	—	—	2	13	11	26
	♂	8	1	—	—	1	13	13	19
M426	♀	23	4	1	—	2	29	32	34
	♂	37	5	—	—	6	35	45	39
M427	♀	42	2	—	—	2	36	30	41
	♂	29	2	—	—	3	37	36	47
M428	♀	31	—	—	1	4	45	29	40
	♂	41	4	—	—	8	40	35	32
M429	♀	37	4	2	—	3	28	41	36
	♂	19	4	—	—	4	33	57	37
Total	♀	471	44	77	15	52	443	445	541
	♂	460	49	—	—	78	461	555	510
Grand total		931	93	77	15	130	904	1000	1051

crossing over gives classes identical in appearance but different in their genetic origin. Thus the non-vortex classes are each composed, theoretically, of progeny from three sources according to whether they represent crossing over in the second, the third, or in both the second and the third chromosome, as may be seen from table 6.

A fortunate simplification of this problem is obtained from a con-

TABLE 6

Classes of eggs produced by a female of the type, $\frac{S}{v_{oII}}\ \frac{D}{v_{oIII}}$, *and of offspring when such eggs are fertilized by sperm of a vortex male.*

Non-crossover (A)				Crossover in II (B)				Crossover in III (C)				Crossover in II and III (D)			
S D	v_{oII} v_{oIII}	S_o v_{oIII}	v_{oII} D	Sv_{oII} D	+ v_{oIII}	Sv_{oII} v_{oIII}	+ D	S Dv_{oIII}	v_{oII} +	S +	v_{oII} Dv_{oIII}	Sv_{oII} Dv_{oIII}	+ +	Sv_{oII} +	+ Dv_{oIII}
S D	v_o	S	D	S D	+	S v_o	D	S D	+	S	D v_o	S D v_o	+	S	D

		♀♀	♂♂			♀♀	♂♂
Vortex	= A =	471	460	Wild-type	= B+C+D =	52	78
Star vortex	= B =	44	49	Star	= A+C+D =	443	461
Dichaete vortex	= C =	77	—	Dichaete	= A+B+D =	445	555
Star dichaete vortex	= D =	15	—	Star dichaete	= A+B+C =	541	510

sideration of the dichaete and the star dichaete classes. Among the males not a single vortex individual of these classes appeared. Since the changing over of genetically vortex into somatically not-vortex is very slight throughout this experiment, this complete absence of males of these classes means that none or only a negligible amount of crossing over occurred between dichaete and the third-chromosome vortex gene.

We must omit from our calculation of the amount of crossing over between dichaete and vortex III the data from the females, since in the females it is known that individuals simply heterozygous for vortex III $\left(\frac{v_{oII}}{v_{oII}}\ \frac{v_{oIII}}{+}\right)$ in some cases show the vortex character. In fact if crossing over between dichaete and vortex III is as rare as is indicated by the male data then all or practically all of the dichaete vortex and star dichaete vortex females were of this heterozygous type. Two of these dichaete vortex and two star dichaete vortex females were tested by crossing to vortex males, and in all cases they proved to be of the heterozygous type (tables 7 and 8).

The complete absence of dichaete vortex males in table 5 is in contrast with their occurrence in the similar experiment of table 2. Instead of quite free crossing over between dichaete and vortex III, as at first supposed to be the case, there is no good evidence of any crossing over at all between them. The dichaete vortex males of table 2 must then have been due to another cause, and certain similar results, to be described in a later section,

TABLE 7

Tests by vortex males of dichaete vortex females from the female back-crosses of star dichaete by vortex.

	v_0	+	D	$D v_0$
M445 (ex 397) ♀	60	—	38	32
♂	68	—	87	—
M446 (ex 397) ♀	78	—	45	30
♂	88	1	93	1

TABLE 8

Tests by vortex males of star dichaete vortex females from the female back-crosses of star dichaete by vortex.

	v_0	$S v_0$	$D v_0$	$S D v_0$	+	S	D	$S D$
M447 (ex 398) ♀	51	41	2	3	—	—	30	27
♂	37	40	—	—	—	—	32	31
M462 (ex 398) ♀	29	16	9	10	—	—	16	14
♂	26	28	—	—	—	—	31	23

will make it apparent that this cause may have been an additional semidominant modifier.

With respect to the linkage between star and the second-chromosome vortex gene, the crossover classes are star vortex and wild-type. When there is considerably more changing over in the male classes than occurred in this experiment there is still no changing over in the females. Four of the wild-type females were tested, and as expected, in no case were they vortexes that had changed over (table 9, cultures 412, 438, 448, 454). The 52 wild-type females of table 5 are therefore all to be considered as true crossovers between star and vortex II. The noncrossover class which corresponds to this wild-type crossover class is the star class of 443 females. Star vortex (44) and vortex (471) are complementary crossover and corresponding non-crossover classes.

Because of the probability of a slight amount of changing over among the males the wild-type males (78) can not be used without correction, which is here of doubtful validity. Of three such wild-type males tested, one was a true wild-type crossover, but the other two were changed over vortex non-crossovers (table 9, cultures M437, M452 and M460).

Males do not show vortex unless they are homozygous for both genes or contain an additional modifier, and all vortex males of table 5 can therefore be used in the calculation. The star vortex class (49) is the

TABLE 9

Tests of wild-type flies from table 5 by out-crosses to vortex.

No.	From	Vortex ♀	Wild-type ♀	Vortex ♂	Wild-type
M412 (♀)	M367	24	16	23	25
M438 (♀)	M367	92	113	109	101
M448 (♀)	M397	84	76	79	93
M454 (♀)	M400	80	72	82	73
M437 (♂)	M366	5	9	13	20
M452 (♂)	M402	136	—	104	20
M460 (♂)	M402	84	—	42	24

crossover and the vortex class is the corresponding non-crossover (460). The total number of crossovers available is 145 (52 + 44 + 49), and the corresponding total of non-crossovers is 1374. The percentage of crossing over between star and vortex II is therefore 9.5. The locus of star is so far to the left in the second chromosome that with a distance of nearly ten units between the star and the vortex loci it seemed far more probable that the locus of vortex II is to the right of star.

THE LOCALIZATION OF VORTEX II BY AID OF STREAK

If, as calculated, the locus of vortex II is about ten units to the right of star then the position of the gene could be more accurately obtained by means of the linkage relations of vortex with the dominant mutant streak. The locus of streak was known to be at about 14.7 units to the right of star, although the data on which that location was based was rather meager in amount. The locus of vortex II was therefore considered to be about five units to the left of that of streak. The most advantageous type of back-cross is that known as "alternated" in which the middle mutant gene is in one chromosome and the two end genes in the other $\left(\frac{S \qquad S_k}{v_o}\right)$. In order to obtain heterozygous females of the required type a crossover star vortex male was taken from the previous experiment and crossed to streak females from stock (M439). The F_1 star streak females were then back-crossed by vortex males (M487). The small proportion of star streak crossovers which occurred among the back-cross offspring were of two types, half were only heterozygous for vortex III while the remaining half were of the desired homozygous type. To eliminate all doubt as to the constitution of the flies used some of the star streak crossovers were tested individually by mating to vortex females. One of these cultures (M520—SS_k♀ 69, ♂ 79; v_o♀ 74, ♂ 76; +♂ 1) of which the father proved to have been homozygous for vortex III gave many star streak offspring, all of which were homozygous for vortex III and heterozygous for vortex II. Fifteen cultures were raised from star streak females of the above constitution (table 10).

TABLE 10

Back-cross tests of $\frac{S\quad S_k}{v_o\text{II}}\ \frac{v_o\text{III}}{v_o\text{III}}$ ♀ *by vortex* ♂.

1918 Oct. 14		S S_k	v_o	S v_o	S_k	S	v_o S_k	S S_k v_o	+
M572	♀	30	28	4	2	—	—	—	—
	♂	25	26	2	3	1	—	—	2
M573	♀	23	23	1	2	—	—	—	—
	♂	21	14	3	1	2	—	—	—
M574	♀	37	50	3	9	1	—	—	—
	♂	57	42	3	5	3	—	—	2
M575	♀	40	36	8	9	1	—	—	—
	♂	44	46	2	7	3	—	—	6
M578	♀	50	53	6	10	2	—	—	—
	♂	42	65	3	5	5	—	—	7
M579	♀	54	61	10	7	2	—	—	—
	♂	50	48	4	8	3	—	—	8
M586	♀	41	41	6	4	—	—	—	—
	♂	34	24	5	2	—	—	—	1
M587	♀	45	55	9	6	3	—	—	—
	♂	41	45	5	8	4	—	—	8
M588	♀	36	40	2	5	3	1	—	—
	♂	43	33	7	6	2	—	—	2
M589	♀	22	31	4	6	—	—	—	—
	♂	22	31	3	5	2	—	—	1
M590	♀	40	43	4	4	2	—	—	1
	♂	44	40	3	10	4	—	—	1
M592	♀	35	32	6	4	2	—	—	—
	♂	38	32	2	7	4	—	—	13
M593	♀	43	38	2	6	—	—	—	—
	♂	23	26	2	2	2	—	—	—
M594	♀	32	34	2	6	3	—	—	—
	♂	30	40	5	6	3	—	—	10
M610	♀	28	44	3	8	1	2	—	—
	♂	27	26	5	14	—	—	—	4
Totals	♀	556	609	70	88	19	3	—	1
	♂	541	538	54	89	38	—	—	65
Grand totals		1097	1147	124	177	57	3	—	66

The classification of the experiment of table 10 was safeguarded by isolating all doubtful flies for at least five days until the pigment of the streak and of the vortex characters was fully developed and until the bubbles (to be mentioned later) characteristic of streak become pronounced. In certain cases the further precaution of actual test matings was taken so that the separations as recorded in table 10 can be regarded as complete. Three males from M593 that were regarded as possible streak were tested but proved to be non-streak. The same result was obtained from tests of several of the vortex males wherever there was suspicion that they might be streak.

Before making the calculations of the amount of crossing over it is necessary to consider the changed-over classes. Extensive experiments involving this region of the second chromosome have shown that the amount of double crossing over within this distance of fifteen units is practically zero, so that there should be no wild-type class. It is doubtful whether the one wild-type female which occurred was such a double crossover or was a changed-over vortex. It seems more probable that she was a vortex female since she occurred in the last count of the culture. We may therefore add this one to the 609 vortex females. Likewise the 65 wild-type males (four of which were tested and proved to be vortex genetically) are to be added to the 538 vortex males bringing the number up to 603 which is then equal to the number of females. Among the males 10.8 percent of the vortex class changed over. If this same proportion of star vortex males changed over then six males should be transferred from the star class to the star vortex class, reducing the star class to 32 and increasing the star vortex to 60.

While the counts of table 10 were being made a striking fact was observed, namely, that the streak vortex class was practically non-existent, although it had been expected to be as large as the star class. Furthermore the vortex present in the three streak vortex flies recorded as such in table 10 was of a different type from the ordinary vortex, being developed only in the anterior pair of vortices as very slight depressions with little pigment and no whorling of the hairs. Morphologically there seemed some slight reason why the presence of the streak character should interfere with the development of the vortex character. The thorax of streak flies is markedly altered, especially with regard to the musculature, which is largely replaced by large bubbles. In fact this character of the thorax is the clearest one for classification. It is to be noticed that the anterior pair of vortices would most often escape the interference by these alterations since the anterior pair of vortices is broadly separated laterally, while the center of the streak disturbance is median and posterior.

Tests, that will be described in a later section, were carried out with these particular streak vortex flies and these tests showed that there was probably present a modifier which favored the development of a vortex among streak flies of these cultures and their descendants.

Not only was the streak vortex class unduly diminished, but correspondingly the streak class was unexpectedly large, being 177 flies while its complementary class star vortex was only 130 flies (corrected for changing over). If the streak vortex flies were included with the streak then we should expect that the number of streak and streak vortex flies should be equal to the sum of the star and the star vortex flies. This is found to be the case, since the sum of the streak flies is 180, while the sum of the star flies is 181. There are two ways of calculating how many flies should be removed from the streak class and added to the streak vortex class. The class of streak vortex should be equal to the complementary class star which is 51 (corrected). This required the transference of 48 flies from the streak to the streak vortex class. The other method is to reduce the size of the streak class to that of its complement (130). This would require the transference of 50 flies from the streak to the streak vortex class. Since these two methods agree the corrected classes may be accepted as 129 streak and 51 streak vortex. The final corrected classes stand as in table 11.

TABLE 11

The classes of table 9 corrected for changing over and for interference by streak.

S S_k	v_o	S v_o	S_k	S	v_o S_k	S v_o S_k	+
1097	1213	129	130	51	51	—	—

On this basis there was 9.7 percent of crossing over between star and vortex, which is in agreement with the value (9.5) obtained from the star dichaete vortex female back-cross tests of table 5. There was 3.8 percent of crossing over between vortex and streak, which is slightly less than that previously calculated from data less extensive. The locus of streak on the basis of the entire data is at about 13.7 units to the right of star.

THE STREAK VORTEX MODIFIER

One of the two exceptional streak vortex flies which occurred in culture 610 was out-crossed to vortex males and gave a considerable proportion (about a quarter) of streak vortex flies of this new type (culture 694, table 12). A streak vortex male from among this progeny out-crossed to vortex females from stock likewise gave this type of streak vortex in the same proportion (M782). This stock has been continued for several generations and gives analogous results. The proportion of streak flies showing vortex in these two cultures and the line descended from one of them is exceptional, since in the other cultures the crossover streak flies which are genetically homozygous for vortex fail to show the vortex character. The new condition which has arisen is probably due to a mutant modifier which has the effect of causing the vortex in streak flies to develop but to develop as a new somatic type. Since this type occurred in two cultures and in out-crossed cultures of their descendants, the modifying gene is a dominant. In the mother of culture 610 this dominant modifier was present in the star streak chromosome not far from streak and probably to the right. When crossing over occurred between vortex II and streak, the streak individuals received the modifier and were then better able to show vortex, but in a modified type. This accounts for the individuals in cultures 610 and 588. When a vortex streak crossover female from 610 was out-crossed, most of the streak descendants should be of the same constitution as the mother, that is, homozygous for vortex II and vortex III and heterozygous for streak and for the dominant modifier. The result showed that only about a quarter of the streak flies developed the new type of vortex. It might have been supposed that crossing over between streak and the modifier had reduced the number of flies containing the modifier and hence showing the new vortex. But in the next generation a

TABLE 12
Selection for vortex streak (new type).

No.	Parentage		S_k	v_o S_k	v_o	+
M694	v_oS_k♀ ex 619 × v_o ♂	♀	23	17	40	—
		♂	19	6	44	8
M812	v_oS_k♂ ex 694 × v_o ♀	♀	31	8	40	2
		♂	17	2	36	7

male of this constitution was out-crossed and results similar to those of the female out-cross were obtained. Since there is no crossing over in the male the similarity of the female and male out-crosses shows that in the female likewise there was probably little crossing over between streak and the modifier. It is evident then that this modifier is able to bring the new vortex to expression in only about a quarter of the flies of the given constitution.

There are many other cases known in which flies of a given constitution may or may not show a certain character. The determining factor is presumably environmental, and has been proved to be such in several of the cases.

FURTHER TESTS OF THE POSITION OF VORTEX III

The other experiments had indicated that the position of vortex III was very close to that of dichaete. In fact no certain crossover had been obtained between these two loci. It was thought advisable to get more extensive data on this point in the hope of finding on which side of dichaete the locus of vortex III is situated. Such an experiment would require the simultaneous use of two known loci in the third chromosome. The stock containing the two dominants dichaete and hairless offered the quickest and most convenient method of obtaining such information. Accordingly a vortex male was crossed to a dichaete hairless female and the F_1 dichaete hairless females heterozygous for vortex II and for vortex III were tested by vortex male from stock (table 13(A)). As in the previous experiment, females that were not homozygous for vortex III showed the vortex character occasionally. Thus among the dichaete females of table 13 (*A*) 16 showed vortex slightly. Tests of one of the vortex dichaete hairless females showed that it was the supposed heterozygous type (table 13 (*C*), culture M450). Such vortex dichaete flies likewise occurred in two parallel tests of F_1 males, and since no crossing over occurs in the males the vortex dichaete flies are clearly of the heterozygous vortex III type (table 13 (*B*)). For this reason it is only among the dichaete males of table 13 (*A*) that real crossing over could be detected. No such dichaete vortex males occurred, which confirms the closeness of vortex III to dichaete, but fails to show the relative order. On the other hand the crossing over between vortex and hairless was of the amount (20.4) to be expected from the known normal distance between dichaete and hairless.

TABLE 13

(*A*) *Back-cross tests of* $\frac{+}{v_o\text{II}}\;\frac{D\quad H}{v_o\text{III}}$ *female by vortex male.*

1918 July 24		v_o	v_o	v_o H	D v_o H	+	D	H	D H
M389	♀	22	—	5	3	21	11	5	43
	♂	12	—	4	—	37	16	9	48
M390	♀	27	—	5	1	36	11	10	37
	♂	21	—	3	—	57	9	8	62
M421	♀	29	—	11	7	41	27	15	61
	♂	24	—	7	—	40	20	13	72
M422	♀	41	—	4	2	38	16	8	74
	♂	30	—	12	—	45	24	7	78
M423	♀	31	—	11	3	40	23	8	62
	♂	29	—	5	—	48	17	11	81
Totals	♀	150	—	36	16	176	88	46	277
	♂	116	—	31	—	227	86	48	341
Grand totals		266	—	67	16	403	174	94	618

(*B*) *Vortex female by heterozygous male.*

M417	♀	34	—	—	4	44	—	—	62
	♂	25	—	—	—	33	—	—	45
M418	♀	14	—	—	9	18	—	—	31
	♂	13	—	—	—	18	—	—	35
Grand totals		86	—	—	13	113	—	—	173

(*C*) *Dichaete vortex hairless female ex M389 by vortex male.*

M450	♀	54	2	18	23	—	22	—	36
	♂	56	—	27	—	9	23	—	60
Grand totals		110	2	45	23	9	45	—	96

THE ISOLATION OF AN ADDITIONAL VORTEX INTENSIFIER

During the course of all these later experiments a sharp outlook was kept for the occurrence of dichaete vortex males such as had been found in the first experiments with the mutant (table 1). In only one of the many cultures was such a male recorded (M446, table 7). This culture was likewise exceptional in the high number of dichaete vortex females of the heterozygous type, over 40 percent of the dichaete females being vortex instead of under 20 percent.

Here was an opportunity to determine whether this male was of a different genetic constitution from ordinary dichaete males which do not show vortex. The male was out-crossed to a vortex female from stock. The absence of dichaete vortex males in the sons of the dichaete vortex male (M508, table 14) proves that he had not been a crossover; that is, that he was not homozygous for vortex III, for in that case practically all of his offspring should have been vortex.

The F_1 culture was exceptional in that there was a very high proportion of vortex among the dichaete females, just as had been the case in the parent culture 446. On the other hand none of the dichaete males showed vortex. So that it may be concluded provisionally that a modifying gene was present which was partially dominant among the females and not obviously dominant among the males. This difference is another expression of the already noted sex-limitation of the vortex character.

TABLE 14

Selection for dichaete vortex males.

No.	Parentage		D	D v_o	v_o	+
M508	v_o♀ × Dv_o♂ ex 446	♀	16	25	48	—
		♂	36	—	16	3
M550	$2Dv_o$♀ × $2D$♂ ex 508	♀	16	22	14	—
		♂	33	8	15	—
M608	$3Dv_o$♀ × $3Dv_o$♂ 550	♀	37	82	30	—
		♂	68	11	34	—

Culture 508 was so similar to 446 that it seemed probable that they were of the same constitution and that the single dichaete vortex male of 446 was a case of the dominance of the modifier, here effective even in the male. Another indication of this dominance is the suppression to a large extent of the changing over of vortex into wild-type in 446.

An F_2 culture was raised from two of the dichaete vortex females mated to two of the dichaete males from 508. While among the dichaete females the proportion of vortex was no higher than in F_1, among the males 8 dichaete vortex males occurred in a total of only 41 dichaetes. These males are presumably to be looked upon as homozygous for the modifier. Three such males were crossed to dichaete vortex sisters and the succeeding generation (M608) was characterized by the highest proportion of dichaete vortex yet observed. More than two-thirds of the dichaete females were vortex, indicating that more flies were homozygous for the modifier than in the previous cross. Another feature of these last two cultures was the absence of wild-type males in contrast to their usual occurrence in cultures free from the modifier. Thus the grade of vortex in all of its types has been raised to a high level by the action of the modifier, but this level is consistently higher in the female than in the male.

The gene for the modifier is known not to be in the third chromosome, unless very removed from dichaete, and the probabilities are that it is in the second chromosome.

The new experiments removed the suspicion that the inheritance of vortex depended upon some unusual chromosome condition. Thus, sterility did not appear in the new experiments, and its occurrence in the first experiments must have been a separate phenomenon. The locus of vortex III proved to be close to that of dichaete and not at the middle of the chromosome as required for "transposition". The other doubtful points have likewise fallen in line with a plural gene explanation.

THE MUTANT CHARACTER, FLIPPER

In culture 367 (table 5) a very small wild-type female was found and tests were made to determine whether she was genetically a dwarf or simply was exceptionally small because of some accident of development. In F_2 from a cross to vortex male no dwarfs reappeared, but a new mutant character appeared in the culture (M466). This new mutation resembled the sex-linked mutation club (see MORGAN and BRIDGES, Carnegie Publication No. 237 for figure). The whole fly was under-sized and was of shrunken appearance. The surface retained a wet appearance. The most obvious feature was the wing which remained in the folded condition in which they were when the fly emerged from the pupa case, and did not expand as wings normally do. These compact wings were held out and curved downwards like flippers.

When these flies began to appear it was observed that most of them were at the same time vortex. Counts were made which show that there was strong linkage betwen vortex and flipper (M466, table 15).

Several attempts were made to mate these flies together in order to obtain a stock of the mutation. All these matings failed except one, which gave 4 flipper females, 1 flipper male, and 1 wild-type male. Probably the wild-type male resulted from non-virginity of the mother.

TABLE 15

F_2 results from the cross of vortex flipper to wild.

	Wild-type	Vortex	Vortex flipper	Flipper
M466	190	17	32	3
M723	232	14	40	7
M724	229	19	25	5
Total	651	50	97	15

Because of the failure of all these matings the character flipper was lost, but it reappeared later in another culture in which vortex was used. In this case also quite extensive matings were made between vortex flipper females and their wild-type brothers. In one case only the mating produced a few wild-type offspring, from which two F_2 cultures were raised (723 and 724, table 15).

A calculation of the position of flipper was made on the basis of the three F_2 cultures of table 15. The flipper class (15) is a crossover class corresponding to the non-crossover vortex flipper class (97). Likewise the vortex class is a crossover class which corresponds to the compound wild-type class. The wild-type class is constituted from 3 non-crossover and 2 crossover classes ($3n + 2x$). The non-crossover class corresponding to the crossover class is calculated as 184 individuals. The total results give 65 crossovers to a total of 346 individuals, or 18.7 percent of crossing over. The amount of crossing over between vortex and flipper is so large that it is improbable that the locus of flipper is to left of vortex since vortex is itself only 10 from the left end of the known chromosome. Flipper can be located approximately at a position 18.7 to the right of vortex or at 28.3 to the right of star.

SUMMARY

The foregoing experiments have shown that the character vortex is dependent upon or is modified by four mutant genes.

Of these genes the most essential one, without which the character is never known to have appeared, is situated in the second chromosome at a position 9.6 units to the right of star. However, this second-chromosome gene is by itself insufficient for the production of the vortex character.

The gene second in effectiveness is situated in the third chromosome very close to the locus of dichaete (11.7). This gene likewise is unable to cause any development of the vortex character when acting alone. But in flies homozygous for vortex II, heterozygosity for vortex III enables about 20 percent of the heterozygous females to show the vortex character although no male of this constitution can show the character. Flies homozygous for both vortex III and vortex II are, if females, practically invariably vortex, while if males they are vortex except that toward the end of old cultures a small proportion of genetically vortex males of this homozygous type "change over" into wild-type. The usual stock of vortex is of this bigenic constitution.

During the experiments a dichaete stock was isolated in which a third gene was present which contributed to the development of the vortex character. In this stock a majority of the females homozygous for vortex II and heterozygous for vortex III showed the character, instead of only about 20 percent as in stocks in which this modifier is not present. A slightly greater percent of such females showed vortex when homozygous for the new modifier: In heterozygous condition this new modifier was almost without effect upon males of the heterozygous type, but in homozygous condition it made vortex show in a considerable proportion of the flies homozygous for vortex II and heterozygous for vortex III, while it eliminated the changing over of homozygous vortex II vortex III flies into wild-type flies. The locus of this modifier is probably in the second chromosome.

In the experiments involving streak a special relation between streak and the vortex was discovered. The vortex character was prevented from developing in streak flies even though such flies were homozygous for both vortex II and vortex III.

However, in the same experiments a special modifier was detected which to a considerable extent reversed this inhibition by streak. This modifier was a dominant situated in the second chromosome quite close to the locus of streak and probably to the right. The streak flies in which the vortex character appeared through the action of the modifier showed a type of vortex different from the usual one.

Throughout all of these experiments and in the various types of vortex a very striking fact was apparent, namely, that the grade of the vortex character and the proportion of flies showing that character was higher in the females than in males of the same genetic constitution.