THE MUTANTS OF THE EXTREME LEFT END OF THE SECOND CHROMOSOME OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

The number of mutant characters known in *Drosophila melanogaster* is continually increasing. Many of the new mutants have no special interest in themselves, but they may serve as tools for the investigation of other problems. Before they can be used with certainty, their general characteristics and linkage relations must be determined.

The important dominant mutant star had been located at the extreme left end of the map of the second chromosome. Four recessive mutants had been found that are very closely linked to star. An account of star, by BRIDGES and MORGAN, has appeared in Carnegie Publication No. 278, pages 259–273. References to the others, namely, telegraph, aristaless, dachsous and expanded, have appeared in several maps and publications, notably "The Genetics of Drosophila," Bibliographica Genetica, 2 : 1–262. The present paper gives, in Part I, descriptions of these mutants and an account of the older data collected by BRIDGES, and, in Part II, the more precise determinations of the locations made recently by STERN. The map based on these determinations is given as figure 1.

FIGURE 1.—Map of the extreme left end of chromosome II of *Drosophila melanogaster*. This map is based on the data of table 21. The symbol (!) denotes the most useful mutants, (+) those nearly as good, while the absence of a mark indicates that the mutant is important only in special connections.

PART I

Star

The mutant star, found by BRIDGES, February 12, 1915, has been probably the most useful mutant in the second chromosome. This was due primarily to its dominance, its excellent viability (roughly 98 percent that of the wild type) and its very favorable location, since it was the mutant farthest to the left among those whose linkage relations were approximately known. The separation of star from the wild type is not as easy or as rapid as desirable, and hence considerable experience in its use is necessary before the classification is reliable in a critical case.

The character star, as seen under a low power of the binocular (about 12 diameters) differs from the wild type in that the eyes are (1) rougher in

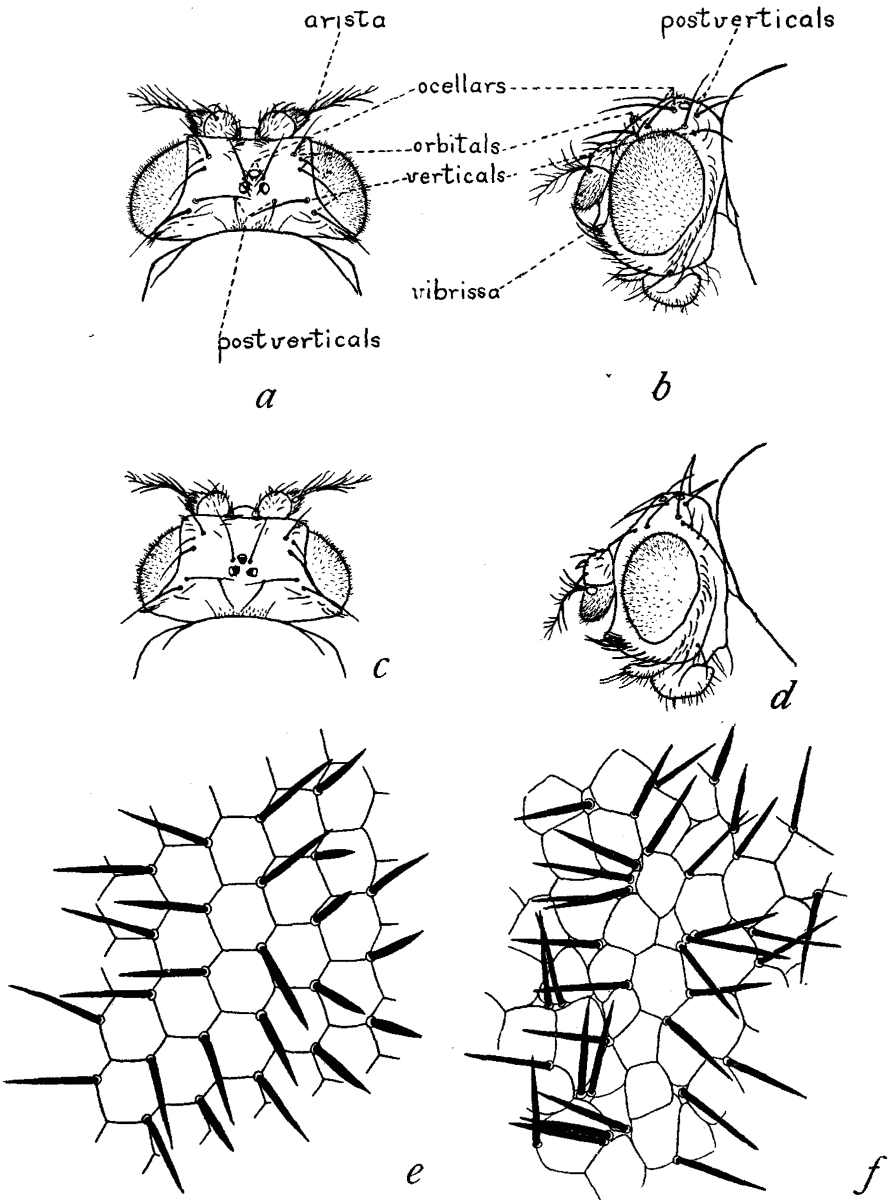

FIGURE 2.—*a* and *b*, top and side views of head of wild-type individual of *Drosophila melanogaster*, with nomenclature of bristles. *c* and *d*, top and side views of the head of the mutant star. *e*, enlarged view of facets and eye-hairs of a wild-type individual (regularity of direction of eye-hairs disturbed by pressure of coverglass). *f*, enlarged view of facets and eye-hairs of the mutant star. (Irregularity in direction of eye-hairs is normal to star.)

texture, (2) somewhat smaller in size, (3) slightly narrower in the antero-posterior direction, (4) slightly darker in color, and (5) faintly scintillating with tiny points of light. (Compare figure 2c with 2a, and 2d with 2b.) A higher power shows that the roughness in texture is due to the ommatidia being arranged irregularly instead of in regular rows, and being of varying size, shape and color (compare figure 2f with 2e). The scintillation is due to reflection of light from the tiny hairs between the facets. In the wild-type eye the hairs stand singly in a perfectly regular latticework pattern and are radially directed. In the star eye the hairs are missing from some spaces and aggregated in two's and three's in others, and point in all directions. (Compare figure 2f with 2e.)

The homozygous condition of the star gene is invariably lethal. The character as described is the heterozygous dominant form.

Since the mutant star had more favorable characteristics, especially with respect to its dominance, than the other mutants of the group at the left end of the map, it has been made the point of reference in locating mutants in its neighborhood. For this same reason the data involving star and the mutants throughout the remainder of the chromosome are greater in amount and better in quality than the data in which the other leftmost mutants are involved. This difference will probably continue for some years and accordingly the locus of star will maintain its prime importance in the region to the left of black.

Reappearances of star

Star, or an allelomorph so similar as not to be distinguished by inspection or in the course of experimentation, has reappeared by mutation on four occasions:

(1) In a cross between two stocks involving only sex-linked characters (cut female by eosin miniature garnet[2] male) BRIDGES found that half the offspring in one pair culture showed a star-like eye (culture 8718, June 8, 1918). Examination of the original stocks showed no star-like individuals present, nor did the pedigrees of those stocks involve star. Tests showed that the new character was a heterozygous dominant, lethal when homozygous, located in chromosome II, and much to the left of black. Crosses between the new star and the original gave in F_1 the typical 2 star : 1 wild type ratio (8756,8757; total 262 star : 132 wild type).

(2) MOHR found star again (July 20, 1920) and has reported the facts for this case (MOHR 1923).

(3) The third reappearance of star occurred in the spring of 1925 in the cultures of L. V. MORGAN. Allelomorphism was established by

the fact that a cross between a stock of the new star balanced by curly (WARD 1922) and a stock of the original star balanced by curly gave in F_1 only flies that were both star and curly, exactly as though either star had been bred to itself in balanced stock. (Unpublished data of L. V. MORGAN.)

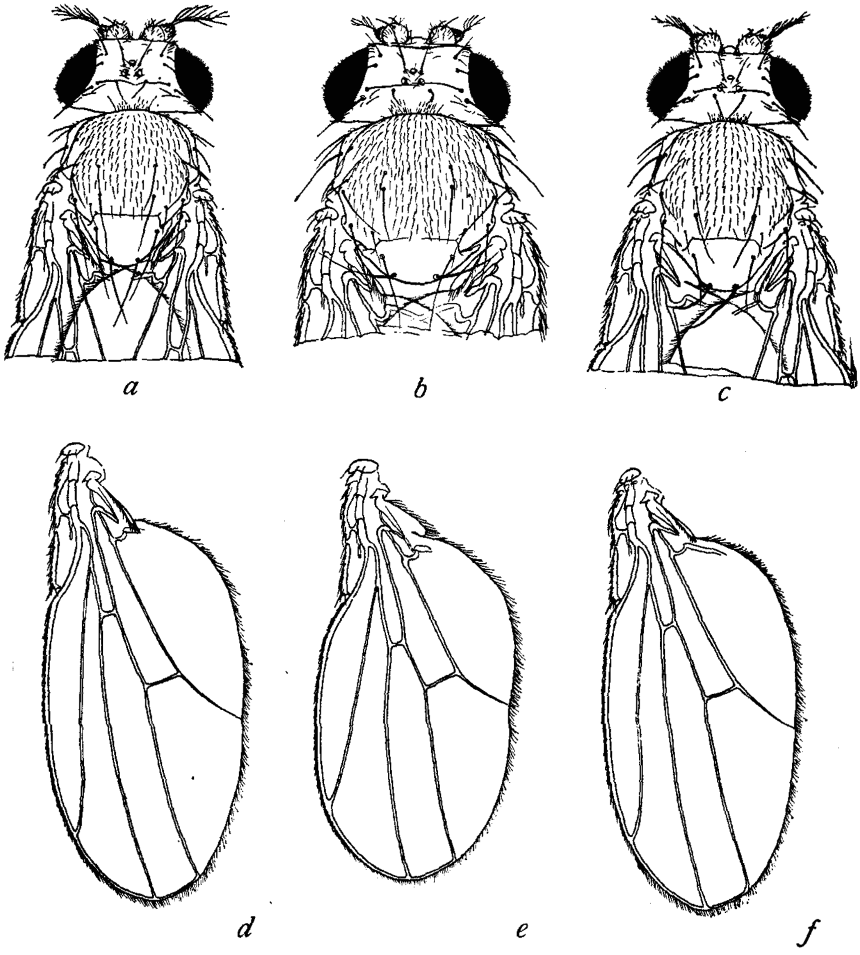

FIGURE 3.—*a* and *d*, dorsal view of head and thorax and a wing of a wild-type individual of *Drosophila melanogaster*. *b*, the mutant aristaless, showing reduced aristae and posterior bristles on the scutellum widely separated and strongly divergent. *c*, the mutant telegraph, showing the slight erection of the posterior bristles of the scutellum. *f*, the wing of the mutant telegraph, showing weak spots in the second longitudinal vein. *e*, the wing of the mutant dachsous, showing the crossveins close together.

(4) In a cross involving the second-chromosome dominant curly Doctor HELEN REDFIELD found one curly female with an eye like star.

The character was found to be dominant and due to a gene in the curly-bearing second chromosome. In crosses to the original star it was found that the new star was lethal in the compounds with the old star, and hence must be an allelomorph of star. (Unpublished data of Doctor H. REDFIELD.)

Telegraph

In establishing a pure stock of the third-chromosome recessive smudge, BRIDGES found a culture (No. 3947, March 27, 1916), in which a majority of the flies showed a break or weak space in the second longitudinal vein (Compare figure 3f with 3d). Many of the flies showed more than one such break, and from this dot-and-dash appearance of the vein the mutant was called telegraph. When telegraph males and females were bred together most of the progeny showed the character. This indicated that the mutant was a recessive for which the stock was homozygous but that the character overlapped the wild type. In some individuals telegraph failed to show, and in others could only be distinguished with difficulty in one wing. Later it was found that many of the telegraph flies showed another characteristic, namely, the posterior scutellar bristles were more or less erect, as in vestigial, instead of pointing backward. (Compare figure 3c with 3a.) This bristle characteristic could often be used as an index of the mutant in those flies in which the breaks were absent or weak.

Telegraph by star backcross

Telegraph males were crossed to star females, and in F_1 the telegraph character was found to be recessive. The F_1 star flies were inbred in pairs for an F_2 generation (table 1). The telegraph character reappeared in

TABLE 1

P_1, telegraph ♂ × star ♀; F_1 star ♀ × F_1 star ♂.

MAY, 1917	TELEGRAPH	STAR	TELEGRAPH STAR	WILD TYPE
7203	86	220	1	1
7204	80	194	3	1
7205	105	210	..	..
Total	271	624	4	2

slightly less than the expected frequency. Only four of the F_2 flies showed both star and telegraph, while 271 showed simply telegraph. This strong linkage showed that the locus of telegraph is in the second chromosome, and also that it is very close to that of star. Only two flies that were not-star failed to show telegraph. These were probably due to crossing over rather than to overlapping of the telegraph character. The percentage of recombination was calculated from the average of the two recombination classes, as compared with the original combination class telegraph

$$\left(\frac{3}{271+3}\times 100=1.1\right)$$

The star class could not be used in the calculation since it was complex, including recombinations as well as original combinations.

From the star class of table 1 virgin females were mated to telegraph males for backcross counts (table 2). Some difficulty in classification was

TABLE 2

Telegraph over star ♀ (from table 1)×telegraph ♂.

MAY, 1917	TELEGRAPH	STAR	TELEGRAPH STAR	WILD TYPE	CONTINUED	TELEGRAPH	STAR	TELEGRAPH STAR	WILD TYPE
7249	107	105	2	2	7323	29	35	1	2
7250	63	63	1	1	7648	161	134	..	7
7251	49	49	..	5	7649	142	161	..	8
7252	215	208	1	4	7654	78	79	..	2
7268	92	89	..	4					
					Total	936	923	5	35

encountered, and the class marked wild type (+) was composed partly of true recombinations due to crossing over but mostly of overlaps from the genotypically telegraph class. The percentage of recombination was at first calculated from the total flies of tables 1 and 2 as 2.0, and in the maps prepared (see especially the maps in BRIDGES 1921 and in MORGAN, BRIDGES and STURTEVANT 1925) the telegraph star distance was given as 2.0. Because the phenotypic class wild type was partly due to overlaps the calculations should have been made on the basis of the telegraph flies alone, and would then have been 0.7

$$\left(\frac{9}{1216}\times 100=0.7\right)$$

Telegraph black stock

A female showing both telegraph and star (from table 2) was crossed to a black male. F_1 heterozygous star females were crossed to telegraph males (culture 7383, telegraph star=71, wild type=79, telegraph=2, star=1). Both of the recombinational telegraph males that occurred were crossed to black of unrelated stock. Half of the offspring were black. These black flies were inbred and in the next generation somewhat less than a quarter of the flies showed telegraph as well as black. These latter were inbred to give a telegraph black stock. The fact that the above procedure was successful in giving a telegraph black stock made it seem probable that the locus of telegraph is to the left of star rather than to the right. If it were to the right, the telegraph flies tested must have resulted from the less frequent double crossing over. A three-point backcross involving telegraph star and black was undertaken to test this point, but difficulties in classification prevented it from being conclusive.

As will be shown in a later section of this paper, the work of STERN has shown that the locus of telegraph is indeed to the left of that of star, and that the distance between them is about 1.3 units.

Aristaless

In examining an F_2 culture (8028, November 7, 1917) involving eosin, vermilion, forked and a new mutant "disbanded" that had arisen in the California wild stock, BRIDGES found that approximately a quarter of the flies showed posterior scutellar bristles that were erect, diverged strongly, and were widely separated from each other (Compare figure 3b with 3a). A pure stock of this recessive was established and freed of the other mutant characters originally present. It was then found that the flies that showed "repelled" bristles were further characterized by great reduction or even complete absence of the aristae (figure 3b). It was anticipated that this mutant, called aristaless, would be especially valuable since it affected a region of the body affected by few other mutants and hence could be used widely without confusion in classification.

Aristaless by star backcross

Aristaless was crossed to star dichaete, and several F_2 cultures were raised. Aristaless occurred freely in the dichaete F_2 flies but apparently not at all in the star F_2 flies. From this it was concluded that the locus of aristaless is in the second chromosome, and also that it is very close to that of star.

From the F_2 flies, star females and aristaless males were bred together for backcross counts (table 3). In two of the cultures the viability of aristaless was very low, but in the other two it was normal. There were three recombinations in a total of 334 flies, or 0.9 percent of recombination.

Since at that time work on the third-chromosome mutants was being carried on practically to the exclusion of other work, as soon as the above data were obtained the mutant was turned into stock without further testing. In making the earlier maps of the second chromosome the locus was put down as to the right of star, since the probability that any mutant should lie to the left of star was thought to be less than that it should be to the right. The recent tests of STERN (see below) have shown that the locus of aristaless is in reality to the left of that of star, and that the distance between them is roughly 1.0 units.

TABLE 3

F_2 aristaless over star ♀ × *F_2 aristaless* ♂.

DECEMBER, 1918	ARISTALESS	STAR	ARISTALESS STAR	WILD TYPE
9029	10	47	..	..
9187	19	59	1	2
9198	43	43	..	..
9263	50	60	..	..
Total	122	209	1	2

Aristaless²

In looking over the second-chromosome stock "fringed," STERN found several flies of both sexes with reduced aristae and erected posterior scutellar bristles (January 1926). Fringed was kept as a balanced stock over curly. The fact that all flies exhibiting the new mutant were fringed not-curly indicated that its locus was in the not-curly second-chromosome. This, together with its characteristics, made it probable that one was dealing with an allelomorph of aristaless. By crossing the mutant flies to aristaless and obtaining only aristaless-like flies in the F_1 generation, the allelomorphism was proven. Aristaless² is a somewhat less extreme character than the old aristaless, both in regard to the reduction of the aristae and the erection of the scutellar bristles. The classification is as easy as in aristaless. If viability tests should show a better viability of aristaless² than of aristaless, then the former would replace the old allelomorph in future work.

Dachsous

In a mixed stock of black and black purple (Culture 9068, November 12, 1917), BRIDGES noticed that a small percentage of the flies had wings which were shorter than normal and in which the crossveins were very close together (figure 3e). The abdomen and legs were also shorter, and the general appearance of the fly was similar to that of the mutant dachs. The "dachsous" flies were inbred, and they produced only dachsous offspring, which showed that the character was a recessive. A dachsous black stock and a dachsous black purple stock were isolated.

Dachsous black backcross

Three cultures of a dachsous black backcross were raised (table 4). They gave respectively 22, 25 and 27 percent of recombination for dachsous and black. This linkage ratio showed that the locus of dachsous is in the second chromosome.

TABLE 4

P_1, dachsous black × wild type; BC., F_1 wild type ♀ × dachsous black ♂.

OCTOBER, 1920	DACHSOUS BLACK	WILD TYPE	DACHSOUS	BLACK
12055	104	118	26	36
12056	86	85	26	32
12061	116	118	40	45
Total	306	321	92	113

Dachsous black purple by lobe² backcross

Dachsous black purple males were crossed to females with the dominant character lobe², whose locus is at 70 in the second chromosome (BRIDGES 1921). In the backcross cultures (table 5) the percentage of recombination for dachsous and black was 36.4, for dachsous and purple 38.9 and for dachsous and lobe² 41.0. That is, the locus of dachsous is farthest from that of lobe and hence is to the left of black. When the locus of dachsous was first calculated on the basis of the data of tables 4 and 5, proper allowance was not made for double crossing over and the locus was entered as probably 12 units to the right of star (see MORGAN, BRIDGES and STURTEVANT 1925, page 92).

TABLE 5

P_1, dachsous black purple ♂ × lobe² ♀; BC., F_1 lobe² ♀ × dachsous black purple ♂.

NOVEMBER 1920	DACHSOUS BLACK PURPLE	LOBE²	DACHSOUS	BLACK PURPLE LOBE²	DACHSOUS BLACK	PURPLE LOBE²	DACHSOUS BLACK PURPLE LOBE²	WILD TYPE	DACHSOUS PURPLE	BLACK LOBE²	DACHSOUS	BLACK PURPLE LOBE²	DACHSOUS BLACK	PURPLE LOBE²	DACHSOUS PURPLE LOBE²	BLACK
12147	98	98	71	49	5	7	21	13	1	..	9	9	3	..	..	..
12154	104	115	54	71	4	4	18	12	..	..	11	9	..	1	1	..
12155	75	114	53	51	4	1	15	14	1	..	5	10	1	3	..	2
12256	95	100	59	59	10	3	17	18	1	2	14	15	3	1	..	..
12157	72	92	42	49	3	3	9	10	4	..	7	9	..	2	..	..
Total	444	519	279	279	26	18	80	67	7	2	46	52	7	7	1	2

Dachsous was given to MOHR for use in studying the relationships of the supposed deficiency "gull," and MOHR reported by letter that he found that dachsous and star gave about 1 percent of recombination. The tests of STERN (see below) have shown that the locus of dachsous is to the left of star by about 1.0 unit.

Dachsous[2]

The stock of speck was found by BRIDGES to be homozygous for a character that resembled dachsous, (April 2, 1925). Crosses between this mutant and dachsous gave only flies like dachsous. In dachsous[2] the crossveins are as close together as in dachsous, but the wings, legs, and body are only slightly shortened.

Dachsous[3]

In a stock of wild flies collected by Doctor D. E. LANCEFIELD at Olympia, Washington, in the summer of 1925, BRIDGES found several males and females that had crossveins very close together (November 5, 1925). Such flies reappeared continuously in several generations, usually late in the course of the culture. Attempts to breed them together failed. Likewise females used in outcrosses produced no offspring. Four such females, that were fully ten days old, were dissected and it was found that two of them contained no ovaries. The oviducts were present and a small tuft of trachae where the ovaries are normally situated. One female differed in that one partly developed egg was present. The fourth had five such eggs. Of these, four stuck out radially from the tuft of trachae and broke away easily. The other egg was partly within the oviduct, wrong end foremost. Of approximately twenty females used in crosses none gave offspring. The males proved fertile and a self-perpetuating stock was obtained by crossing them to curly, and then inbreeding the curly F_1 flies. The fact that the F_2 flies in this stock were all of two classes, simple curly and those with close-crossveins but not-curly, showed that the locus of the mutant is in the second chromosome.

Dachsous[3] by star backcross

A backcross with star gave less than one percent of recombination (table 6). Crosses of close-crossvein to dachsous gave only flies with close-crossveins. Accordingly the new mutant is an allelomorph of dachsous and is called dachsous[3]. The compound between the two allelomorphs dachsous and dachsous[3] had wings nearly as long as dachsous[3], abdomen occasionally shortened but not so strikingly as in dachsous. The viability and fertility of the compound were apparently normal.

In viability dachsous[3] was very poor, 50 percent that of star in table 6, while dachsous has always been excellent (99 percent that of wild type).

TABLE 6

P_1, *dachsous*^{3}$\times$*star*; *BC*., F_1 *star* ♀$\times$*dachsous*^{3} ♂.

DECEMBER 1925	DACHSOUS^3	STAR	DACHSOUS^3 STAR	WILD TYPE
15233	53	96	..	1
15236	77	167	1	1
Total	130	263	1	2

Dachsous^3 was delayed in emergence; few dachsous^3 flies appeared until the not-dachsous^3 flies had been hatching for five days. The first dachsous^3 flies to emerge had no bristles on the scutellum, or around the ocelli, and the scutellum was shortened. The wings were spread slightly and often lacked the anterior crossveins. The flies that hatched later had normal head and scutellum and there were even one or two extra bristles on the scutellum. The wings and abdomen of dachsous^3 were not shortened as much as those of dachsous or dachsous^2.

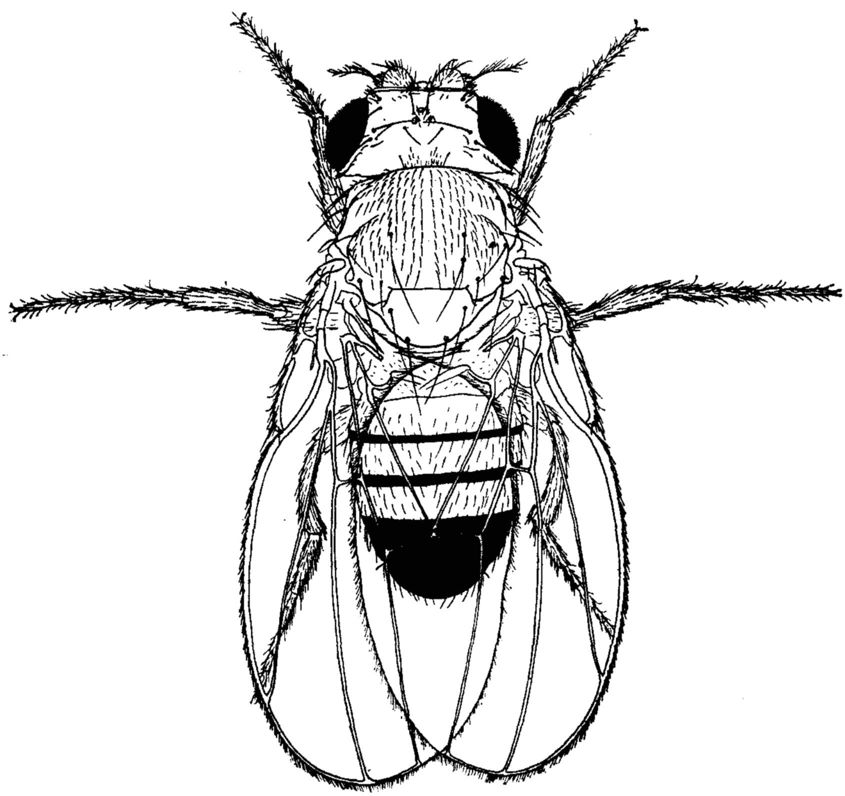

FIGURE 4.—The mutant expanded, showing large, broad wings.

Expanded

In determining the locus of the sex-linked lethal, lethal 15, BRIDGES found that one culture (8118, November 21, 1917), was giving a few flies with wings very broad, and similar in posture to curved, namely, extended rather widely at the sides and held above the back, except that a curvature of the wing brought the tips of the wings down to the normal level (figure 4). The crossveins were also closer together than normal. The eyes were somewhat reduced and roughish in texture. The flies were larger than normal.

Expanded by star backcross

"Expanded" males were out-crossed to star dichaete females, and in F_1 gave only not-expanded progeny, from which the character was known to be recessive. F_1 star dichaete females were crossed to expanded males that had been secured by breeding together expanded males and females from the original culture (table 7).

TABLE 7

P_1, expanded ♂×star (D) ♀; BC., F_1 star (D) ♀×expanded ♂.

NOVEMBER 1917	EXPANDED	STAR	EXPANDED STAR	WILD TYPE
8250	70	80	3	9
8295	44	52	..	8
8365	219	237	2	4
8391	150	157	16	2
8393	134	149	6	4
Total	617	675	27	27

The different cultures gave contradictory results. The individuals classified as recombinations were probably mostly errors due to unfamiliarity with the character and to confusion between the roughish eye of expanded, and the roughish eye of star. The apparent percentage of recombination was 4.0, and accordingly the locus of expanded was set down tentatively as about 4 units to the right of star.

STERN (see below) found that the reduction in the eye of expanded tends to increase as the cultures become older; while on the other hand the wing character becomes less extreme in older cultures. Trustworthy classifications could be made for expanded and star after familiarity with the interaction of the two had been acquired. The locus was found by STERN to be about 1.2 units distant from star, and to be to the left, not to the right.

PART II

Preliminary tests of BRIDGES, reported in Part I, had shown that the dominant character star and the four recessive characters telegraph, aristaless, expanded, and dachsous form a group of genes at the left end of the map of the second chromosome. There still remained to be determined, first, the sequence of the loci of these genes within the group, this being the most important and constant feature represented by a chromosome map, and second, the relative distances between the loci.

The first step in the procedure was to find more exactly, by a series of two-point backcross experiments, on a fairly large scale, the distance of each of the recessives from the locus of star, which, on account of its dominance, was the easiest point of reference.

Thus, the general procedure in the first series of tests was to cross star to a given recessive, and then to mate several of the F_1 star double heterozygous females

$$\frac{r \qquad +^s}{+^r \qquad S}$$

each to a male of the recessive stock. Since star is a dominant, the male of the recessive stock is in effect a double recessive

$$\frac{r \qquad +^s}{r \qquad +^s}.$$

The results of the series of distance tests should eliminate certain sequences of loci as improbable and limit the number of subsequent tests.

Telegraph by star backcross

On inspection of the telegraph stock it was seen that about half of the flies failed to show any break in the longitudinal vein. And in the telegraph by star backcross results this same overlap was observed. In calculating recombination percentages for cases in which one of the mutant characters overlaps wild type, fairly correct values may be obtained by disregarding all flies that fail to show the character in question and using only those flies in which the character was seen. This method was followed in Part I in calculating the telegraph star recombination percent.

However, in the F_1 of the telegraph by star cross it was seen that about 6 percent of the flies heterozygous for telegraph showed breaks in the longitudinal veins. In the backcross results the flies in which the breaks were due to dominance of the character could not be distinguished from the flies in which the breaks were the homozygous recessive form. Consequently the calculations could not be made on the basis of the flies that showed the break. It was found that the other characteristic of the telegraph mutant, namely, erected scutellar bristles, was strictly recessive and accordingly in making the classifications telegraph flies were identified solely by use of this index. The erect-bristle character also overlapped the wild type by about 50 percent and in making the calculations all not-erected flies have been disregarded. The fifteen cultures (table 8) produced a total of 1169 flies with erected bristles, of which 11, or 0.9 percent were star and were therefore recombinations due to crossing over between the loci of telegraph and star.

TABLE 8

P_1, telegraph ♀ × star ♂; BC., F_1 star ♀ × telegraph ♂ (Jan. 1925).

TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR
56	..	95	..	98	1	43	1
49	..	70	1	56	..	99	..
41	1	70	1	97	..	134	3
85	1	68	2	97	..		
						1158	11

Aristaless by star backcross

The character aristaless, like telegraph, has two prominant characteristics, namely, the aristae are reduced or even absent, and the posterior scutellar bristles are erected, strongly divergent and situated abnormally far apart. It was found that the arista character, though fluctuating in the amount of reduction, is a safe index of the homozygous recessive character. But the scutellar bristles character was occasionally

TABLE 9

P_1, aristaless ♀ × star ♂; BC., F_1 star ♀ × aristaless ♂. (Jan. 1925).

ARISTALESS	STAR	ARISTALESS STAR	WILD TYPE	ARISTALESS	STAR	ARISTALESS STAR	WILD TYPE
118	131	..	1	64	67	1	..
61	57	3	2	131	173	3	1
73	87	1	1	149	157	4	1
69	119	..	..	61	72	..	1
120	120	2	1	99	108	1	1
115	126	4	1	124	136	3	2
85	110	1	2	18	32	1	1
148	154	2	1	37	34	2	..
166	168	1	4	188	176	1	2
53	64	2	2				
156	162	1	1	2035	2244	33	25

apparent in heterozygous flies, and in homozygous flies was sometimes difficult to distinguish. The classification throughout has been on the basis of the arista characteristic of "aristaless."

Two sets of backcross cultures (table 9; 1, the first seven cultures and 2, the remainder) were raised, and these agreed in the percentages of recombination (1.4 and 1.3). The total number of recombinations in the two sets was 58 in a total of 4337 flies, or 1.3 percent.

Dachsous by star backcross

The new dachsous by star backcross (table 10) gave a total of 4269 flies of which 31, or 0.7 percent, were recombinations.

TABLE 10

P_1, dachsous ♀ × star ♂; BC., F_1 star ♀ × dachsous ♂. (Jan. 1925).

DACHSOUS	STAR	DACHSOUS STAR	WILD TYPE	DACHSOUS	STAR	DACHSOUS STAR	WILD TYPE
173	175	1	4	170	166	2	2
159	174	..	1	190	205	..	2
96	123	1	3	225	209	2	3
156	163	1	1	203	219	1	1
193	176	..	1	130	161	2	..
157	144	..	1				
235	236	1	1	2087	2151	11	20

Expanded by star backcross

The new expanded by star backcross (table 11) gave a total of 3060 flies, of which 46, or 1.5 percent, were recombinations.

TABLE 11

P_1, expanded ♀ × star ♂; BC., F_1 star ♀ × expanded ♂. (Jan. 1925).

EXPANDED	STAR	EXPANDED STAR	WILD TYPE	EXPANDED	STAR	EXPANDED STAR	WILD TYPE
149	118	1	3	190	179	4	1
153	138	6	2	297	238	5	5
169	169	2	1	206	204	2	1
162	173	2	5	148	191	2	2
68	62	1	1				
				1542	1472	25	21

The results of the above tests with star confirmed the earlier finding of BRIDGES that the loci of the five mutants lie very close together (table 12). The loci were found to be even closer together than expected. No certain conclusion as to the relative distances could be drawn, since the probable errors of such small percentages are high, and the possible effects of linkage modifications in the different experiments could not be excluded.

TABLE 12

Summary of two-point data of Part II.

LOCI	TOTAL	PERCENT RECOM.
Aristaless-star	4337	1.3
Telegraph-star	1169	0.9
Expanded-star	3060	1.5
Dachsous-star	4269	0.7

The next step was to build double recessive stocks of the recessive mutants. By use of such stocks, together with star, a series of three-point experiments could be made that would give decisive evidence as to the sequence of the three loci of each experiment and also relative distances found under the same condition with respect to crossover modifiers. In the process of building the double recessives, evidence could be obtained as to the relative order of certain of the genes.

Telegraph expanded double recessive

The first double recessive sought was telegraph expanded. From the telegraph by star backcross of table 8 recombinational telegraph star flies were obtained which were mated to expanded. The F_1 star females were then crossed to telegraph males. If expanded were on the opposite side of star from telegraph

$$\frac{t_g \quad S \quad +}{+ \quad + \quad e_x},$$

as seemed probable from the preliminary results of BRIDGES, then the telegraph not-star flies which occurred in the offspring of this experiment as a result of crossing over between telegraph and star should have the constitution

$$\frac{t_g \quad e_x}{t_g \quad +}.$$

By breeding together such recombinational telegraph flies, the double recessive telegraph expanded should be obtained as about 25 percent of the telegraph offspring. But when this mating was made it was found that none of the offspring were expanded. This showed that the assumed order was incorrect, and that, in fact, the loci of telegraph and expanded are both on the same side of star. The loci of telegraph and expanded must therefore be very close to each other, but there was no evidence as to which of the two is further from star. On the supposition that the order is expanded telegraph, an expanded star was crossed to telegraph, and the F_1 star female was backcrossed to expanded (table 13). The F_2 expanded flies were crossed to telegraph, but none gave telegraph offspring.

TABLE 13

P_1, expanded star×telegraph; BC., F_1 star ♀×expanded ♂. (July 1925).

EXPANDED STAR	EXPANDED	EXPANDED STAR	EXPANDED	EXPANDED STAR	EXPANDED	EXPANDED STAR	EXPANDED
99	1	43	1	63	2	73	..
156	..	89	..	93	2	40	1
142	1	49	..	79	1	70	..
96	..	62	..	77	3	88	1
80	1	66	1	90	..	93	1
35	..	87	..	79	1	56	..
30	1	86	2	72	..		
						2143	21

Similarly, on the supposition that the order is telegraph expanded, a telegraph star fly was crossed to expanded, and the F_1 star females were backcrossed to telegraph males (table 14). The F_2 telegraph not-star flies that resulted from crossing over were crossed to expanded. In the off-spring of this cross (F_3) expanded flies were produced, which were inbred and which gave telegraph expanded in the next generation (F_4). The order is then established as telegraph, expanded, star (or star, expanded, telegraph). The stock of telegraph expanded was lost in traveling, and the three-point experiment could not therefore be carried out.

TABLE 14

P_1, telegraph star×expanded; BC., F_1 star ♀×telegraph ♂. (July 1925).

TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH
82	..	37	..	74	1	59	..
82	1	52	2	31	..	87	..
106	..	34	..	34	..	35	..
62	1	35	..	35	..	26	..
38	..	51	1	84	2	37	..
14	..	33	3	51	..	62	1
37	..	23	..	82	4	49	..
30	2	45	..	67	4	39	..
30	..	90	3	77	..		
						1810	25

Telegraph dachsous double recessive

Telegraph flies were also crossed to dachsous, and the F_1 star females were backcrossed to telegraph males (table 15). The F_2 recombinational

TABLE 15

P_1, telegraph star×dachsous; BC., F_1 star ♀ ×telegraph ♂. (Mar. 1925).

TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH
74	..	51	3	72	1
83	..	56	1	65	1
65	..	79	1		
				545	7

telegraph not-star flies were bred together, and in the next generation telegraph dachsous flies were obtained. This fact shows that dachsous is either on the opposite side from telegraph

$$\frac{t_g \qquad + \qquad d_s}{+ \qquad S \qquad +},$$

or, if telegraph and dachsous are on the same side of star, that dachsous is nearer to star than is telegraph

$$\frac{t_g \qquad + \qquad S}{+ \qquad d_s \qquad +}.$$

It had been supposed that the telegraph dachsous double recessive might be too difficult to distinguish from simple dachsous, since dachsous itself causes a slight erection of the scutellar bristles. However, it was found that often the bristles of the double form were extremely erected, even directed forward, and that telegraph could be identified in dachsous flies in about the same percentage ($50\pm$) as simple telegraph could be distinguished from wild type.

Telegraph star by dachsous backcross

The three-point backcross involving telegraph, dachsous and star (table 16) showed that telegraph and dachsous are on the same side of star, since the crossing over between them was much less frequent than between either and star. The telegraph dachsous recombination percentage was 0.1, while the telegraph star recombination percentage was 1.2 and the dachsous star 1.1. The telegraph star percentage was greater than the dachsous star percentage, which agrees with the order telegraph, dachsous, star. In a three-point backcross the class due to double crossing

TABLE 16

P_1, telegraph star×dachsous; BC., F_1 star ♀ ×telegraph dachsous ♂. (April 1925).

TELEGRAPH STAR	TELEGRAPH DACHSOUS	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH DACHSOUS	TELEGRAPH	TELEGRAPH STAR	TELEGRAPH DACHSOUS	TELEGRAPH
75	1	2	92	..	1	53	..	..
88	..	..	62	..	2	73	..	1
58	..	..	92	..	1	88	..	1
75	..	..	73	..	2	64	..	1
71	1	1	81	..	..	114	..	1
69	..	1	61	..	..			
58	..	2	88	..	..	1435	2	16

over is the smallest class, and where the loci are very close together, as in the present case, it is not expected to be realized at all. The absence of flies of the telegraph dachsous star class shows that this was the double crossover class, and that the orientation of the genes was

$$\frac{t_g \qquad + \qquad S}{+ \qquad d_s \qquad +}.$$

Aristaless dachsous double recessive

A dachsous star fly (from table 10) was crossed to aristaless and the F_1 star females were crossed to dachsous. The dachsous not-star flies that occurred as the result of crossing over were inbred. None of their progeny were aristaless. From this it was evident that the locus of aristaless is not on the opposite side of star from dachsous

$$\frac{d_s \qquad S \qquad +}{+ \qquad + \qquad a_1}$$

but that it is on the same side. A second method consisted in crossing an aristaless star (from table 9) to dachsous and again mating the F_1 star females to aristaless males. The recombinational aristaless not-star flies were inbred, and in some cases there were produced the double recessive aristaless dachsous. The production of the double from the cross showed that the orientation of the genes was

$$\frac{a_1 \qquad + \qquad S}{+ \qquad d_s \qquad +}.$$

Aristaless star by dachsous backcross

The three-point backcross experiment (table 17) confirmed this order, since the double crossover classes, not realized, were aristaless dachsous star and wild type, and the orientation of the genes was accordingly

$$\frac{a_1 \qquad + \qquad S}{+ \qquad d_s \qquad +}.$$

TABLE 17

P_1, *aristaless star×dachsous; BC., F_1 star ♀ ×aristaless dachsous ♂.* (*April 1925*).

ARISTALESS STAR	DACHSOUS	ARISTALESS DACHSOUS	STAR	ARISTALESS	DACHSOUS STAR	ARISTALESS STAR	DACHSOUS	ARISTALESS DACHSOUS	STAR	ARISTALESS	DACHSOUS STAR
130	153	..	1	1	1	116	165	..	1	2	2
106	170	1	..	..	2	159	204	..	1	1	1
139	158	..	..	1	2	145	190	1	1	1	1
148	155	2	..	..	2	76	77	..	3	..	1
65	91	..	..	..	..	169	213	..	..	1	2
67	112	..	..	1	2	158	184	..	..	1	2
91	139	1	..	1	1						
137	149	..	..	2	3	1706	2160	5	7	12	22

The recombination percentages from table 14 were aristaless dachsous 0.3, aristaless star 1.2, and dachsous star 0.9 percent.

Aristaless expanded double recessive

An expanded star fly, from table 11, was crossed to aristaless, and the F_1 star flies were backcrossed to expanded. The expanded not-star flies that occurred were then crossed to aristaless. None of the offspring of this later type of cross showed aristaless. From this result it was clear that aristaless and expanded lie on the same side of star; for if the orientation had been

$$\frac{e_x \quad S \quad +}{+ \quad + \quad a_1} \text{ or } \frac{a_1 \quad + \quad +}{+ \quad S \quad e_x}$$

then all the recombinational expanded flies would have produced aristaless.

The two-point data summarized in table 12, showed that the loci aristaless and expanded must be very close indeed to each other since the expanded star recombination percent was 1.5, and the aristaless star percent 1.3, which is very little different.

Aristaless star by expanded backcross

A second experiment was started by mating a female that carried aristaless and star in one chromosome and expanded in the other to aristaless males. Twenty-nine of the recombinational aristaless not-star flies were mated to expanded, and at least two gave expanded flies. These were inbred, and in the next generation, aristaless expanded flies appeared. This fact showed that the orientation of the loci was

$$\frac{a_1 \quad + \quad S}{+ \quad e_x \quad +} \text{ or } \frac{S \quad + \quad a_1}{+ \quad e_x \quad +}.$$

Two three-point backcrosses (table 18) confirmed this order and gave an aristaless expanded recombination percent of 0.05, and an expanded star recombination percent of 1.7. The data in the second experiment (right column) gave unusually little expanded star crossing over, except in one culture (the last) that gave unusually much.

TABLE 18

P_1, aristaless star×expanded; BC., F_1 star ♀×aristaless expanded ♂. (June 1925).

ARISTALESS STAR	EXPANDED	ARISTALESS EXPANDED	STAR	ARISTALESS	EXPANDED STAR	ARISTALESS STAR	EXPANDED	ARISTALESS EXPANDED	STAR	ARISTALESS	EXPANDED STAR
85	97	..	..	1	1	51	52	..	1	..	1
25	31	..	..	..	1	28	25	..	..	1	1
61	62	..	..	..	3	87	70	..	..	1	..
106	86	..	..	1	2	109	96	..	..	..	..
13	17	..	..	..	..	18	10	..	..	..	..
116	89	..	..	4	1	46	37	..	..	..	..
87	57	..	..	2	3	50	76	..	..	..	..
27	26	..	..	1	2	104	114	..	..	7	3
17	16	..	..	1	..						
75	71	..	..	..	..	1105	1032	..	1	19	18

Expanded dachsous double recessive

The previous experiments had shown that aristaless and dachsous lie on the same side of star; likewise that aristaless and expanded lie on the same side of star. From this it follows that expanded and dachsous are on the same side of star.

The data of table 12 had given 1.5 percent of recombination for expanded star and 0.7 for dachsous star. Accordingly the attempt to obtain an expanded dachsous stock was made on the assumption that the locus of expanded is further from star than is the locus of dachsous. Expanded star flies were bred to dachsous and the F_1 star females were backcrossed to expanded males. The expanded not-star flies produced as the result of crossing over were inbred (4 ♀×4 ♂). It was expected that part of the expanded flies should be of the constitution

$$\frac{e_x \qquad d_s}{e_x \qquad +},$$

coming from crossing over between the loci of two recessives in the

$$\frac{e_x \qquad + \qquad S}{+ \qquad d_s \qquad +}$$

mother. The remainder should be of the constitution

$$\frac{e_x \quad +}{e_x \quad +}$$

coming from crossing over between the loci of dachsous and star. If two of the first type be bred together, then the double recessive would be produced in the next generation. The inbreeding did not produce the double type. Evidently if there were present flies of the first type they had mated only with flies of the second type. The flies were then remated to dachsous, and in the offspring of some, dachsous flies appeared

$$\frac{e_x \quad d_s}{+ \quad d_s}$$

These were inbred and the double recessive was obtained. This fact showed that the order is, as assumed, expanded, dachsous, star (or star, dachsous, expanded).

It had been anticipated that there would be considerable difficulty in distinguishing the double recessive expanded dachsous from expanded, since the main characteristic of dachsous is crossveins close together, and this feature is likewise present in expanded. However, it was found that the shortness of wings in dachsous was very apparent in the double form, which had wings practically as broad as long. The expanded dachsous flies were small and rather low in viability and inclined to sterility, so that much trouble was experienced in carrying on stocks and securing crosses. This difficulty was solved by crossing expanded dachsous to curly, a second-chromosome dominant character that is lethal when homozygous and whose gene occurs in a chromosome that also carries crossover suppressors. The F_1 curly flies, when bred together, gave a stock that was mostly curly flies heterozygous for expanded dachsous with a few expanded dachsous homozygotes.

Expanded star by dachsous backcross

For determining the recombination percentages for expanded dachsous and star, an

$$\frac{e_x \quad + \quad S}{+ \quad d_s \quad +}$$

backcross was carried out (table 19). The males used in backcrossing were usually curly over expanded dachsous, and the curly offspring were discarded before making the separations for the three other characters. The data gave 0.2 as the expanded dachsous, and 1.2 as the dachsous star recombination percent.

TABLE 19

P_1, expanded star × dachsous; BC., F_1 star ♀ × expanded dachsous curly. (July 1925).

EXPANDED STAR	DACHSOUS	EXPANDED DACHSOUS	STAR	EXPANDED	DACHSOUS STAR	EXPANDED STAR	DACHSOUS	EXPANDED DACHSOUS	STAR	EXPANDED	DACHSOUS STAR
121	129	1	..	3	3	17	28	..	..	1	..
57	61	..	..	..	1	48	51	..	..	1	..
58	80	..	..	2	..	53	64	..	..	..	..
42	64	..	..	..	..	49	54	1	..	..	1
52	61	..	1	..	..	56	45	..	..	..	2
44	57	..	..	1	1	61	64	..	..	4	1
60	71	..	1	..	2	62	68	..	..	1	..
30	44	..	..	..	..	51	64	..	..	1	..
65	89	..	1	1	..	55	70	..	..	..	..
46	57	..	1	..	..	43	66	..	..	..	2
48	67	..	..	1	..						
						1118	1354	2	4	16	13

THE ORDER OF THE LOCI

The three-point experiments reported in Part II allowed the order of the loci telegraph, aristaless, expanded, dachsous and star to be deduced. Dachsous was found to lie on the same side of star as telegraph

$$\frac{t_g \quad + \quad S}{+ \quad d_s \quad +},$$

table 16, as aristaless

$$\frac{a_1 \quad + \quad S}{+ \quad d_s \quad +}$$

table 17, and as expanded

$$\frac{e_x \quad + \quad S}{+ \quad d_s \quad +}$$

table 19. Furthermore, in each case it was found to lie nearer to star than does each of these other loci. Similarly, expanded lies on the same side of star as does telegraph

$$\frac{t_g \quad + \quad S}{+ \quad e_x \quad +}$$

table 14 and aristaless

$$\frac{a_1 \quad + \quad S}{+ \quad e_x \quad +},$$

table 18 and is nearer than is either telegraph or aristaless.

Aristaless dumpy by star backcross

The final point to be determined was whether the group of the loci aristaless, telegraph, expanded and dachsous lies to the left or to the right of star. Star had been regarded as the zero point, and the other genes of the second chromosome arranged in order to the "right" of it. By using in a three-point backcross with star any member of the group in question and any locus known to be to the right of star, the relative order could be determined. Accordingly, an aristaless dumpy by star backcross was carried out (table 20). None of the more than 2000 flies of this backcross

TABLE 20

P_1, *star×aristaless dumpy; BC., F_1, star ♀×aristaless dumpy ♂. (Nov. 1925).*

ARISTALESS DUMPY	STAR	ARISTALESS STAR	DUMPY	ARISTALESS	STAR DUMPY
83	105	2	2	17	16
96	93	..	..	19	7
111	131	..	1	14	12
113	101	..	..	22	16
101	137	2	2	16	16
147	164	1	..	20	9
120	107	1	..	12	11
147	118	1	..	12	13
918	956	7	5	132	100

showed all three characters or were wild type. Therefore these classes constitute the double crossover classes, and the order of loci is

$$\frac{a_1 \quad + \quad d_p}{+ \quad S \quad +}.$$

Thus, star is no longer the zero point of the second chromosome. On the basis of the data of this paper a new map may now be constructed.

CONSTRUCTION OF MAP

In table 21, the recombination data of the preceding sections have been summarized.

In determining the distances between loci two lots of data have been disregarded, namely, all recombination percentages in which the untrustworthy character telegraph is involved, (telegraph dachsous and telegraph star), and the expanded star data of table 7, that had been collected before sufficient familiarity with expanded and its interactions with star had been acquired.

TABLE 21
*Summary of recombination data**.

LOCI	EXPERIMENT	TABLE NO.	TOTAL	RECOMBINATIONS	PERCENT RECOMBINATION
Telegraph dachsous	[tel. star/dach.	16	1453	2	0.1]
Telegraph star	[tel./star	1	274	3	1.1]
	[tel./star	2	941	5	0.5]
	[tel./star	8	1169	11	1.0]
	[tel. star/exp.	14	1835	25	1.4]
	[tel. star/dach.	15	552	7	1.3]
	[(tel. star/dach.	16	1453	18	1.2)]
Aristaless expanded	aris. star/exp.	18	2175	1	0.05
Aristaless dachsous	aris.star/dach.	17	3912	12	0.3
Aristaless star	aris./star	3	334	3	0.9
	aris./star	9	4337	58	1.3
	aris.dumpy/star	20	2118	12	0.6
	Total		6789	73	1.1
	(aris.star/exp.	18	2175	37	1.7)
	(aris.star/dach.	17	3912	46	1.2)
	(Total		6087	83	1.4)
	Grand total		12876	156	1.2
Expanded dachsous	exp. star/dach.	19	2507	6	0.2
Expanded star	[exp./star	7	1346	54	4.0]
	exp./star	11	3060	46	1.5
	tel./exp. star	13	2164	21	1.0
	Total		5224	67	1.3
	(exp.star/dach.	19	2507	35	1.4)
	Grand total		7731	102	1.3
Dachsous star	dach./star	10	4269	31	0.7
	tel. star./dach.	16	1453	16	1.1
	aris. star/dach.	17	3912	34	0.9
	exp. star/dach.	19	2507	29	1.2
	dach.^{3}/star	6	396	3	0.8
	Total		12537	113	0.9
Star dumpy	aris.dumpy/star	20	2118	232	11.0

*The data enclosed by square brackets [] are considered untrustworthy. The data enclosed by parentheses () are "secondary" and are not used in calculating distances.

There is no certain evidence as to the relative order of aristaless and telegraph. But since aristaless is a trustworthy character that will be used extensively, and telegraph is relatively very poor, aristaless has been chosen as the zero of the new map. The locus of telegraph is probably of the order of 0.1 unit distant, and has been arbitrarily put to the right at $0.0\pm$.

The aristaless expanded recombination percent was 0.05, based on 2715 flies. Since at least two flies in the 29 tested in securing the aristaless expanded double recessive, proved to be crossovers between aristaless expanded, an independent calculation of the aristaless expanded distance can be made ($2 : 29 :: x : 1.3; x = 0.09$). In view of the high probable errors of such small percentages, an accuracy to 0.1 unit is beyond reach, so the nearest tenth, namely, 0.1, has been taken as the locus of expanded.

The next locus to the right is dachsous, which is 0.3 to the right of aristaless (3912 flies) and 0.2 to the right of expanded (2507 flies). These two determinations agree at the value $+0.3$ as the locus of dachsous.

The dachsous star data (12,537 flies) place star at 0.9 to the right of dachsous, or at 1.2 ($0.3+0.9$). The expanded star data (5,224) place it at 1.3 to the right of expanded, or at 1.4 ($0.1+1.3$). The aristaless star data (6,789) place star at 1.2 ($0.0+1.2$). The weighted average of these data places the locus of star at $+1.3$ (to the nearest tenth).

The final map is given in figure 1.

SUMMARY AND EVALUATION OF THE MUTANTS

The serial order and relative distances have been established for a group of five mutant loci, namely, star, telegraph, aristaless (aristaless^2), dachsous (dachsous^2, dachsous^3) and expanded. These linkage relations are expressed in the map given in figure 1, in which aristaless is the new zero point of the second chromosome and the locus of star is at 1.3.

The most useful of these mutants is star, primarily because of its dominance, which enables the mutant to be followed through extended and complex experiments with a minimum of labor in preparing stocks.

The next most useful mutant is aristaless, primarily because its locus is the one furthest to the left in the map. A second advantage is that it can be used more successfully than star with plexus, which is a very important mutant and which is partly suppressed by star. A disadvantage of aristaless is that its viability is poorer than that of star, being roughly 90 percent that of the wild type. In speed of classification aristaless is very high, in which respect star is fairly poor.

Dachsous had the best viability (99 percent) of any of the mutants at the left end, and dachsous[2] is probably slightly better even than dachsous. The classification of each is certain and fairly rapid. The disadvantage of the dachsous mutants is that dumpy, which is the most important mutant between star and black, also has crossveins that are close together, and it is probable, though not definitely known, that dachsous would be unworkable with dumpy. Dachsous[3] has very poor viability (50 percent) and hence will not be used except in situations in which its female-sterility is an advantage.

Expanded offers no advantages over aristaless or dachsous, though its viability is high (97 percent) and its general characteristics good.

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