X Chromosome Inversions and Meiosis in Drosophila melanogaster

G. W. Beadle, and A. H. Sturtevant

PNAS 1935;21;384-390
doi:10.1073/pnas.21.6.384

X CHROMOSOME INVERSIONS AND MEIOSIS IN DROSOPHILA MELANOGASTER

BY G. W. BEADLE AND A. H. STURTEVANT

WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated April 27, 1935

It has long been obvious that inversions, like other chromosome aberrations, should be useful in studying the nature of meiosis. Previous studies of inversions in Drosophila have not led to a general theory of the behavior of inversions that is subject to quantitative tests. Our experiments together with the published cytological observations on plants have led to such a theory, outlined below. The detailed data on which our conclusions are based will be published elsewhere.

Single Exchange.—One of the reasons no general theory has been developed is that it has been inferred that single crossovers within the inverted section are rare (e.g., Sturtevant,[1] Gershenson[2]). The results now to be presented show that this conclusion is incorrect.

With regard to single exchanges between segments inverted relative to one another we know the following facts:

1. Single exchange probably occurs in all inversions with which we have worked. (See table 1 for list and description of these.) For long inversions the frequency of single exchange apparently approaches the frequency characteristic of the same segments normally arranged. The arguments in support of this conclusion follow.

We can regard Inv. scute-8 as practically a normal chromosome with the spindle attachment transposed from the right to the left end (table 1). From Inv. scute-8 over Inv. scute-4, a slightly shorter inversion, single crossovers are recovered and their frequency can be measured with fair accuracy. The observed frequency of recombinations is approximately normal; hence we can argue that Inv. scute-4 against a normal chromosome gives approximately the normal frequency of single crossovers but that they are not recovered. Inversion scute-8 over Inv. scute-7, a relatively short inversion, gives, in the common inverted segment, single crossovers which can be recovered by mating to males which give duplications and deficiencies of appropriate length. Here the frequency cannot be accurately measured but our data suggest that single exchange frequency is reduced in Inv. scute-7 to approximately one-half to two-thirds of the normal for the segment which is inverted. In a similar way we have demonstrated the occurrence of single crossovers in the common inverted segment of several other combinations of inversions although in many cases the relative inviability of the crossovers makes an accurate measure of their frequency difficult.

A second method that we have used involves the use of attached X females heterozygous for inversions. Such females heterozygous for Inv. yellow-4 give, by single exchange in the inversion, either (1) a closed X chromatid which can be recovered, or (2) a chromatid with two spindle attachments forming a chromatid tie between the egg nucleus and the second polar body nucleus. From the distortion of the sex ratio in the progeny it is inferred that the second result gives inviable eggs. The distortion of the sex ratio is therefore itself a measure of the frequency of single exchange. Both the number of recovered closed chromatids and the distortion of the sex ratio show that single crossovers occur with approximately normal frequency. Attached X females heterozygous for Inv. scute-7 give results as above, except that the closed chromatid cannot be recovered directly. Distortion of the sex ratio gives results in approximate agreement with those from Inv. scute-8/Inv. scute-7 discussed above. The sex ratio in $\widehat{XX}$ females heterozygous for Inv. delta-49 indicates that single crossovers within the inversion occur, but with a low frequency.

2. Single exchange must result in one chromatid with two spindle attachments and one with none, leaving two non-crossover normal chromatids. This is shown directly by cytological observations in Zea[3] and Trillium.[4] These cytological observations are in agreement with the genetic evidence given in the paragraph above in indicating that single crossovers occur within inverted segments.

3. Chromatids with two spindle attachments must form a tie between the first division nuclei, or between one pair of second division nuclei following a three-strand double, one exchange of which is inside, the other outside the inversion.

4. In Drosophila such a tie either does not break, or if it does the fragments are rarely or never included in the egg nucleus. This conclusion follows from the fact that duplication- or deficiency-carrying zygotes do not occur in the progeny of females heterozygous for inversions known to give single crossovers.

5. A single chromatid tie between first division nuclei does not result in the production of an inviable egg or a zygote inviable at a later stage. This conclusion comes from experiments in which egg and larval-pupal mortality were measured. Inversions known to give a high frequency of single crossovers give low egg mortality, and a mortality at later stages not higher than normal controls. Observed egg mortalities for certain inversions are as follows:

Inv. scute-8/+	6.8%
Inv. scute-4/+	7.5%
Inv. yellow-4/+	7.7%

If single exchanges gave rise to inviable eggs in half the cases, we should expect for these inversions approximately 50 per cent inviability.

The above facts, together with the fact that the second meiotic division spindles in the Drosophila egg lie with their axes approximately on the same straight line and form four nuclei likewise in a line, the inner one of which becomes the egg nucleus (Huettner[5]), lead us to propose the following scheme:

1. A single chromatid tie between two spindle attachments, one in each first division nucleus, prevents the tied daughter spindle attachments from going into the end nuclei but results in conditions (possibly mechanical orientation) such that the two normal chromatids always go to the end nuclei, one to each (Fig. 1). The chromatid with no spindle attachment goes to neither pole and is lost.

2. When both daughter spindle attachments of a chromosome in one first division nucleus are tied to spindle attachments in the other nucleus, nothing from the particular tetrad under consideration can go to the end nuclei during the second division (Fig. 1).

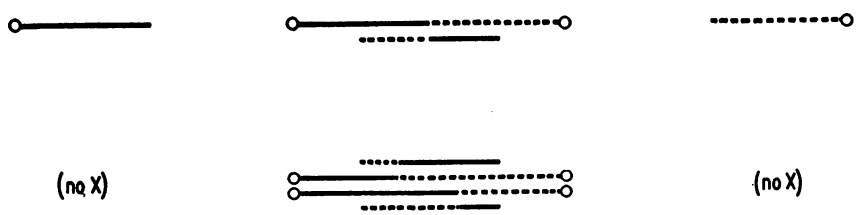

FIGURE 1

Above: the products of meiosis in the egg following a single exchange within a heterozygous inverted segment. Below: as above following a four-strand double exchange within the inverted segment. In both cases only the *X* chromosome tetrad is figured; each product, of course, contains one set of autosomes not shown in the diagrams. Either of the end products can be taken to represent the egg nucleus.

3. When the egg nucleus contains a spindle attachment with a chromatid tie to a spindle attachment in the second polar body nucleus, the egg is inviable, i.e., gives rise to a zygote which dies in the egg stage.

Applying the above hypotheses to the heterozygous inversion case, it is seen that: (1) Single exchange tetrads undergo reduction in such a way that only non-crossover chromatids are distributed to the end nuclei; the crossover chromatids are selectively eliminated. In half the cases of single exchange in the inversion accompanied by single exchange outside (three-strand doubles), one pair of second division nuclei will be connected by a chromatid tie,[4] which if it involves the inner nucleus, will result in an inviable egg. Except for the case of Inv. scute-7, lethal eggs from this source would be of negligible frequency in the inversions considered here. (2) Two-strand double exchange tetrads undergo reduction in the normal way; no chromatid ties are formed. (3) Three-strand double exchanges are equivalent mechanically to single exchanges but give rise to an egg nucleus

containing either a non-crossover or a double crossover chromatid. (4) Four-strand double exchanges give nothing but no-*X* nuclei. (5) Triple exchanges of the various possible types give results which can readily be worked out; the results of all of the types, occurring with frequencies determined by random exchange, together with a summary of the results from singles and doubles, are as follows:

EXCHANGE	NON-CROSSOVERS	DOUBLE CROSSOVERS	NO-*X* EGGS
None	1		
Single	1		
Double	3	3	2
Triple	7	21	4

The relations discussed below can be considered as tests of the above scheme.

1. There should be a quantitative relation between recovered double crossovers and patroclinous males. Inversions which give no double crossovers should give a low frequency of patroclinous males. (Normal females give some, presumably as the result of an independent mechanism.) With longer inversions the frequency of doubles increases and likewise the frequency of patroclinous males should increase. The frequencies of patroclinous males from several inversions are given in table 1.

TABLE 1

INVERSION	ENDS OF INVERSION (MAP POSITION)	PRIMARY EXCEPTIONS FEMALES %	PRIMARY EXCEPTIONS MALES %	SECONDARY EXCEPTIONS FEMALES %	SECONDARY EXCEPTIONS MALES %
+/+ (standard control)		0.02	0.08	4.3	4.3
Scute-7/+	0+ to 14±	0.09	0.28	12.9	15.1
Delta-49/+	11± to 42±	0.00	0.20	45.4	41.9
ClB/+	6± to 59.4	0.25	0.47	36.6	37.8
Bobbed-df/+	9± to 64±	0.00	1.95	13.7	15.9
Yellow-4/+	0− to 61±	0.05	2.34	6.8	8.9
Scute-4/+	0+ to 64±	0.02	5.75	4.3	13.4
Scute-8/+	0+ to 66	0.02	3.20	9.9	13.8*
Scute-4/delta-49		0.00	0.17		
Scute-8/delta-49		0.26	0.34	47.3	43.5
ClB/delta-49		0.32	0.16		
Delta-49/yellow-4		0.32	0.13	53.2	53.2

Scute-7 inversion and Inv. delta-49 give few or no doubles; patroclinous males are very low in frequency. ClB inversion gives recovered doubles with a low frequency; patroclinous males are low but higher than from Inv. scute-7 or Inv. delta-49. The other inversions give appreciable frequencies of doubles. The relation between doubles and patroclinous males from experiments where practically all doubles could be detected in the males and where, at the same time, patroclinous males could be detected, are as follows:

CONSTITUTION	REGULAR MALES	CROSSOVER MALES	PATROCLINOUS MALES
Bobbed-df/+	3261	93	66
Yellow-4/+	2063	57	51
Scute-4/+	1090	108	63
Scute-8/+	1677	93	57
Scute-8df/+ (lethal in males—numbers corrected)	422	60	31
Total		411	268

From double exchanges we expect 3 double crossover males to 2 patroclinous males. None of the counts shows significant deviations from this ratio and the total gives a ratio of 3.22 to 2, a deviation from 3:2 not statistically significant. From the longer inversions we expect a low frequency of triple exchanges which would change the ratios to 3 +:2. Actually the inversions giving the highest frequencies of crossovers show the greatest deviation from a 3:2 ratio; these deviations are in the expected direction.

2. Reducing crossing-over in a long inversion by putting it over a shorter one should decrease the frequency of patroclinous males as well as of double crossovers. We know that, from the combinations given in table 1, no double crossovers are recovered. Patroclinous males are very low in frequency.

3. The frequency of exceptional females should be as low as that from normal females and should not vary with crossing-over. From table 1 it is seen that this expectation is approximately realized.

4. The frequency of inviable eggs from females carrying an inversion should be equal to the frequency of patroclinous males (no-X eggs fertilized by Y and X sperms should account for these two classes). The egg mortality data from inversions considered in relation to more or less comparable normal controls (strictly comparable controls cannot be had), show that this is so.

It is clear, then, that the hypothesis is in reasonably good agreement with the available evidence from heterozygous inversions.

There is another test which can be applied, namely, the behavior of heterozygotes and homozygotes for a closed X chromosome. These have been studied by L. V. Morgan.[6] Comparing the published data with expectation according to the above scheme, we find two important discrepancies: (*a*) The non-crossover classes from X/X^c are not equal, there being an excess of X over X^c individuals. (*b*) The inviable eggs from X/X^c are much higher in relation to doubles than expected. Experiments using a second closed X chromosome found by Mr. R. D. Boche of this laboratory show that both of these discrepancies are due to viability effects. Data from both heterozygotes and homozygotes for this new closed X chromosome will be considered in detail in another publication, but we can say here that all of the data are in substantial agreement with the scheme under consideration.

Secondary Non-Disjunction.—Heterozygous inversions give secondary non-disjunction with a frequency which shows a very strong negative correlation with crossing-over. This can readily be seen to be true in table 1. Inversions ClB and delta-49 which show low crossing-over for the whole chromosome give high frequencies of secondary exceptions. Combinations of long against short inversions likewise give high frequencies. Since XXX and YO zygotes are lost the frequency of exceptional gametes will differ from that of exceptional zygotes; where q is taken as the proportion of exceptional zygotes and p the proportion of exceptional gametes, $p = \frac{2q}{1+q}$. From the data given in table 1 and from additional data not included here, p appears to approach 0.667 as a maximum. Since the $X-Y$ synapsis scheme proposed by Bridges[7] gives a maximum p value of 0.5, clearly below that indicated by the inversion data, it becomes necessary to search for a new interpretation of secondary non-disjunction. We know that secondary exceptional gametes practically always come from non-crossover X tetrads. Such tetrads in the presence of a Y chromosome evidently undergo a preferential segregation similar to that studied by Sturtevant[8] for the fourth chromosome. Non-crossover X tetrads plus a Y must give a higher frequency of XX-Y than X-XY separation. The data are inadequate to measure accurately the extent of this preference. The constant value 0.667 (limiting value for certain types of fourth chromosome arrangements) gives results that are not unreasonable. Additional experimental data are needed.

General Conclusions.—It has been shown above that crossovers between X chromosome segments inverted with respect to one another influence the orientation of the meiotic tetrads in such a way as to eliminate the single crossover chromatids, leaving a normal non-crossover one in the reduced egg nucleus. The more complex results expected from multiple exchange are analyzed and shown to give results in agreement with observation. It seems probable that the scheme outlined in this paper will apply for the case of inversions that do not include the locus of the spindle attachment and for other chromosome aberrations in which crossing-over gives rise to chromatids with two spindle attachments, and where the conditions of meiosis are such that (*a*) the meiotic spindles are oriented so that the reduced nuclei lie approximately on a single straight line, and (*b*) only one of the terminal nuclei functions in further development. These conditions obtain in megasporogenesis in most seed plants, as well as in oögenesis in most animals.

Discussion of the bearing of this scheme on the mechanism of normal disjunction, on the evolutionary significance of inversions, and similar elaborations, is omitted for lack of space.

[1] Sturtevant, A. H., *Pub. Carnegie Inst. Wash.*, **421**, 1–27 (1931).
[2] Gershenson, S., *Jour. Genet.*, **30**, 115–125 (1935).
[3] McClintock, B., *Z. Zellforsch.*, **19**, 192–237 (1933).
[4] Smith, S. E., *Jour. Genet.*, **30**, 227–232 (1935).
[5] Huettner, A. F., *Jour. Morph. Physiol.*, **39**, 249–265 (1924).
[6] Morgan, L. V., *Genetics*, **18**, 250–283 (1933).
[7] Bridges, C. B., *Ibid.*, **1**, 1–50 (1916).
[8] Sturtevant, A. H., *Proc. Nat. Acad. Sci.*, **20**, 514–518 (1934).

LINKAGE STUDIES OF THE RAT (RATTUS NORVEGICUS)

BY HELEN DEAN KING AND W. E. CASTLE

WISTAR INSTITUTE AND BUSSEY INSTITUTION, HARVARD UNIVERSITY

Communicated April 18, 1935

The mutated genes of the rat now on record are as follows:

A, a	agouti, non-agouti
B, b	black, chocolate
C, c^r, c	fully colored, ruby-eyed, true albino
Cu, cu	curly, normal coat
Cu_2, cu_2	curly, normal coat
D, d	intense, dilute
H, h^I, h	self, Irish, hooded
Hr, hr	normal coat, hairless
K, k	normal coat, kinky
P, p	fully colored, pink-eyed yellow
R, r	fully colored, red-eyed yellow

Of these *A*, *C* and *H* were found by Castle to be independent of each other, but *P*, *R* and *C* were found by Castle and Wright, Dunn and Wachter to lie in a common linkage system. Subsequently Roberts discovered genes *d* and *hr* and showed that they are probably independent of the genes previously known (*A C H P* and *R*). More recently King has discovered the dominant mutation, curly (*Cu*), and the recessive mutation, brown or chocolate (*b*). Linkage studies on these newly discovered genes will be reported in this paper. Gregory has recently described a second dominant curly mutation apparently distinct from the curly described by King; and Feldman (unpublished data) has found a recessive mutation called *kinky*, which even more strongly than the "curly" mutations has a tendency to shorten the hair and make it curly. We are indebted to Dr. Feldman for an opportunity to study the kinky mutation in advance of his forthcoming publication concerning it.

The increasing number of mutated genes increases the probability that additional genetic linkages will be discovered, if search is made for them,