THE TETRASOMIC FOR CHROMOSOME 4 IN DROSOPHILA MELANOGASTER

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DROSOPHILA with one more or less 4th chromosome than the normal pair were first described by BRIDGES (1921a,b). Haplo-4 flies with only one 4th chromosome were discovered because of their obvious complex of abnormal characters. Most conspicuously they are Minute; that is they have short slender bristles. Triplo-4 flies do not differ greatly from normal flies in phenotype, but according to BRIDGES they do have smaller, smoother eyes, narrower and more pointed wings, darker body color and less pattern on the thorax than wild-type flies. Triplo-4 flies of both sexes are vigorous and fertile.

One half of the gametes of triplo-4 flies are expected to carry two 4th chromosomes. A zygote resulting from the union of an egg nucleus possessing two 4th chromosomes with a sperm also carrying two 4th chromosomes will have four 4th chromosomes (tetra-4). BRIDGES (In MORGAN, BRIDGES and STURTEVANT 1925) interpreted genetic data from parents each of which were triplo-4 to indicate lethality of the tetra-4.

LI (1927) made egg counts of triplo-4 females mated to triplo-4 males. Thirty to 40 percent more progeny from this cross died in the egg and larval stages than from crosses in which one or both parents were diplo-4. He concluded that all tetra-4 Drosophila die before reaching adulthood. He believed that most of them do not survive the embryonic stages and the remainder die during larval life. The resolution afforded by LI's or BRIDGES' experiments does not exclude infrequent survival into the adult stage.

BRIDGES (1935) reported that he found one female pupa with four 4th chromosomes in oogonial cells. This indicated that tetra-4 Drosophila survive at least on occasion into the pupal stage. SCHULTZ (1935) states that he may have seen a tetra-4 adult with four doses of the 4th chromosomes mutant, shaven. He described the fly as having extra bristles, but offered no genetic or cytological evidence that it was indeed a tetra-4.

The difficulty in proving by genetic methods that flies are tetra-4 arises from the lack of suitable genetic markers on chromosome 4. To circumvent this difficulty, translocations of the 4th chromosome with other chromosomes can be used. The markers on the other chromosome parts of the translocations serve to indicate the presence of the 4th chromosomes. This method has been used in the experiments reported here to demonstrate that flies carrying the genetic material of four 4th chromosomes survive, even though infrequently, into the adult stage.

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MATERIALS AND METHODS

Chromosomes: T(1;4)w^{m5} T(1;4)B^S, w^{m5} *v* B^S was obtained by D. L. LINDSLEY and E. S. VON HALLE (personal communication) by crossing over in a female heterozygous for T(1;4)w^{m5} and T(1;4)B^S *y cv v* B^S (see BRIDGES and BREHME 1944 for definition of mutant symbols). T(1;4)w^{m5} is a reciprocal translocation between the X and 4th chromosomes with breaks at salivary gland chromosome map positions 3C2-3 and 101 F (BRIDGES and BREHME 1944). T(1;4)B^S has breaks at 16A1 (BRIDGES and BREHME 1944) and 102F (LEWIS 1956). The derivation of the crossover product is shown diagrammatically in Figure 1. The crossover produced three centric chromosome fragments with the genetic material of an X chromosome and two 4th chromosomes.

T(1;4)w^{m5} T(1;4)h2 was produced by a crossover in a female heterozygous for T(1;4)w^{m5}, w^{m5} *v* and T(1;4)h2, *y*. T(1;4)h2 is a reciprocal translocation between the X and 4th chromosomes with a break at salivary gland map position 9A in the X chromosome (D. L. LINDSLEY, personal communication). The derivation of T(1;4)w^{m5} T(1;4)h2 is shown in Figure 2. In T(1;4)h2 the break in

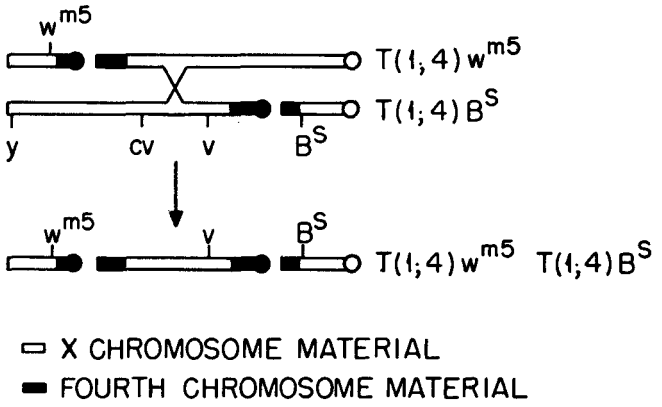


FIGURE 1.—Derivation of T(1;4)w^{m5} T(1;4)B^S.

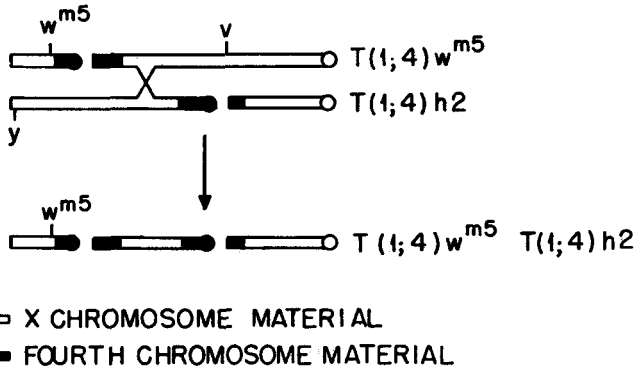


FIGURE 2.—Derivation of T(1;4)w^{m5} T(1;4)h2.

the X chromosome was near the locus of vermilion (v). A recombinant which was y^+ w^{m5} v^+ from the heterozygous female carried an X chromosome in three fragments with two 4th chromosomes on these fragments.

Two slightly different X chromosome balancers were used. They were derivatives of FM4 (MISLOVE and LEWIS 1954) and had the inversions of FM4. Both carried an X-ray-induced white allele, w^{55f}, not present in the original FM4 and in one of the chromosomes B has been replaced by f. The complete designations of the balancers were then FM4, y^{31d} sc^8 w^{55f} dm B and FM4, y^{31d} sc^8 w^{55f} dm f. The FM4 inversions were effective crossover suppressors and it is assumed that there can be no recombination between the balancer and the translocations.

The object of the crosses shown in Figure 3 was to derive a stock in which the females were heterozygous for T(1;4)w^{m5} T(1;4)B^S and a balancer and males that were hemizygous for the X;4 translocations. The ci^D-marked 4th chromosome was used in order to select against a free 4th chromosome in the stock. It was not essential to have eliminated the free 4th chromosome, but it was done to avoid possible ambiguity. The only 4th chromosomes in the stock were therefore those involved in the translocations. Both males and females were then diplo-4.

T(1;4)w^{m5} T(1;4)h2 was balanced with FM4, y^{31d} sc^8 w^{55f} dm B using the same sort of crosses used to balance T(1;4)w^{m5} T(1;4)B^S.

The cytological preparations were squashes stained in lacto-aceto-orcein as described by NICOLETTI (1959). The preparations were analyzed and photographed with a Zeiss Photomicroscope.

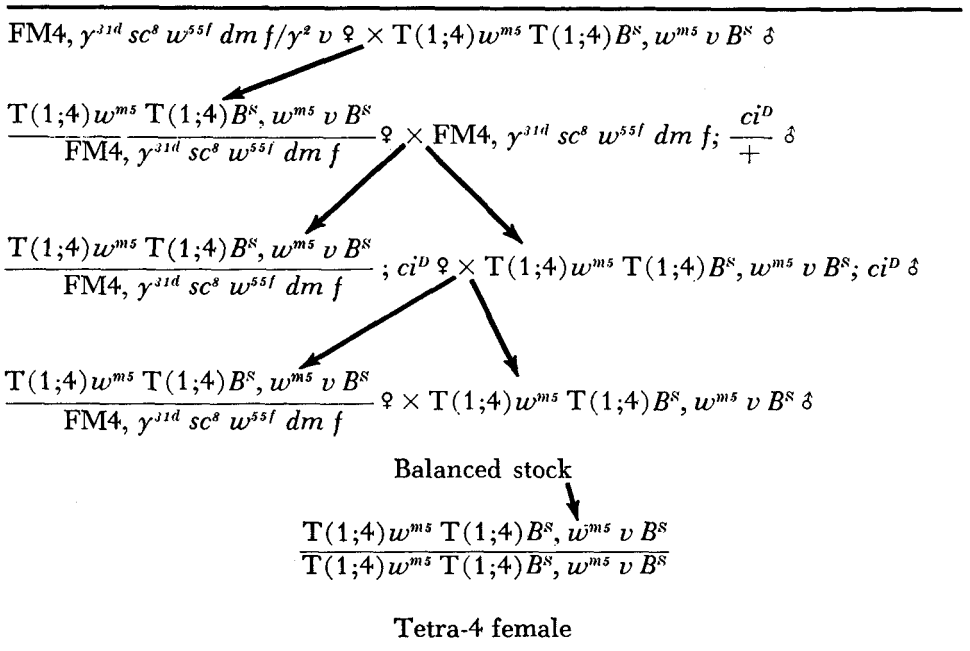


FIGURE 3.—Production of balanced stocks of T(1;4)w^{m5} T(1;4)B^S and the origin of tetra-4.

RESULTS

Production of tetra-4 females: The two balanced stocks gave rise to flies homozygous for T(1;4)w^{m5} T(1;4)B^S and T(1;4)w^{m5} T(1;4)h2. These homozygotes were females since they carried the material of two X chromosomes. They also had to be tetra-4 and carry the chromosome material of four 4th chromosomes. They occurred in the stocks with a frequency of one to two homozygotes per hundred heterozygous females. The genetic markers were such that identification of the homozygote was certain. First, the homozygote for T(1;4)w^{m5} T(1;4)B^S, w^{m5} v B^S had vermilion eye color with some mottling of w^{m5}. The size of the eyes was greatly reduced by the presence of homozygous B^S, but enough facets were present to make the vermilion eye color determination with certainty. On the other hand, the heterozygote for the translocation and the balancer had wild-type eye color. Secondly, the sc^s inversion in the FM4 balancer causes a variegation for Hairy-wing which results in the presence of a few hairs in the mesopleural region of the thorax. This Hairy-wing effect is dominant and is observed in the heterozygote of the translocations and the balancer but not in the homozygote. Both these pieces of evidence demonstrate that the balancer was not present in flies identified as homozygotes (and tetra-4's).

The possibility that some portion of the translocation was missing from the homozygote may be excluded for the following reasons: (1) A deficiency of the middle portion (3C2–3 to 16A) was clearly inviable. (2) Deficiencies for the distal portion (1 to 3C2–3) or the proximal portion (16A1 to the centromere) were also inviable and if they had survived they would have been expected to show Minute phenotypes; the flies identified as homozygotes were not Minute.

The homozygote of T(1;4)w^{m5} T(1;4)h2,w^{m5} was phenotypically distinguishable from the heterozygote of the translocations and the FM4, y^{31d} sc^8 w^{55f} *dm* B balancer by the B^+ phenotype and by the absence of the Hairy-wing effect on the mesopleura of the homozygote. The possibility that a part of the translocations was absent could again be excluded for the aforementioned reasons with the other translocation homozygote.

Cytological observations: The cytological observations on chromosomes in brain squashes and salivary glands confirmed the conclusions based on genetic evidence. Mitotic metaphases in larval brain tissue of tetra-4's had four large chromosomes and six smaller chromosomes. The large chromosomes were the second and third chromosomes and the six smaller ones made up the two X chromosomes and the four 4th chromosomes.

Salivary gland preparations (Figure 4) clearly show the three parts of the X chromosome. The sections from the tip to 3C2 and 9A to the centromere extend out of the chromocenter. The region between 3C2 and 9A forms a loop with both ends in the chromocenter. Unfortunately, it has not been possible to identify the translocated 4th chromosomes in salivary gland preparations.

Characteristics of tetra-4 females: The most noticeable abnormality of the tetra-4 females was the shape of the wings. Compared to diplo-4 females the wings were slightly longer and more pointed (see Figures 5 and 6). BRIDGES found that triplo-4's also have narrow wings and this character is accentuated in

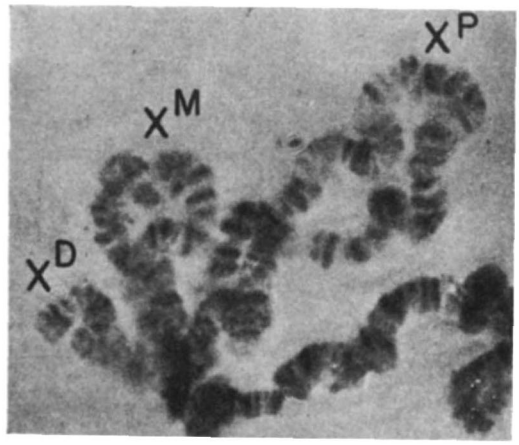
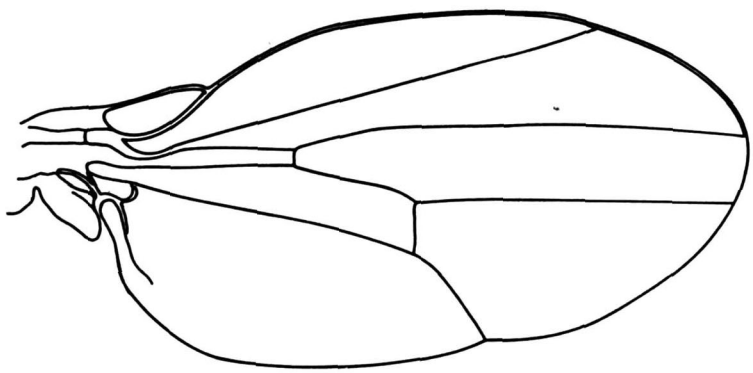


FIGURE 4.—T(1;4)w^{m5} T(1;4)h2 Salivary gland chromosomes. X^D is region from the tip of the X chromosome to 3C2, X^M is the region of the X chromosome between 3C3 and 9A, X^P is the region between 9A and the centromere.



WING OF DIPLO–4 FEMALE

FIGURE 5.—Drawing of wing from normal diplo-4 Drosophila female.

tetra-4's. The abdomens of tetra-4's were slightly longer and narrower than they were in diplo-4's. One reason for the narrow abdomen is that the tetra-4's did not contain as many eggs as normal females. The fertility of tetra-4 females was very low. They produced about ten progeny per female. If tetra-4 females are mated to males carrying the same X;4 translocations, a stock should be established in which all females are tetra-4. Nearly all of the progeny of such matings were males. The tetra-4 females were not produced in sufficient numbers even to replace their mothers.

Their low frequency of appearance in these translocation stocks may be attributed to their low viability. Another factor probably tending to reduce the number was the segregation of the chromosome fragments in their parents. Tetra-4's arise from the union of two gametes, both of which carry the three parts

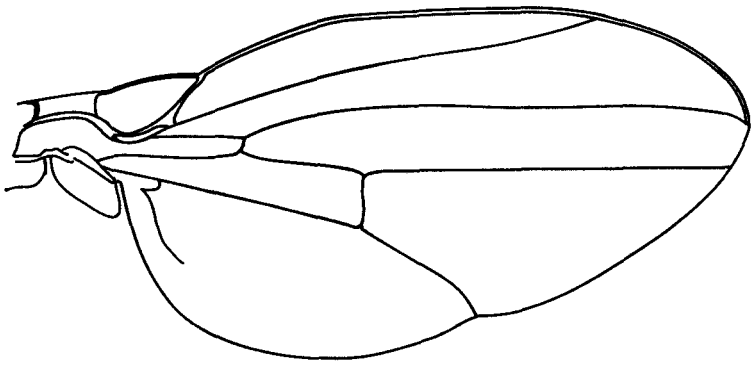


FIGURE 6.—Drawing of wing from tetra-4 Drosophila female.

of the double translocation. Such gametes were not common because parts on 4th chromosome centromeres probably tended to pass to opposite poles in the first meiotic division.

DISCUSSION

The tetrasomics for chromosome 4 described in this report are not the classical sort of multisomic since the multisomic chromosome is involved in rearrangements. There seems to be no reason to doubt that all of the chromosomal material of chromosome 4 is present in these animals in four doses, but the breaks in the chromosomes may have an effect on the viability of these tetra-4's. BRIDGES (1925) and LI (1927) were dealing with intact 4th chromosomes and could not find adult tetra-4's. The rearranged chromosomes may be less deleterious to the animals than the normal chromosomes. On the other hand their methods were not sensitive and these workers may have overlooked a rare tetra-4 adult.

Only tetra-4 females have been produced although chromosomes were available to yield tetra-4 males. There may be a difference in the viability of male and female tetra-4's. In the case of haplo-4's, the males are more viable than females.

Using modifications of the translocation technique just described the effect of higher degrees of multisomy could be tested without difficulty. Since tetra-4's seem to be so inviable, any higher degree of multisomy for chromosome 4 in otherwise diploid *D. melanogaster* is likely to be completely lethal before the adult stage is reached.

SUMMARY

1. The tetrasomic for chromosome 4 has been produced in *Drosophila melanogaster*. Females homozygous for T(1;4)w^{m5} T(1;4)B^{S} or T(1;4)w^{m5} T(1;4)h2 have both X chromosomes divided into three parts. Translocated onto each

tripartite X are two 4th chromosomes. Females with two tripartite X's then carry the chromosmal material of four 4th chromosomes.

2. The tetra-4 females have low viability and fertility. They are phenotypically somewhat abnormal. Their most characteristic feature is wings that are longer and more pointed than normal.

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