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Partial Sterility in Drosophila Melanogaster; Schemes for Complex Chromosome Rearrangements

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ABSTRACT — These investigations are pilot studies of possible chromosome rearrangements to effect sterility in insects. Techniques of oviposition and partial sterility in *Drosophila melanogaster* are described. Partial sterility is measured for a number of different crossing combinations using wild-type stocks, heterozygous translocation stocks, and homozygous translocation stocks: the highest percent partial sterility occurring when F-1 intercross translocation stocks are used as one of thparents. Three different crossing schemes to gain more complex chromosome rearrangements and their outcomes are reported, though tests indicated negative results in these. Methods devised to distinguish between simple and complex translocation stocks in *Drosophila* are deemed important for future work in this area.

Research in control of insect pests through chromosome osome rearrangements has increased considerably in recent years. The significance of such research lies within economic entomology. Although progress continues in the development of insecticides, the use of active chemicals engenders problems due to their toxic effects and pollution properties. Among the various ideas that have surfaced, there exists considerable interest in the possible use of chromosome rearrangements for this control. The data being presented here have been accumulated by a team of undergraduate student researchers at Saint Mary's College in Winona, Minnesota. Studies in chromosome translocations in *Drosophila melanogaster*, are described, along with schemes to synthesize more complex chromosome rearrangements, and the techniques used to distinguish between simple and complex translocations.

Oviposition and Sterility

Drosophila stocks T(2; 3)g163d and T(2; 3)175 were obtained from Phillip T. Ives, Amherst College; all T(2;3) 63 and 64 series from Claude Hinton, College of Wooster; and all wild-type and genetic marker stocks from the *Drosophila* Stock Center, Bowling Green University. All stocks were maintained on Formula 4024 standard medium obtained from Carolina Biological Supply Company in control chambers at 21 C. with a 12 hours light/12 hours darkness regime. Virgin female and male flies of the desired crosses were placed into culture bottles with standard medium for two days. The flies were then transferred to bottles containing only cotton for six hours. These bottles were then inverted onto the surface of petri dishes containing an oviposition medium which is composed of 1.5% agar blackened with powdered charcoal. The agar surface was wiped with red wine and sprinkled evenly with brewer's yeast. The flies were removed and the eggs were counted after every 24-hour period. After an additional 48 hours the percentage of eggs hatch was tallied. Transfer of the flies of a particular cross to fresh oviposition medium was repeated an average of four times.

Table 1 shows the degree of sterility, calculated by the number of unhatched eggs, obtained from a variety of different types of crosses (Howard 1974; Pitzen and Mescher 1974; Neuroth 1975; Mikos 1975). Wild-type flies crossed amont themselves, gave the lowest degree of sterility at 9.5 percent. Stocks having a reciprocal chromosome translocation in a homozygous arrangement showed much higher partial sterilities when crossed with wild-type flies.

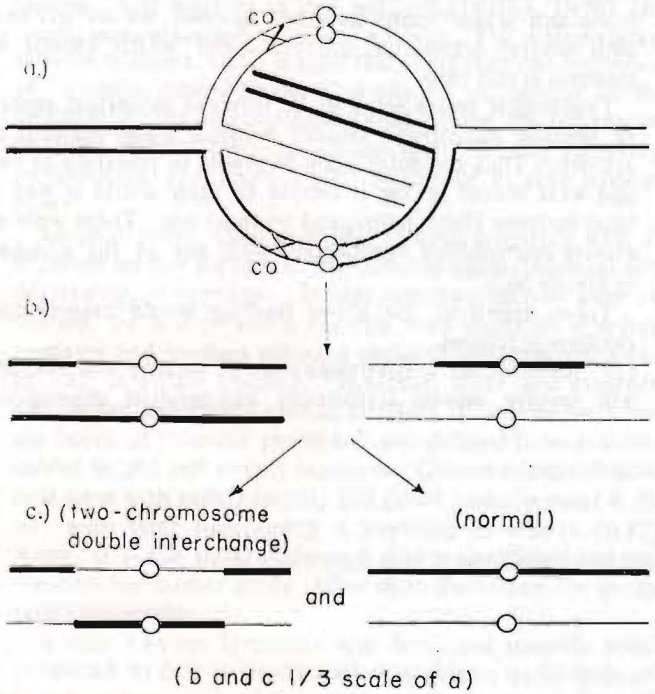

Figure 1. - Theoretical pachytene configuration of an intercross between stocks that each possess a translocation involving the same chromosomes, but with different points of exchange. The configuration is shown with single strands for clarity. (a) simultaneous crossing over in both differential segments; (b) the four chromosomes that result from these crossover events; (c) viable products of either a two-chromosome double translocation or the normal arrangement that can result from segregation at meiosis-II.

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TABLE 1 – PERCENT PARTIAL STERILITIES AS DETERMINED BY EGG HATCH FROM CROSSES INVOLVING WILD-TYPE AND SINGLE TRANSLOCATION STOCKS.

Type of Cross	Number of Eggs	Larval Hatch	Number of Unhatched Eggs	Percent Partial Sterility
Wild-type (X) Wild-type				
+/+(X)+/+	1735	1571	164	9.5
Homozygous Translocation Stocks (X) Wild-type				
T63-6/T63-6(X)+/+	708	508		28.2
T63-18/T63-18(X)+/+	106	79	27	25.5
Tg163d/Tg163d(X)+/+	227	142	85	37.4
*T175/T175 (X)+/+	-	-	-	-
Teterozygous Translocation Stocks (X) Wild-type				
T63-6/+(X(+/+	761	353	408	53.6
T63-18/+(X)+/+	953	541	412	43.2
Tg163d/+(X(+/+	387	119	268	69.3
T175/+(X)+/+	1042	502	540	51.8
Heterozygous Translocation Stocks (X) Heterozygous Translocation Stocks				
T63-6+(X)T63-6/+	366	61	305	83.3
T63-18/+(X)T63-18/+	285	111	174	61.1
Tg163d/+(X)Tg163d/+	496	121	375	75.6
T175/+(X)T175/+	539	171	368	68.3
F_1 Intercross Translocation Stocks (X) Wild-type				
T63-6/T63-18(X)+/+	864	224	640	74.1
T63-16T63-18(X)+/+	1756	262	1494	85.1
T64-37/T63-18(X)+/+	1876	344	1532	81.7
T63-13/T63-16(X)+/+	1179	162	1017	86.3

*Insufficient Data

Higher partial sterilities were later obtained when heterozygous translocation stocks were crossed with wild-type. Most organizms average approximately 50 percent partial sterility in this situation and *Drosophila* appears to conform at least with the particular translocation stocks used in this study. Both adjacent types of chromosome segregation lead to duplication/deficient gametes and zygotic lethality whereas alternate segregation maintains chromosome balance. These data indicate that alternate segregation and the two types of adjacent segregation approximate each other in frequency during these tests. Heterozygous translocation stocks crossed with each other would then be expected to display an even higher partial sterility and this did result in the tests performed. If both parents showed 50 percent partial sterility, the overall probability of sterility in a cross of this type would be 75 percent. The results in these tests ranged from 61.1 percent to 83.3 percent. The lowest partial sterility of 61.1 percent occurred with the T63-18 stock which also had the lowest sterility when it was heterozygous and crossed with wild-type. The highest partial sterilities consistently occurred in tests between F-1 intercross progeny and wild-type; that is, progeny from crosses between two different translocations that were reared and crossed with the wild-type stock. These partial sterilities ranged from 74.1 percent to 86.3 percent. Chromosome pairing during pachytene in meiosis in the intercross progeny theoretically forms the two-cross configuration depicted in Figure 1. In addition to the segregation patterns that result in duplications and deficiencies, single crossovers in the looped area (differential segment) lead to unbalanced gametes in every instance; consequently, the partial sterility is increased.

Synthesizing more complex translocation stocks.

Several breeding schemes to establish a double translocation within two non-homologous chromosomes were attempted. Burnham (1968) first proposed the cytogenetic scheme for this possibility in corn and he termed these lines "two-chromosome double interchanges". Several double interchanges (translocations) have been reported previously, but these were composed of segments from three to four non-homologous chromosomes rather than from only two chromosomes (Brink and Cooper, 1932; McDonald and Rai, 1970). Figure 1 shows the cytogenetic relationships that theoretically allow for the synthesis of the more complex double translocation. It can presumably arise by simultaneous crossing over in the two differential segments that are formed when intercrosses between single translocations are made. Three separate attempts were made using different combinations of *Drosophila* single translocation stocks in each case.

Breeding schemes for double translocations :

The T(2;3) g163d/T(2;3) 175 experiment presented several fundamental problems (Richards, 1976). Recognition of a double translocation stock, should it occur, posed the greatest problem. It was therefore decided to use particular single translocation stocks which also displayed a visible phenotypic characteristic due to the presence of the homozygous translocation. T(2;3)g163d is called glassy and has an eye that is white and shiny in appearance. T(2;3)175 is called smudge and it has a smudge-like slight depression of the eye. The breeding scheme began with an intercross between the two single translocation stocks. Glassy and smudge served as markers when homozygous. The scheme needed to take into account the lack of crossing over in the male. It also needed to circumvent the problem of not being able to discern smudge flies when they are also glassy; therefore, the steps outlined in Figure 2 were followed.

The T(2;3)63-6/T(2;3)63-18 experiment was executed in a slightly different way (Lichtenfels, 1976). The T63-6 translocation stock is characterized by obliquely creased wings in 44 percent of the flies and 50 percent of the T63-18 flies have trough-like wings (Carroll, 1974). Intercrosses were again established between the two stocks. The progeny were heterozygous for each of the single translocations and

TABLE 2 – RESULTS OF THREE SEPARATE BREEDING EXPERIMENTS IN ATTEMPTS TO GAIN A TWO-CHROMOSOME DOUBLE TRANSLOCATION.

EXPERIMENT					
glassy x smudge		trough x creased		trough x glassy	
phenotype	number of progeny	phenotype	number of progeny	phenotype	number of progeny
glassy scored within smudge phenotype	0	trough	613	trough	346
		creased	969	glassy	253
wild-type scored within smudge phenotype	20821	wild-type	16386	wild-type	7944
		trough-creased	16	trough-glassy	0
TOTAL	20821	TOTAL	17984	TOTAL	8543

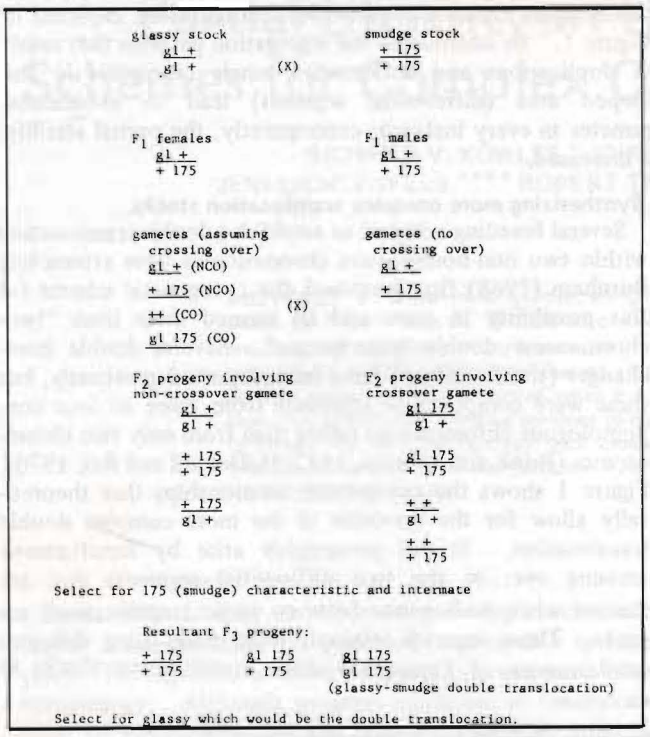

Figure II. - The glassy-smudge breeding scheme. The desired crossover occurs in the F_1 female.

appeared wild-type because the breakpoint expressions follow a recessive mode of inheritance. Virgin females were selected from the initial intercross and mated with virgin-wild-type males. The reciprocal cross, male intercross progeny with female wild-type, would not be effective for this purpose due to the lack of crossing over in the male. The majority of the progeny from this cross were heterozygous for the single translocations; however, if the desired simultaneous crossovers did occur, there would also be the synthesis of a heterozygous two-chromosome double translocation. The progeny from the latter cross were allowed to mass mate in an attempt to establish a homozygous double translocation line. At this point they would be discernible with a creased/trough phenotype. These progeny were scored, and the frequency of each phenotype was tallied.

The T(2;3)63-18/T(2;3)g163d experiment was performed in exactly the same manner as the previous experiment (Lichtenfels, 1976). The only difference being that the phenotype which would indicate the double translocation would be a glassy eye/trough-wing combination.

The results displayed in Table 2 show absolutely no double translocation progeny as determined by phenotypes in the glassy/smudge and the trough/glassy schemes. The results with the glassy and smudge stocks are comparable to those previously obtained by other researchers using the same two stocks (Curtis and Robinson, 1972). In the trough/creased scheme, 16 progeny were scored as trough-creased progeny out of a total of 17984 flies. These were, therefore, putative double translocation stocks. Because of extreme difficulty in scoring the trough-wing, it was deemed necessary to set up tests that would serve to confirm the actual existence of the new chromosome rearrangement.

The first step was taken by making cytological observations of the polytene chromosomes in the salivary glands of larvae from crosses between the putative double translocation stocks and wild-type. Theoretically, a two-cross configuration similar to that depicted in Figure 1 should form due to the somatic pairing in these chromosomes. This proved to be equally inconclusive since one of the breakpoints involved is located close to the centromere (Rychlik, 1975). In salivary cells this area amounts to an amorphous chromocenter which disallowed the discernment of any chromosome exchange at this point. The other exchange point was clearly visible which denoted the presence of at least one of the translocations.

Different genetic tests designed

Two different genetic tests were designed to offer more definitive information as to whether these new stocks, arising from the trough/Creased experiment, were truly a new double translocation type or simply one of the parental single translocation stocks. In the first test, putative double translocation flies were first made heterozygous and then were crossed with wild-type in a reciprocal manner. Partial sterility was calculated from these reciprocal crosses. Hypothetically, it was expected that the heterozygous double translocation females crossed with wild-type males would result in a higher partial sterility than heterozygous double translocation males crossed with wild-type females. This is due to the increased sterility that results from crossing over in the differential segment of the cross-configuration (Burnham, 1968). Since there is a lack of crossing over in male Drosophila, it follows that differences should exist in the degree of sterility between reciprocal crosses. The data from these crosses are given in Table 3. Note that the higher sterility (59.0 percent) occurred when the male was heterozygous for the chromosome rearrangement, not the female (52.7 percent) as it would have been expected if a double translocation truly existed. In addition, statistical comparisons between the partial sterilities of the putative double translocation stock and the T(2;3)63-6 (creased) single translocation stock that was used in the original breeding scheme, showed no significant difference. ($p>.05$).

In a final genetic test, putative double translocation stocks were subjected to recombination studies with marker genes. Two different putative double translocation stocks were backcrossed with stocks that possessed the linked marker genes black body (b), vestigial (vg), and brown eyes (bw). Progeny were then scored for these three characters and recombination was calculated for the regions b-vg and vg-bw. Backcross linkage data were also obtained for the single translocation stocks %(2;3) 63-18 (trough) and T(2;3)63-6 (creased) and with wild-type for reference data. The single translocation T63-18 has a breakpoint iin the b-vg region while the single translocation T63-6 breakpoint is in the vg-bw region. Translocation breakpoints cause areas of asynapsis that result in reduced crossing over. The recombination data obtained for the single translocation stocks demonstrate this reduction. In T63-6, a decrease from .358 to .129 is observed in the vg-bw region where the breakpoint is located. In T63-18 a decrease from .180 to .121 was observed in the b-vg region where that breakpoint is located. If the double translocation stock was actually isolated, there should be reduced crossing over in both the b-vg and the vg-bw regions in those recombination tests. In actuality, this is not the case. Examination of the data in Table 4 reveals reduced crossing over in only the vg-bw of both of the putative double translocation stocks tested. Statistical tests show no significant idfferences in the amount of crossing ove in the b-vg and vg-bw regions between the putative double translocation stocks and the single translocation stock T63-6.

TABLE 3 – PARTIAL STERILITY OF PUTATIVE HETEROZYGOUS DOUBLE TRANSLOCATION STOCK CROSSED WITH WILD-TYPE STOCK

Trials	Number of Eggs	Number of Unhatched Eggs	Percent Partial Sterility
Putative heterozygous double translocation females crossed with wild-type males			
1	173	90	52.0
0	693	364	52.5
3	744	380	51.1
4	1059	569	53.7
TOTALS	2669	1403	52.7
Wild-type females crossed with putative heterozygous double translocation males			
1	66	34	51.5
2	213	130	61.0
3	403	226	56.1
4	345	216	62.6
TOTALS	1027	606	59.0

All of the sterility and recombination data presented are strongly indicative that the stocks in question contain one of the original single translocations from stock T63-6, and not the double translocation arrangement.

Breeding schemes and genetic tests continue in efforts to gain more complex chromosome arrangements in this well-known genetic organism, *Drosophila melanogaster.* In this way, fundamental information about chromosome behavior can be accumulated. All of which might play an eventual role in genetic manipulation for pest control.

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TABLE 4 RECOMBINATION RESULTS FROM BACKCROSS DATA WITH WILD-TYPE.

Stock: Backcross with b-vg-bw	Progeny Counted	b-vg Region	vg-bw Region	Double Crossover	C Value
Wild-type	1,468	0.180	0.358	0.049	0.761
T63-6 (creased)	1,413	0.151	0.129	0.021	1.077
T63-18 (trough)	1,033	0.121	0.356	0.035	0.814
CR/TR-5 (putative double translocation)	2,881	0.191	0.137	0.020	0.769
CR/TR-7 (putative double translocation)	3,755	0.218	0.143	0.029	0.935