NON RANDOM ASSORTMENT OF NON-HOMOLOGOUS CHROMOSOMES IN DROSOPHILA MELANOGASTER[1]

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NON-HOMOLOGOUS chromosomes assort independently at meiosis. This principle has been repeatedly confirmed by genetical and cytological experiments. Under abnormal or special conditions exceptions to this principle have been reported (GERSHENSON 1940; LONGLEY 1944; MITCHIE and WALLACE 1953; SANDLER and NOVITSKI 1956).

In the experiments described in this paper a markedly non random assortment of non-homologous chromosomes of *Drosophila melanogaster* has been discovered. A normal Y chromosome and a normal fourth chromosome have been found under certain conditions to segregate from each other with a frequency as high as 92 percent. The details of the experiments and the assortment results are presented below.

MATERIALS AND METHODS

Non random assortment of the sex chromosomes and the fourth chromosomes has been studied principally in XXY females heterozygous for a translocation between the third and fourth chromosomes. Such females will be referred to as Type A females. The extra Y chromosome of Type A females was derived from a Canton-S stock and will be designated Y^{C-S}. The principal translocation used in Type A females was obtained from E. B. LEWIS and is known as T(3;4)86D (GRELL 1956). It is a reciprocal translocation between the right arm of the third chromosome and the basal portion of the right arm of the fourth chromosome. Salivary gland chromosome analysis by LEWIS shows a break in the third chromosome just after 86D1–2 and a break in the fourth chromosome at 101F. The stock of this translocation and the stock of T(3;4)88B, which is described below, were cleared of extra fourth chromosomes prior to the start of the experiments. In order to select against triplo-4 flies in preliminary crosses involving either translocation, all free fourth chromosomes present in such crosses were marked with dominants.

Type A females were obtained among the progeny of the following cross (Cross No. 1):

$$\frac{y^2 v}{\text{FM3}, y^{s1d} sc^s dm\ B\ l};\ Y^{C-S};\ \frac{In(2L)Cy,Cy + In(2R)bw^{VDe1},\ bw^{VDe1}}{bw};\ \frac{ci\ sv^n}{ci\ sv^n}\ \text{♀} \times$$

$$y^2 v;\ Y^{C-S};\ Y^{C-S};\ \frac{In(2L)Cy,Cy + In(2R)bw^{VDe1},\ bw^{VDe1}}{bw};\ \frac{\text{T}(3;4)86\text{D},\ bx^{34e} e^4}{+;\ ey^D}\ \text{♂}$$

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where FM3 symbolizes a complex X chromosome balancer known as "First Multiple 3" GRELL. (The FM3 balancer (GRELL 1954) was derived from X-irradiation of males carrying two X chromosome inversions scute-8(sc^8) and delta-49(dl-49), and the mutants y^{31d}, *dm* and *B*. It carries a lethal of unknown locus. Salivary gland chromosome analysis (LEWIS 1954) shows three new breakage points in regions 3EF, 16AB and in the proximal heterochromatin (19F–20) superimposed on those of sc^8 and delta-49).

The presence in the parents of Cross No. 1 of a chromosomal rearrangement associated with dominant brown variegation, namely $In(2R)bw^{VDe1}$ served as the means for identifying the presence of the extra Y chromosome in those parents. It is well known that such variegation is virtually completely suppressed in the XXY female or the XYY male (GOWEN and GAY 1933; SCHULTZ 1936). The reasons for the other markers will become apparent below.

The non-Bar, non-Curly, non-eyeless^D virgin daughters of Cross No. 1 were individually mated to XY males heterozygous for bw^{VDe1}. This constitutes Cross No. 2:

$$\frac{y^2v}{y^2v};\ (Y^{c\text{–}s})\,(Y^{c\text{–}s});\ \frac{bw}{bw};\ \frac{T(3;4)86D,\ bx^{34e}e^{k}}{+\ ;\ ci\ sv^{n}}\ \text{♀}\ \times\ v\ ;\ \frac{In(2R)bw^{VDe1},bw^{VDe1}}{SM1,\ al^2Cy\ sp^2}\ \text{♂}$$

The number of Y chromosomes present in the parental females of Cross No. 2 is expected to vary from none to two. Females which carry one extra Y are Type A; females which carry no Y will be referred to as Type B and females which carry two extra Y's will be referred to as Type C.

The method of determining the number of Y chromosomes in the parental females of Cross No. 2 was to observe the proportion of regular bw^{VDe1} (non-Curly) progeny which showed suppression of the brown variegation.

If the parental female of Cross No. 2 is Type A ($XXY^{c\text{–}s}$) then the bw^{VDe1} regular progeny are expected to consist of approximately equal numbers of suppressed and unsuppressed types. That is, approximately one-half of the regular progeny of XXY females carry an extra Y chromosome.

If the parental female of Cross No. 2 is Type B (XX) then all of the bw^{VDe1} regular progeny are expected to have unsuppressed brown variegation. That is, there will normally be no extra Y's in the progeny of XX females.

If the parental female of Cross No. 2 is Type C ($XXY^{c\text{–}s}Y^{c\text{–}s}$) then most of the bw^{VDe1} regular progeny are expected to have suppressed brown variegation. This follows from STERN's analysis (1929) which showed that approximately 98 percent of the regular progeny of XXYY females carry an extra Y chromosome.

The detection of the suppression of variegation is facilitated by the presence of the mutants vermilion (*v*) and brown (*bw*). Thus, $\frac{v}{v};\ \frac{bw^{VDe1}}{bw}$ females or *v*; Y; $\frac{bw^{VDe1}}{bw}$ males have nearly white eyes, whereas the addition of an extra Y chromosome to either of these genotypes gives a bright red color virtually indistinguishable from that of *v*. For this reason, *v* and *bw* were introduced into Crosses Nos. 1 and 2.

In order to follow the disjunction of the X chromosomes in the progeny of Cross No. 2, the parental female was made homozygous for the body color mutant, yellow^2. The regular progeny of Cross No. 2 thus consist of y^+ females and y^2 males, whereas the nondisjunctional progeny consist of y^2 females and y^+ males.

Since the parental females of Cross No. 2 were heterozygous for a translocation involving the third and fourth chromosomes, there will be an appreciable number of offspring nondisjunctional for the fourth chromosomes. That is, the progeny of Cross No. 2 will be composed of haplo-4 and triplo-4 flies as well as the regular diplo-4 flies.

Haplo-4 types, which are late-hatching, were scored directly on the basis of their characteristic complex of dominant effects (Minute bristles, small body size, pale body color, pronounced trident pattern and spread wings).

As triplo-4 flies are not noticeably different phenotypically from diplo-4 flies, it is necessary to distinguish these types from one another by progeny tests. The tester stock employed is *Ubx*/+; *M-4*/*ey*^{D} where *Ubx* is Ultra-bithorax; *M-4* is Minute-4; and *ey*^{D} is eyeless-Dominant. Samples of 105 suppressed and 391 unsuppressed *bw*^{VDe1} non-haplo-4 progeny from Cross No. 2 were selected for the test. Such flies are expected to consist of three classes,

1. Diplo-4 flies not carrying T(3;4)86D.
2. Diplo-4 flies heterozygous for T(3;4)86D.
3. Triplo-4 flies heterozygous for T(3;4)86D and carrying two free fourth chromosomes.

When a Class 1 fly is mated to the tester stock, it is expected to produce only diplo-4 flies. Therefore all the non-eyeless-Dominant offspring are expected to be Minute-4 in phenotype.

Class 2 flies when mated to the tester stock, although diplo-IV in composition, are expected to produce some triplo-4 offspring as the result of nondisjunction induced by T(3;4)86D. Non-eyeless-Dominant flies that are triplo-4 are easily recognized because they have complete suppression of the Minute-4 phenotype. The problem, then, is to distinguish Class 2 flies from Class 3 flies since the latter will also produce triplo-4 progeny. This can be done, as will be shown below, by first identifying the translocation-bearing progeny of Class 2 and Class 3 flies. The presence of the dominant marker *Ubx* in the tester stock serves this purpose. Translocation (3;4)86D carries a rarely separable marker, bithorax^{34e} (*bx*^{34e}) which is a recessive pseudoallele of *Ubx*. The "trans" form of the double heterozygote *bx* + / + *Ubx* has a large wing-like haltere which is clearly distinguishable from ++ / + *Ubx*. Moreover, if a rearrangement is present in the vicinity of the bithorax mutants, a further modification of the trans-type position effect occurs. This consists of the development of a hairy band of tissue across the mesonotum and is known as the "transvection effect" (LEWIS 1954). The presence of this extra band of tissue indicates that the marker *bx*^{34e} is still associated with the translocation.

Class 2 and Class 3 flies may now be distinguished from each other by examining their translocation-bearing progeny. If the parent is Class 2 (diplo-4), a

maximum of 50 percent of the progeny which carry the translocation are expected to carry two free fourth chromosomes. Thus a maximum of 50 percent of the translocation-bearing progeny are expected to show suppression of the Minute phenotype. If the parent is Class 3 (triplo-4), 90 percent or more of the progeny which carry the translocation are expected to carry two free fourth chromosomes. This is based on STURTEVANT's observation (1936) that the two normal fourth chromosomes are recovered together in about five percent of the progeny of females which carry an X-4 duplication in addition to two normal fourth chromosomes. In the case of a Class 3 parent, over 90 percent of the translocation-bearing progeny are expected to show and did show suppression of the Minute phenotype.

In order to follow the segregation of the fourth chromosomes more directly, i.e., without the necessity of progeny testing, two additional experiments were devised. The first of these (Cross No. 3) is identical with Cross No. 2 except that the free fourth chromosome of the female parent carries the dominant marker cubitus-interruptus-Dominant (ci^D) instead of *ci* sv^n. The absence of the dominant marker in one class of progeny provides a means of directly identifying it as the diplo-4, translocation-bearing class.

In the second experiment (Cross No. 4) the parental female carries a dominantly marked translocation as well as a free fourth chromosome marked with ci^D. It was easier to use a different translocation already containing a dominant marker than to attempt to introduce a dominant marker into T(3;4)86D. The translocation chosen was T(3;4)88B which carries the virtually inseparable dominant marker *Ubx*. Salivary gland chromosome analysis of T(3;4)88B by LEWIS shows a break in 3R after 88B and a break in 4R at 101. Although in other respects the genotypes of the parents in Cross No. 4 are identical with those in Cross No. 2, the presence of the two dominant markers enables one to follow results of the segregation of the fourth chromosomes in all classes of progeny.

The female parent which was used in the final cross (Cross No. 5) was not of Type A. Instead, a female (Type D) was constructed which was homozygous for T(3;4)86D, $bx^{34e}e^4$ and which carried in addition a free fourth chromosome marked with ci^D or sparkling Cataract (spa^{Cat}). Otherwise the genotypes of the parents were again identical with those used in Cross No. 2. All of the progeny from Cross No. 5 are heterozygous for T(3;4)86D and the segregation of the free fourth chromosome is easily followed by observing which progeny are ci^D or spa^{Cat} (triplo-4) and which progeny are non-ci^D or non-spa^{Cat} (diplo-4).

RESULTS AND ANALYSIS

If the assortment of the sex chromosomes and the fourth chromosomes are independent of each other in Type A (XXY;heterozygous for a 3–4 translocation) females, suppressed (extra Y) and unsuppressed (no extra Y) progeny are expected to appear in equal numbers among each of the two products of regular disjunction and each of the two products of nondisjunction of the fourth chromosomes. The progeny of Type A females from Cross No. 4 will be considered first since all of the classes of progeny are phenotypically identifiable. An examination

of Cross No. 4 progeny (Table 3) shows that the ratio of suppressed to unsuppressed classes in the haplo-4 group of flies is 53:1 and that the ratio in the triplo-4 flies is 1:62. Both ratios represent highly significant deviations from the expected 1:1 ratio. Furthermore, the ratio of suppressed to unsuppressed classes in the diplo-4, nontranslocation flies is 1:1.6. This is a statistically significant deviation from the expected 1:1 ratio ($P < .002$). The ratio of suppressed to unsuppressed classes among the diplo-4, translocation flies shows no significant deviation from a 1:1 ratio.

The progeny of Type A females from Cross No. 2 (Table 3) will be considered next. In the haplo-4 group, the only group in which the number of fourth chromosomes can be determined directly, suppressed and unsuppressed classes are found in the ratio of 17:1. The proportions of flies in the other three groups were determined by progeny tests. All of the fertile samples were successfully classified according to the criteria outlined under Materials and Methods. The results of the tests (Table 3) show that the ratio of suppressed to unsuppressed classes in the triplo-4 group is 1:24; that the ratio of suppressed to unsuppressed classes in the diplo-4, nontranslocation group is 1:2.4; and that the ratio of suppressed to unsuppressed classes in the diplo-4, translocation group is 1.8:1. Each ratio represents a statistically highly significant deviation from 1:1.

Lastly, the progeny of Type A females from Cross No. 3 (Table 3) contain two groups in which the fourth chromosome content can be directly determined. In the first of these, the haplo-4 group, suppressed and unsuppressed classes are found in the ratio of 22:1. In the second or diplo-4, translocation-bearing group, suppressed and unsuppressed classes are found in the ratio of 2:1. The third or triplo-4 group is indistinguishable phenotypically from the fourth or non-translocation diplo-4 group. Among the combined third and fourth groups, however, the suppressed and unsuppressed classes are phenotypically distinguishable and have the ratio of 1:5.6. For comparison, the ratio of the suppressed and unsuppressed classes when the latter two groups from Cross No. 2 are summed is 1:4.9 and similarly for Cross No. 4, the corresponding ratio is 1:3.8.

The data from Crosses Nos. 2, 3, and 4 are rearranged in Table 4 with respect to the types of segregation involved and the classes of progeny that result from each segregation type. There are four types of segregation each of which produces two complementary classes which should be numerically equal if the viability of each class is the same. Type-I and Type-II segregations are defined as those nondisjunctional for the fourth chromosomes. The difference between them is that in Type-I the extra Y chromosome goes to the opposite pole from the normal fourth chromosome and the translocation while in Type-II the extra Y goes to the same pole. Both segregations lead to haplo-4 and triplo-4 flies. As haplo-4's are known to have a low and erratic viability, the two complementary classes from Type-I and Type-II segregations are not expected to, and do not, appear in equal numbers. The relative frequency of Type-I and Type-II can be measured by comparing the number of haplo-4 flies from Type-I with those from Type-II or by comparing the number of triplo-4 flies from the same segregations. The frequency of

Type-I based on haplo-4 flies varies, depending on the cross, from 17 (Cross No. 2) to 53 (Cross No. 4) times the frequency of Type-II and based on triplo-4 flies from 24 (Cross No. 2) to 62 (Cross No. 4) times the frequency of Type-II. This means that from Type-I and Type-II segregations, the normal fourth chromosome and the translocation are recovered without the extra Y chromosome some 17 to 62 times as frequently as they are recovered with the extra Y.

Type-III and Type-IV segregations are defined as those arising from the regular disjunction of the fourth chromosomes. They lead to diplo-4 progeny, 50 percent of which are expected to carry the translocation and 50 percent of which are expected to carry a normal third and fourth chromosome. The distinction between Type-III and Type-IV segregation is again in the assortment of the Y chromosome which goes to the same pole as the translocation in Type III and to the same pole as the normal third and fourth chromosomes in Type-IV. The two complementary products from Type-III show a significant departure from equality in Cross No. 4 but not in Cross No. 2. The 44 percent deficiency that is observed in the extra Y, translocation-bearing class from Cross No. 4 may be due to reduced viability for this type of zygote or to a meiotic event which leads to a reduction in the number of viable extra Y, translocation-bearing gametes. Type-IV segregation produces two approximately equal complementary classes from Cross No. 2 and from Cross No. 4. Cross No. 3 does not permit a comparison of classes from Type-III or Type-IV for only one class is identified in each case.

The relative frequency of Type-III and Type-IV segregations can be measured by comparing the numbers of translocation progeny from each type or by comparing the numbers of nontranslocation progeny from each type. When the former criterion is used, Type-III is found to be 1.8 times as frequent as Type-IV segregation in Cross No. 2 and twice as frequent as Type-IV in Cross No. 3. In Cross No. 4, Type-III is only .77 times as frequent as Type-IV. The cause of the apparent inconsistency is the 44 percent deficiency of the extra Y, translocation-bearing class in Cross No. 4. A comparison of Type-III and Type-IV segregations based on the diplo-IV, nontranslocation classes shows that the frequency of Type III varies depending on the cross, from 1.6 (Cross No. 4) to 2.4 (Cross No. 2) times that of Type-IV. This means that from Type-III and Type-IV segregations, the normal fourth chromosome is recovered without the extra Y chromosome some 1.6–2.4 times as frequently as it is recovered with the extra Y chromosome and that the translocation is recovered with the extra Y chromosome some .77–2 times as frequently as it is recovered without it.

The total frequency with which the extra Y chromosome and the normal fourth chromosome are recovered together in the progeny of Type A females can be estimated for each cross by totaling the non-extra Y progeny from the cross and then determining what percent of this total is represented by flies which do not carry the normal maternal fourth chromosome. The calculations are based on the non-extra Y classes in order to eliminate the large haplo-4 extra Y bearing class whose viability is low and erratic. The small haplo-4, non-extra Y bearing class is necessarily included in the progeny from which the estimates are derived.

A viability correction of 3.0 is assigned to this class in each cross since this is the value which is needed to equalize the complementary haplo-4 and triplo-4 classes from Type-I segregation in Cross No. 2 (Table 4) where the discrepancy between them is greatest. The values for the recovery of the extra Y and fourth chromosome together are as follows:

Cross No. 2, 16.8 percent = [(2.4·3.0) + 34.5] ÷ [(2.4·3.0) + 118 + 88 + 34.5]
Cross No. 3, 16.2 percent = [(5·3.0) + 104] ÷ [(5·3.0) + 616 + 104]
Cross No. 4, 22.4 percent = [(2·3.0) + 83] ÷ [(2·3.0) + 186 + 123 +83]

Since the expectation of recovery of the Y and fourth chromosome together is 50 percent if their assortment is random, the values obtained are obviously much under the expectation. Of interest is the similarity in the values (16.2 and 16.8 percent) which are obtained when the same translocation is used in different experiments (Cross No. 2 and No. 3) and the difference in value (22.4 percent) when a different translocation is used (Cross No. 4).

The frequency with which the extra Y chromosome and the translocation are recovered together in the progeny of Type A females can also be estimated for Cross No. 2 and No. 4. The values for the recovery of the Y and translocation together are as follows:

Cross No. 2, 38.5 percent = [(2.4·3.0) + 88] ÷ [(2.4·3.0) + 118 + 88 + 34.5]
Cross No. 4, 32.4 percent = [(2·3.0) + 123] ÷ [2·3.0) + 83 + 123 + 186]

The values are again certainly much under the expectation of 50 percent. On the basis of BROWN's data (1940) for a heterozygous 3–4 translocation with a break at 87E3–87F1, the nontranslocated fragment of the third chromosome pairs and disjoins regularly with the normal three. The translocated fragment of three shows a high rate of nondisjunction with the normal three. Presumably it is this fragment translocated to the four centromere which assorts non randomly with the extra Y chromosome.

The difference in the frequency of recovery of Y,4 and Y, translocation together in the two translocations which were used may be correlated with the degree to which the translocation has impaired pairing between the two fourth chromosomes. In the case of T(3;4)86D, the genetic data indicate that the free fourth chromosome assorts nearly randomly with respect to its homologue involved in the translocation so that 22 percent haplo-4, 22 percent triplo-4 and 56 percent diplo-4 progeny are produced. The observed percentage of haplo-4 flies from Type B females (XX;T(3;4)86D/ +) from Cross No. 2 (Table 1) is 8.62. An estimate of the viability of the haplo-4 class has been made by comparing the number of suppressed haplo-4 flies from Type A females, which were observed directly, with the number of complementary unsuppressed triplo-4 flies from the same segregation (Type I segregation) which were determined by progeny tests (Table 4). On this basis the viability of the haplo-4 flies appears to be approximately one-third of the triplo-4 flies and a viability correction of about 3 for the haplo-4 class is in order. The corrected value for haplo-4 flies from Type B females of Cross No. 2 is 22.05 percent ($3 \times 8.62 \div 100 + 2 \times 8.62$) indicating a nearly random assortment of the fourth chromosomes.

TABLE 1

Results from Cross No. 2. Progeny of y^2v/y^2v; bw/bw; *T(3;4)86D,* bx^{34e} $e^4/+$; ci sv^n *females which carry no Y (Type B), one Y (Type A) or two Y's (Type C) chromosomes and* v; In(2)bw^{VDe1}, bw^{VDe1}/SM1, al^2 Cy sp^2 *males*

	Regular disjunction of X chromosomes Suppressed				Nonsuppressed			
	♀		♂		♀		♂	
Type of ♀ parent	Diplo- or Triplo-4	Haplo-4	Diplo- or Triplo-4	Haplo-4	Diplo- or Triplo-4	Haplo-4	Diplo- or Triplo-4	Haplo-4
Type A (1 Y)	906	230	877	448	2,173	15	1,917	25
Type B (no Y)	...	...	...	...	733	53	733	86
Type C (2 Y)	392	27	372	58	27	0	17	0
	Primary or secondary nondisjunction of X chromosomes							
Type A (1 Y)	42	5	...	...	...	...	63	2
Type B (no Y)	3	...	...	...	...	...	3	...
Type C (2 Y)	3	...	4	...	...	...	6	...
	Percent regular progeny		Percent nondisjunctional progeny				Percent haplo-4's	
Type A (1 Y)	98.3		1.7				10.9	
Type B (no Y)	99.6		.4				8.6	
Type C (2 Y)	98.6		1.4				9.5	

In the case of T(3;4)88 *Ubx* the number of haplo-4 and triplo-4 progeny from Type B females is observed directly (Table 2). The viability of the haplo-4 class is equal to one-third of that of the triplo-4 class (26:78). The corrected sum of haplo-4 and triplo-4 flies is about 19 percent ($2 \times 78 \div 764 + 2/3 \times 78$) of the progeny. The assortment of the fourth chromosomes appears to be much more regular in this case.

TABLE 2

Results from Cross No. 4. Progeny of y^2 v/y^2 v; bw/bw; *T(3;4)88B,* Ubx/+; ci^D *females which carry no Y (Type B) or one Y (Type A) chromosome and* v; In(2)bw^{VDe1}, bw^{VDe1}/SM1, al^2 Cy sp^2 *males*

	Regular disjunction of X chromosomes Suppressed								Nonsuppressed							
	♀				♂				♀				♂			
Type of ♀ parent	*Ubx*	ci^D	*Ubx* ci^D	Haplo-4	*Ubx*	ci^D	*Ubx* ci^D	Haplo-4	*Ubx*	ci^D	*Ubx* ci^D	Haplo-4	*Ubx*	ci^D	*Ubx* ci^D	Haplo-4
Type A (1 Y)	32	41	1	41	32	37	2	64	37	61	81	1	46	62	105	1
Type B (no Y)	..	..	..	..	..	..	..	..	157	188	45	13	147	168	33	13
	Primary or secondary nondisjunction of X chromosomes															
Type A (1 Y)	1	2	0	1	..	..	..	..	..	..	..	..	4	3	0	0
Type B (no Y)	..	..	..	1	..	..	..	..	..	..	..	..	..	..	2	..
	Percent regular for X's				Percent nondisjunctional for X's								Percent Haplo-4's			
Type A (1 Y)	98.3				1.7								16.3			
Type B (no Y)	99.6				.4								3.5			

TABLE 3

Progeny of Type A females from Crosses No. 2, 3 and 4 arranged to show the assortment of the Y chromosome in the four kinds of progeny which result from regular disjunction and nondisjunction of the fourth chromosome

		Cross No. 4 T(3;4)88Ubx/+; ci^D			Cross No. 2 T(3;4)86D/+; *ci* sv^n			Cross No. 3 T(3;4)86D/+; ci^D		
		Y	O	Ratio Y:O	Y	O	Ratio Y:O	Y	O	Ratio Y:O
Nondisjunction of fourth chromosomes	Haplo-IV	105	2	53:1	678	40	17:1	109	5	22:1
		...	...	...	39.9†	2.4†	...	...	...	...
	Triplo-IV	3	186	1:62	(5)	(192)	1:24	...	...	...
		...	...	...	...	118*	...	...	...	...
								110	616	1:5.6
Regular disjunction of fourth chromosomes	III; IV	78	123	1:1.6	(37)	(143)	1:2.4	...	...	...
		...	...	...	...	88.1*	..	...	...	...
	Translocation	64	83	.77:1	(63)	(56)	1.8:1	204	104	2:1
		...	...	...	...	34.5*	...	...	...	...

Numbers in parenthesis indicate determination by progeny tests.

* Corrected value. Correction is necessary because the suppressed and unsuppressed flies were not progeny tested in the proportion in which they are produced. Correction factor equals 0.616=Ratio Y:O in total sample of non-haplo-4's/Ratio Y:O in progeny tested samples.

† Number of haplo-4 flies expected to be produced in a sample containing the number of suppressed non-haplo-4 animals that were progeny tested.

TABLE 4

Progeny from Type A females from Crosses No. 2, 3 and 4 arranged with respect to the four types of segregation involved and the two complementary classes which result from each type

	Segregations nondisjunctional for 4th chromosomes			Segregations regular for disjunction of 4th chromosomes	
	Type I Segregation (XY3/XT4)	Type II Segregation (X3/XYT4)		Type III Segregation (XYT/X34)	Type IV Segregation (XT/XY34)
			Diplo-4		
Haplo-4	Class 1 (Y)	Class 1 (no Y)	Translocation	Class 1 (Y)	Class 1 (no Y)
Cross #2	39.9†	2.4†	Cross #2	(63)	34.5*
Cross #3	109	5	Cross #3	204	104
Cross #4	105	2	Cross #4	64	83
Triplo-4	Class 2 (no Y)	Class 2(Y)	Diplo-4 (3;4)	Class 2 (no Y)	Class 2 (Y)
Cross #2	118*	(5)	Cross #2	88.1*	(37)
Cross #3	...	...	Cross #3	...	...
Cross #4	186	3	Cross #4	123	78
Ratio of Haplo-4 Type I:Type II			Ratio of Translocation Type III:Type IV		
Cross #2 17:1			Cross #2 1.8:1		
Cross #3 22:1			Cross #3 2.0:1		
Cross #4 53:1			Cross #4 .77:1		
Ratio of Triplo-4 Type I:Type II			Ratio of 3;4 Type III:Type IV		
Cross #2 24:1			Cross #2 2.4:1		
Cross #3 ...			Cross #3		
Cross #4 62:1			Cross #4 1.6:1		

For meaning of symbols, see Table 3.

Type D (XXY;T(3;4)86D/T(3;4)86D/ci^D or spa^{Cat}) which is the designation given to the female used in Cross No. 5, carries a homozygous translocation instead of the heterozygous translocation which is present in the Type A female. The regular progeny from Cross No. 5 are heterozygous for the translocation and are genotypically similar except for those differences which result from the assortment of an extra Y and an extra fourth chromosome, i.e., the presence or absence of a Y chromosome, a fourth chromosome or both chromosomes. All classes of progeny are identifiable for the free fourth chromosome is marked with ci^D or spa^{Cat} and the presence of the Y is evidenced by its suppression of the dominant-brown variegation. Since the female parent in Cross No. 5 is triplo-4, the progeny are either diplo-4 or triplo-4 and thus Y-4 segregation can be measured without the need for a viability correction for haplo-4 flies.

If, in the female used in Cross No. 5, the assortment of the sex chromosomes and the free fourth chromosome are independent of each other, suppressed (extra Y) and unsuppressed (no extra Y) progeny are expected to appear in equal numbers among the diplo-4 and triplo-4 groups of flies. The results from Cross No. 5 are given in Table 5.

The data from Cross No. 5 are arranged in Table 6 with respect to the types of segregations involved and the classes of flies which result from each segregation type. Type-I segregation is defined as that in which the extra Y chromosome and the fourth chromosome assort to opposite poles and Type-II segregation is defined as that in which the Y chromosome and the fourth chromosome assort to the same pole. The relative frequency of Type-I and Type-II segregation can be measured by comparing the number of diplo-4 flies from Type-I with those from Type-II or by comparing the number of triplo-4 flies from Type-I with those from Type-II. The frequency of Type-I:Type-II segregation based on diplo-4 flies is 10:1 and the frequency based on triplo-4 flies is 13:1. The frequency with which the Y

TABLE 5

Results from Cross No. 5. Progeny of Type D females (y^2 v/y^2 v; Y^{C-S}; bw/bw; *T(3;4)86D*, bx^{34e} e^4/*T(3;4)86D*, bx^{34e} e^4/ci^D or spa^{Cat}) *and* v; In(2)bw^{VDe1}, bw^{VDe1}/SM1, al^2 Cy sp^2 *males*

Type of female parent	Regular disjunction of X chromosomes: Suppressed ♀ Triplo-4	Suppressed ♀ Diplo-4	Suppressed ♂ Triplo-4	Suppressed ♂ Diplo-4	Nonsuppressed ♀ Triplo-4	Nonsuppressed ♀ Diplo-4	Nonsuppressed ♂ Triplo-4	Nonsuppressed ♂ Diplo-4
ci^D	14	83	4	70	94	5	71	6
spa^{Cat}	10	148	8	148	150	18	164	15
Totals	24	231	12	218	244	23	235	21
	Secondary nondisjunction of X chromosomes							
ci^D	...	4	...	...	...	...	7	2
spa^{Cat}	...	6	...	...	...	...	5	3
Totals	...	10	...	...	...	...	12	5
	Percent regular for X's: 97.4				Percent nondisjuctional for X's: 2.6			

TABLE 6

Progeny of Type D females arranged with respect to the two types of segregation involved and the complementary classes which result from each type

	Type I Segregation (TY/T4)	Type II Segregation (T/TY4)
	Class 1 (Y)	Class 1 (no Y)
Diplo-4	449	44
	Class 2 (no Y)	Class 2 (Y)
Triplo-4	479	36
	Ratio Diplo-4 Type 1:Type 2 10:1	
	Ratio Triplo-4 Type 1:Type 2 13:1	

chromosome and the fourth chromosome are recovered together (Type-II progeny/Total progeny) is 7.9 percent whereas the expected frequency is 50 percent.

Only progeny which are products of the regular disjunction of the X chromosomes have been considered in the above results. Progeny which arise from nondisjunction of the X's comprise less than 4 percent of the total flies from each cross and their numbers are too small to analyze in detail.

A comparison of the total amount of nondisjunction of the X chromosomes in the four kinds of females under consideration presents a consistent pattern. The presence of a heterozygous translocation (Type B female) increases the amount of primary nondisjunction of the X's from .05 percent (BEADLE and STURTEVANT 1935) to .4 percent for T(3;4)86D and .4 percent for T(3;4)88B (Tables 1 and 2). The addition of a Y chromosome to a female which carries two normal X's is known to produce about 4.3 percent secondary nondisjunctional progeny (BEADLE and STURTEVANT 1935) or to have about eleven times the effect of these heterozygous translocations. The simultaneous presence of a Y and a heterozygous translocation (Type A female) does not have an additive effect but instead decreases the percentage of secondary nondisjunctional progeny to 1.7 percent for T(3;4)86D and 1.7 percent for T(3;4)88B (Tables 1 and 2). It is reasonable to expect that two Y chromosomes (which presumably form a bivalent), and a heterozygous translocation will reduce the percent of progeny nondisjunctional for the X chromosomes more than a homozygous translocation in the presence of one Y. This is borne out by the experimental results. When two Y chromosomes and heterozygous T(3;4)86D are present (Type C female), 1.4 percent secondary nondisjunctional progeny are observed (Table 1) whereas the presence of homozygous T(3;4)86D and one Y chromosome (Type D female) results in 2.6 percent secondary nondisjunctional progeny (Table 5).

DISCUSSION

Although it has been postulated that regions of homology may exist in the basal heterochromatin among all the chromosomes of *Drosophila melanogaster*

(GERSHENSON 1940; MULLER and PAINTER 1932; PROKOFYEVA-BELGOVSKAYA 1935) and although the possibility of associations formed in such regions between non-homologous chromosomes has been suggested as the mechanism responsible for certain enigmatic genetic data (COOPER, ZIMMERING and KRIVSHENKO 1955; SANDLER and NOVITSKI 1956), direct evidence in support of such associations is rather sparse.

Ideally, such evidence should be of two kinds. It should consist of observed cytological pairing between non-homologous chromosomes at the first meiotic metaphase and of genetic data clearly indicating the subsequent segregation of the paired non-homologues to different gametes in a significant frequency.

As the cytology of the meiotic events, particularly in the female of *Drosophila melanogaster* continues to be fairly refractory to analysis, no direct cytological evidence is available. Direct genetic evidence has been limited to segregations involving the sex and fourth chromosomes since zygotes aneuploid for entire autosomes other than chromosome four are lethal. Several types of gametes aneuploid for the sex and fourth chromosomes are recoverable as aneuploid zygotes, but under normal conditions pairing between homologues is so complete that there is no opportunity to observe whether the sex and fourth chromosomes possess the potentiality for non-homologous association and the consequent formation of these aneuploid types. It has been possible, however, through the use of unbalanced sets of chromosomes or structurally abnormal chromosomes to observe segregations which strongly suggest that such associations occur.

SANDLER and NOVITSKI (1956) have shown that the observed percentage of primary nondisjunction of X's is increased from .21 percent in sv^n diplo-4 flies to .68 percent in sv^n triplo-4 flies. Presumably, the extra four has interfered with the normal pairing of the X's by forming its own association with an X to cause the increase.

Similarly, LINDSLEY and SANDLER (1956) have reported the effect of an X duplication on the disjunction of the fourth chromosomes. Among 4524 progeny from attached-X females which also carry an X duplication, 29 haplo-4 flies were observed and 27 of these, or .59 percent, also carry the duplication. The non-homologous association in this case appears to be between the X duplication and a fourth chromosome and the amount of non-homologous pairing is related to the amount of nondisjunction of the fours.

In the two instances cited above one chromosome or a part of a chromosome has been added to the normal diploid complement. In each case, a potential univalent is now present and may interfere with the disjunction of a normal diploid pair. The frequency of nondisjunction of this pair is a measure of the degree of interference exerted by the extra chromosome or fragment.

It is only a step from this situation to one in which two extra chromosomal elements are present. Thus, GERSHENSON (1940) introduced three different duplications into triplo-4 males. Two of the three duplications did not assort randomly with the extra fourth chromosome but instead were recovered with a highly significant frequency in the diplo-4 progeny. The two duplications were of X-ray

origin, and although they carried the left tip of X, the remainder of the microchromosome was heterochromatic. The fragments were not distinguishable from the fourth chromosome on the metaphase plate. GERSHENSON points out that among 20 fragments that he had available, the three that were tested were selected because they had no significant effect on the disjunction of the X and Y in the male. Their effect on the disjunction of the X's in the female falls into GERSHENSON's minimal group and this effect does not appear to be significantly higher than that found by SANDLER and NOVITSKI to be caused by an extra fourth chromosome. It may well be that the fragments were in fact selected because they possessed a fourth chromosome centromere instead of an X chromosome centromere.

The present experiments make use of a much more favorable set of conditions for the study of this phenomenon, namely, an extra sex chromosome in the form of a Y and a 3–4 translocation. The translocation is expected to reduce pairing between the fourth chromosomes. For the first set of experiments, Type A females were developed to include a Y chromosome and a heterozygous 3–4 translocation. The two non-homologues (the Y and the normal fourth chromosome) go to opposite poles with a frequency of 78–83 percent instead of 50 percent as expected. The Y and the translocation go to opposite poles with a frequency of 62–68 percent instead of 50 percent. In other words, under these conditions, the Y chromosome and the fourth chromosome show a strong association. This association is stronger between the Y and the normal four than between the Y and the fourth chromosome which is involved in the translocation.

The situation in the second set of experiments (Type D females) is still more advantageous for the detection of nonrandom segregation of the Y and the fourth chromosomes. Type D females carry a Y, a free four and a homozygous 3–4 translocation instead of the heterozygous 3–4 translocation present in Type A females. BROWN (1940) found that in homozygous 3–4 translocations similar to T(3;4)-86D, crossing over and disjunction are normal. If the pairing requirements of the translocation are satisfied in the homozygous condition, the translocation would not be expected to become involved with the Y and segregate from it as it appears to do in the heterozygous condition. An increased frequency of Y-4 pairing and segregation to opposite poles might be expected. The results show that the Y and the fourth chromosome are recovered apart with a frequency of 92 percent in the progeny of Type D females (T(3;4)86D/T(3;4)86D/ci^D) in Table 5 as compared to a frequency of 85 percent in the progeny of Type A females (T(3;4)86-D/ci^D) in Table 3 if uncorrected for haplo-4 viability reduction. In other words, nondisjunction of the Y and the fourth chromosome occurs eight percent of the time when the mother is Type D and 15 percent of the time when the mother is Type A. This is a highly significant difference. When corrected for viability difference the corresponding percentages are eight percent and 16 percent.

If all pairing and subsequent disjunction, including heterochromatic pairing, implies homology, then the results obtained in these experiments may be taken to indicate that homologous regions are present in the Y and fourth chromosomes of

Drosophila melanogaster. On the other hand, since heterochromatin is often characterized by its ability to pair unspecifically with other heterochromatin, the observed Y-4 segregations may result from the general pairing affinity of all heterochromatin.

SUMMARY

1. The segregation of the sex chromosomes, the translocation and the free fourth chromosome has been simultaneously followed in females of *Drosophila melanogaster* which carry a normal Canton-S Y chromosome and which are heterozygous for a 3–4 translocation. A marked non random assortment between the Y and the free four and between the Y and the translocation has been observed for the two types of translocations which were used. When the translocation is one in which disjunction between the translocation and the free four is nearly random (T(3;4)86D), the Y and the free fourth chromosome are found to segregate to opposite poles with a frequency of 83 percent; the Y and the translocation segregate to opposite poles with a frequency of 62 percent. When the translocation is one in which disjunction between the translocation and the free fourth chromosome is more regular (T(3;4)88B), the Y and the free fourth chromosome are found to segregate to opposite poles with a frequency of 78 percent; the Y and the translocation segregate to opposite poles with a frequency of 68 percent.

2. The segregation of the sex chromosomes, the translocation and the free fourth chromosome has been followed in triplo-4 females which carry a normal Canton-S Y chromosome and which are homozygous for a 3–4 translocation (T(3;4)86D). The non random assortment of the Y and the free four is more pronounced in this case. The Y and the fourth chromosomes are found to segregate to opposite poles with a frequency of 92 percent as compared to a frequency of 83 percent when the same translocation is heterozygous.

3. The observed non random assortment between the Y chromosome and the free fourth chromosome or between the Y chromosome and the translocation is interpreted as arising from the association between and subsequent disjunction of the Y and the fourth chromosome or the translocation. Whether the postulated association implies homology remains open to question.

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