PREFERENTIAL SEGREGATIONS IN TRIPLOID FEMALES^{1,2}

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Received September 12, 1960

RECENT work has shown that regular preferential segregations involving the following chromosome pairs can occur in diploid females: Y-II (OKSALA 1958), Y-IV (GRELL 1957, 1959b), X-II (FORBES 1960), Dup(X)-IV (GRELL and GRELL 1960), and X-IV (GRELL 1959a). In all these experiments specific chromosomes were made to behave as univalents with respect to their homologues by the use of translocations and inversions (see OKSALA 1958, for an interesting interpretation of these preferential segregations based on the formation of a polarized bouquet stage in prophase). The particular advantage of triploid females is that the same type of preferential segregations can be observed without the necessity of employing gross chromosomal rearrangements. New information is presented here on chromosome segregation in triploid females which provides additional evidence for interactions between non-homologous chromosomes.

Triploid females provided the first indication that the four different chromosomes in *Drosophila melanogaster* did not always segregate independently of each other. In triploids with three free X chromosomes (referred to in the future as free X triploids) those recovered eggs with two X chromosomes almost always had one set of autosomes, and the recovered eggs with one X chromosome almost always had two sets of autosomes (BRIDGES, in BRIDGES and ANDERSON 1925). Further work with respect to preferential segregations in triploids has been limited to the observations of BEADLE (1934, 1935) on triploid females with two attached-X chromosomes and one free X chromosome (referred to in the future as attached-X triploids). These females showed the same type of preferential segregation observed in free X triploid females, but it was much less marked.

BEADLE discovered the occurrence of X chromosome nondisjunction in attached-X triploid females (1935) and pointed out that here also the distribution of the X chromosomes was not random with respect to the autosomes. The nullo X eggs almost always had two sets of autosomes (resulting in X3A patroclinous metamales when fertilized by X1A sperm) and rarely had one set of autosomes (resulting in X2A patroclinous diploid males when fertilized by X1A sperm). On a random basis, the two types of eggs should have been equally frequent.

¹ From material submitted to the Graduate School of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

² This investigation was supported in part by a Public Health Service Predoctoral Fellowship (9140) from the National Heart Institute, United States Public Health Service.

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In earlier work (1934) many of the attached-X triploid females studied by BEADLE carried a Y chromosome. As a result of secondary nondisjunction, patroclinous diploid males were very frequent, and this was one of the criteria used by BEADLE to separate the Y-bearing from the non-Y-bearing triploid female parents. Neither regular metamales (resulting from X2A eggs and Y1A sperm) nor patroclinous metamales were found by BEADLE (viability of metamales is low and variable), so the relative frequency of 01A eggs *versus* 02A eggs could not be determined. When regular disjunction of the X chromosomes occurred the segregation of the X chromosomes appeared to be nearly random with respect to the autosomes.

Attached-X triploid females were also studied in the present experiments. The use of a marked Y chromosome permitted a study of the segregation of the Y chromosome itself, as well as a more complete analysis of the influence of the Y chromosome on X chromosome segregation. The influence of a free X chromosome carrying multiple inversions on the frequency of primary nondisjunction was also studied. In addition some new information is presented on the types and frequencies of autosomally unbalanced eggs formed by attached-X triploid females.

METHODS

In the basic triploid stock used in these experiments the triploid females carried attached-X chromosomes and a free X chromosome. The attached-X chromosomes were homozygous for the genes yellow² (body color), scute (removes postscutellar bristles), apricot (eye color), and echinus (large rough eyes), and the free X chromosome carried the markers yellow^{31d}, scute⁸, diminutive (small body and bristle size), and Bar (shape of eye). The free X chromosome was the FM4 (First Multiple 4) chromosome of MISLOVE (MISLOVE and LEWIS 1954). This chromosome carried an X-ray induced inversion superimposed on two other inversions already present, *In(1)sc⁸*, and *In(1)dl-49*. The females used to start the stock had been tested to be sure they did not carry a Y chromosome (certain exceptions are noted below). The males in the basic stock carried the FM4 chromosome and a Y chromosome carrying a short duplication of the tip of the X chromosome with the wild type allele of yellow (the *sc⁸·Y*).

To obtain triploid females with normal free X chromosomes, the stock females were mated to males with normal X chromosomes carrying yellow and white (eye color). To obtain triploid females with a Y chromosome, the stock females were mated to males carrying the appropriate X chromosome (FM4, or γw) and both the normal Y and the *sc⁸·Y*. Triploid offspring carrying the *sc⁸·Y* could be picked out because of their "not yellow" phenotype.

In experimental matings the males carried an X chromosome with yellow. If the triploid female carried a *sc⁸·Y* chromosome the males carried a normal Y chromosome, if the triploid females did not have a Y chromosome, the males carried a *sc⁸·Y* chromosome. The scheme for identifying chromosomal origins among the offspring went thus: yellow *versus* wild type determined the absence

or presence of a sc^s Y chromosome, white (or apricot or Bar) *versus* wild type determined maternal *versus* paternal origins of the X chromosomes. All crosses were made at $25 \pm 1^\circ\text{C}$ and offspring were counted from eggs laid during two consecutive five-day periods except for the experiments shown in Table 2, no. 1 and no. 6, where 3–5 such transfers were made. The latter two experiments also furnished data for Table 1.

TABLE 1

Effect of age on segregation patterns shown by triploid females

Type of triploid parent and age in days	Total 2N females produced	Percent attached-X 2N females	Percent exceptional 2N males	Percent exceptional metamales
$\hat{X}X,X$				
1–5	2883	56.1	0.1	0.0
6–10	2556	54.3	0.4	0.1
11–15*	432	54.9	0.0	0.5
Total 1–10	5439	55.2	0.2	0.0
$\hat{X}X,FM4$				
1–5	4035	60.1	1.0	..†
6–10	3789	59.1	1.0	..†
Total 1–10	7824	59.6	1.0	..†
$\hat{X}X,FM4,sc^s.Y$				
1–5	367	48.5	10.1	3.3
6–10	277	52.0	13.7	2.9
11–15	234	48.3	10.7	5.1
Total 1–15	878	49.5	11.4	3.6

* Several experiments involving $\hat{X}X,X$ triploids were pooled, but only one provided data for the 11–15-day age range.
 † This class was not counted.

Methods used for the crosses from which the data for Table 6 was obtained have been reported by FROST (1961). All other information recorded in this paper was obtained from crosses using single triploid females per one ounce creamer. The results shown for no. 1 of Table 2 were obtained in crosses where the possibility of the triploid female parents carrying a Y chromosome had not yet been excluded. However such females could usually be detected because of the high rate of secondary nondisjunction. One female producing five exceptions was excluded and another female producing three exceptions was also excluded as the exceptional males were fertile. Of the remaining 303 females, 32 produced the total of 36 exceptions recorded in Table 2, four triploid females producing two exceptions. Exceptional diploid males from seven different triploid females were tested, the males from only the one female mentioned above being fertile.

Analysis of autosomally balanced eggs

Effect of age: There are four different types of segregation that produce autosomally balanced eggs, two which result in regular eggs, $X1A-\hat{X}X2A$ and

TABLE 2
Autosomally balanced egg types produced by different types of triploids

Triploid type	X Chromosome constitution of eggs and recovered zygotes										Total
	Regular X chromosome segregations					Nondisj. X chromosome segregations					
	X1A — X2A	X2A	X2A	X3A	X3A	X1A — X2A	X2A	X2A	X3A	X3A	
1. $\hat{X}\hat{X},X$ Percent	4365 19.8	3956 18.0	2842 12.9	1683 7.6	8 0.0	5389 24.5	3722 16.9	33 0.1	15 0.1	8 0.0	22034
2. $\hat{X}\hat{X},FM4$ Percent	133 17.8	113 15.1	84 11.3	59 7.9	..	191 25.6	139 18.6	5 0.7	2 0.3	1 0.1	746
3. $\hat{X}\hat{X},FM4^*$	188	222	..	..	..	320	..	..	5	..	782
4. $\hat{X}\hat{X},X$ $sc^e.Y$ Percent	151 16.4	163 17.7	173 18.8	85 9.2	..	122 13.3	162 17.6	16 1.7	23 2.5	5 0.5	919
5. $\hat{X}\hat{X},X^+$ $sc^e.Y$ Percent	267 22.3	194 16.2	210 17.5	119 9.9	2 0.2	174 14.5	201 16.8	12 1.0	10 0.8	2 0.2	1200
6. $\hat{X}\hat{X},FM4$ $sc^e.Y$ Percent	208 21.6	131 13.6	172 17.9	77 8.0	..	111 11.5	177 18.4	1 0.1	40 4.2	8 0.8	963

* In this experiment only diploid offspring and exceptional metamales were counted.
+ In this experiment the triploid females were mated to FM4,Y males instead of to Y,Y males.

$\hat{X}X1A-X2A$, and two which result in nondisjunctional eggs, $01A-\hat{X}XX2A$ and $\hat{X}XX1A-02A$. In Table 1 the following four types of offspring are used as a measure of the relative frequency of the four types of segregation: free X diploid females ($X1A$ egg, $X1A$ sperm) for the $X1A-\hat{X}X2A$ segregation, attached-X diploid females ($\hat{X}X1A$ egg, $Y1A$ sperm) for the $\hat{X}X1A-X2A$ segregation, exceptional diploid males ($01A$ egg, $X1A$ sperm) for the $01A-\hat{X}XX2A$ segregation, and exceptional metamales ($02A$ egg, $X1A$ sperm) for the $\hat{X}XX1A-02A$ segregation. Metamales are the most viable type of offspring produced by this last segregation. $02A$ eggs fertilized by Y-bearing sperm form lethal zygotes, as do $\hat{X}XX1A$ eggs fertilized by X-bearing sperm. $\hat{X}XX1A$ eggs fertilized by Y-bearing sperm form diploid metafemale zygotes which are much less viable than meta-male zygotes. The total number of free X and attached-X diploid females produced is used as a base line with which to compare the relative frequencies of offspring coming from the regular and nondisjunctional segregations. For any one experiment in Table 1, only those triploid females producing offspring in all age categories are compared.

Table 1 shows no effect of age on the pattern of segregations occurring in triploid females, at least for the 1–15 day period studied. REDFIELD (1930) also found that crossing over frequencies in triploids were not affected by age.

Nondisjunction of the X chromosomes: Practically all of the autosomally balanced eggs recovered from attached-X triploid females with a normal free X chromosome come from the two regular segregations. BEADLE found that only 0.2–0.3 percent of the total offspring came from the two nondisjunctional segregations, a result that agrees well with the data presented here in Table 2 no. 1. The proportion of exceptional offspring can be raised markedly, however, by introducing a Y chromosome (Table 2 no. 4 and no. 5), as BEADLE showed with respect to the production of exceptional diploid males.

What Table 2 shows in addition to previously known results is that the frequency of nondisjunction can also be raised by introducing a free X chromosome with multiple inversions and that the pattern of nondisjunction shown in this case is distinctly different from that shown when a Y chromosome is present. If attached-X triploid females with a normal free X chromosome (no. 1) are compared to those with the FM4 chromosome (no. 2) it can be seen that the frequency of exceptional diploid males is increased very slightly from 0.1 percent to 0.3 percent, while the frequency of exceptional metamales is increased from 0.1 percent to 2.5 percent. Rounding off the percent figures to the nearest tenth obscures some of the difference. Comparing the number of exceptions to the total number of regular diploid offspring, there is a 50–70-fold increase in frequency of exceptional metamales, but only a 4–6-fold increase in frequency of exceptional diploid males. There is almost a tenfold difference in frequency of exceptional diploid males *versus* exceptional metamales in the offspring of triploids carrying the FM4 chromosome (no. 2). Thus almost the entire increase in nondisjunction frequency is attributable to the $\hat{X}XX1A-02A$ segregation rather than the $01A-\hat{X}XX2A$ segregation. On the other hand, in triploid females carrying a

Y chromosome (no. 4, no. 5, and no. 6) much of the increase in frequency of nondisjunction is also due to the 01A-XXX2A segregation.

Estimated frequencies of the four types of segregations: Because of differences in viability, it is difficult to properly assess the relative frequency of the nondisjunctional segregations by simply examining the numbers of offspring recorded in Table 2. For this reason estimated frequencies of both the regular and nondisjunctional types of segregation are given in Table 3. The methods used in making these estimations are discussed below in detail since they also illustrate why, in certain cases, conclusions regarding the relative frequency of the two regular segregations are not justified.

One assumption used in making the estimations for Table 3 was that the two types of eggs resulting from any one segregation were equally frequent. Whether this is actually true cannot be determined. For example, in the columns under the X1A-XX2A segregation in Table 2, triploid females in no. 1 and no. 2 occur less frequently than the diploid males or females. This difference could be interpreted in terms of lower viability of triploid females or in terms of a preferential orientation of the first meiotic division so that the XX2A nucleus was preferentially included in the polar body. Estimations based on the latter interpretation would change the frequencies shown in Table 3 very little, at least for those triploid females without the Y chromosome. Even marked preferential orientations occurring in the nondisjunctional segregations would not affect their estimated frequency greatly. If, because of a preferential orientation, 01A eggs were twice as frequent as XXX2A eggs, an estimate of the total number of eggs based on the recovery of 01A eggs only, but which assumed equality of the two types of eggs, would be one and one-third times too large.

Other assumptions used in making the estimates for Table 3 were that X and Y sperm fertilized eggs in a 1:1 ratio, and that diploid offspring (with one exception) were 100 percent viable. Thus for the X1A-XX2A segregation the diploid

TABLE 3

Estimated frequencies in percent of the four segregation types resulting in autosomally balanced eggs

Type of triploid female	Viability of metamales	Regular X chromosome segregations		Nondisjunctional X chromosome segregations	
		X1A-XX2A	XX1A-X2A	01A-XXX2A	XXX1A-02A
XX,X	.006	36	47	0	17
	.130	43	56	0	1
XX,FM4	.026	12	18	0	70
	.130	27	41	0	32
XX,X,sc ^s .Y*	.068		72	4	24
	.130		81	5	14
XX,FM4,sc ^s .Y	?				
	.130		73	7	20

* These estimates are based on the results shown in Table 2, No. 4.

males and females were added together and multiplied by two, for the $\widehat{XX}1A-X2A$ segregation the number of attached-X diploid females was multiplied by four, and for the $01A-\widehat{XXX}2A$ segregation the number of exceptional diploid males was multiplied by four.

A correction was made however, in the number of attached-X diploid females produced by triploid females carrying a Y chromosome. In Table 2 in the columns under the $\widehat{XX}1A-X2A$ segregation, free X intersexes occur less frequently than attached-X diploid females among offspring of triploid females without a Y chromosome. This would be expected since intersexes have lowered viability, but for the triploid females with a Y chromosome the situation is reversed. The most probable explanation seems to be that the viability of attached-X diploid female offspring receiving the maternal Y chromosome, as well as the paternal Y chromosome, is greatly lowered, and most attached-X diploid females do receive the maternal Y chromosome (see the next section). NOVITSKI (1954) discovered a similar effect when studying a tandem acrocentric attached-X chromosome. The latter chromosome had most of the Y chromosome attached to it and NOVITSKI found that twice as many attached-X female offspring were recovered when these females were mated to X·Y/0 males rather than to X/Y males, an effect which he ascribed to the lowered viability of attached-X females hyperploid for the heterochromatin of the X and Y. COOPER (personal communication) has studied $\widehat{XX}-sc^s \cdot Y$ nondisjunction in attached-X females and finds that exceptional female offspring are recovered 3–5 times more frequently when the females are mated with X·Y/0 males rather than with X/Y males, although other experiments of his indicate that it is not simply hyperploidy for the Y chromosome that is responsible for these altered frequencies. The above findings suggest that if the triploids with the Y chromosome had been mated to X·Y/0 males, attached-X diploid female offspring would have been recovered with a much higher frequency, and that in these triploid crosses the viability of attached-X females receiving both Y chromosomes is low. An alternate explanation based on a preferential orientation of the first meiotic division is discussed in the next section, but such an explanation would not alter the conclusions reached at the end of this section since the estimated number of $\widehat{XX}1A-X2A$ segregations would be lowered and the relative frequency of the nondisjunctive segregations would be raised. Since most intersexes do not receive the maternal Y chromosome it was assumed that the viability of free X intersexes was unaffected by the presence of a Y chromosome in the triploid female parent. The expected number of attached-X female offspring could then be derived from the relative frequency of attached-X females and free X intersexes observed among the offspring of triploid females without a Y chromosome.

Table 2 shows another possible influence of the Y chromosome that would lead to errors in estimating the number of $X1A-\widehat{XX}2A$ segregations, but for which no corrections were made. From triploid parents without the Y chromosome (no. 1 and no. 2), triploid offspring are recovered with a somewhat lower frequency than diploid males, but from triploid parents with the Y chromosome

(no. 4, no. 5, and no. 6) triploid offspring are somewhat more frequent than diploid males. Estimates of the frequency of the $X1A-\hat{X}X2A$ segregation would vary according to one's interpretation of these differences, i.e. as changes in the relative viability of the diploid males, or as an influence of the Y chromosome on the preferential orientation of the first meiotic division. The possible errors here would not greatly affect the relative magnitude of the nondisjunctional segregations *versus* the regular segregations, but they would affect estimates of the relative magnitude of the two regular segregations. For this reason the estimates of the two regular segregations made for Table 3 are not shown separately.

BEADLE (1934) had observed that in triploid females without a Y chromosome attached-X diploid female offspring were 1.6 times as frequent as free X diploid females, while in triploid females carrying a Y chromosome, attached-X diploid female offspring were slightly less frequent than free X diploid females. This led to his observation that the segregation of the X chromosomes appeared to be nearly random with respect to the autosomes in triploid females carrying a Y chromosome. The present experiments show that it is not yet possible to say whether the two different regular segregations in these females are equally frequent or not.

The only type of offspring consistently recovered from the $\hat{X}XX1A-02A$ segregation is the exceptional metamale (X1A sperm and 02A egg) and to estimate the frequency of this segregation one must estimate the viability of metamales. Metamales also result from X2A eggs and the number of these regular metamales can be compared to the number of attached-X diploid females since they both come from the same type of segregation. In the case of those triploid females carrying a Y chromosome the number of regular metamales was compared to the corrected number of attached-X diploid females. Viabilities calculated in this way are shown in Table 3 (the first figure given for each type of triploid female) and were the basis for determining the number of $\hat{X}XX1A-02A$ segregations in each experiment. These viabilities may be compared with values of 0.13 and 0.09 calculated from the data of BRIDGES (BRIDGES and ANDERSON 1925) and BEADLE (1935) respectively, and to give an idea of the possible range of error the value of 0.130 is taken as a maximum estimate of metamale viability. Consequently, Table 3 also shows the estimated frequency of $\hat{X}XX1A-02A$ segregations calculated on the latter basis. Whether viability of regular metamales represent good estimates of viability of exceptional metamales is particularly in doubt for those triploid females carrying the FM4 chromosome. Exceptional metamales are wild type or yellow only, while regular metamales are yellow^{31d} (or wild type), scute^s, diminutive, and Bar. Some evidence for the lower viability of FM4 metamales is provided by Table 2 no. 5 where the triploid female parents had been mated to FM4 males instead of to γ males. Consequently the exceptional metamales would carry the phenotype associated with the FM4 chromosome. No exceptional metamales were recovered but the recovery of two exceptional diploid females suggests that the $\hat{X}XX1A-02A$ segregation had been relatively frequent.

Despite the large errors Table 3 makes clear the following points:

1. There is an appreciable frequency of $\hat{X}X1A-02A$ segregations even when no Y chromosome is present and the free X chromosome is structurally normal. BEADLE's 1935 data yield an estimate of four percent for the frequency of the $\hat{X}X1A-02A$ segregation and thus permit the same conclusion.

2. When the FM4 chromosome is substituted for the structurally normal free X chromosome more than 30 percent of the segregations leading to autosomally balanced eggs are due to the $\hat{X}X1A-02A$ type.

3. In the absence of a Y chromosome the frequency of the $01A-\hat{X}X2A$ segregation is negligible, and while the introduction of a Y chromosome causes substantial increases in the frequency of the $01A-\hat{X}X2A$ segregation, $\hat{X}X1A-02A$ segregations are 3–6 times more frequent.

Preferential segregation of the Y chromosome: Table 4 shows the distribution of the Y chromosome among the progeny of the Y carrying triploid females listed in Table 2 no. 4, no. 5, and no. 6, the results for no. 4 and no. 5 being pooled together. The Y chromosome is recovered about 90 percent of the time from eggs with one set of autosomes, a distribution suggesting that when a sex chromosome had not paired with a homologue it had usually paired with an autosome. The only exception to the rule that the Y chromosome is recovered preferentially at the 1A pole is the $\hat{X}X1A-02A$ segregation where the Y chromosome is almost always recovered at the 02A pole. The behavior of the Y chromosome in this case is that predicted from its role in producing nondisjunction. The reasons for coming to the above conclusions are discussed in detail later. Table 4 gives no indication that the Y chromosome pairs preferentially with one of the X chromosomes. If it did the distribution of the Y chromosome with respect to the autosomes should have been markedly different in the two types of regular X chromosome segregations.

Table 4 provides more information regarding the effect of the presence of the Y chromosome on the number of attached-X diploid female offspring produced by triploid females. For every $\hat{X}X1A$ egg without a Y chromosome there should be an $X2A$ egg with a Y chromosome. Thus for each segregation type listed in Table 4 the percentage of eggs with a Y chromosome should add up to 100, but for the $\hat{X}X1A-X2A$ segregation there are marked deficiencies of 8–12 percent. Assuming that mortality of $\hat{X}X2A$ zygotes receiving the maternal and paternal Y chromosomes is the principal cause of the discrepancy, one can calculate that a mortality rate for such zygotes of 48–62 percent, respectively would account for the observed difference. Explanations based on preferential orientation of the first meiotic division are more complicated. One can account for the low number of attached-X diploid females by assuming that the presence of the Y chromosome causes the $X2A$ pole to become the future egg nucleus in preference to the $\hat{X}X1A$ pole, but to explain the distribution of the Y chromosome one would have to assume that the preferential orientation is much more marked when the $X2A$ pole does not receive the Y chromosome.

TABLE 4
Y chromosome segregation in attached-X triploid females

Triploid type	Chromosome constitution of eggs and recovered zygotes										Total			
	Regular X chromosome segregations					Nondisj. X chromosome segregations								
	X1A — $\hat{X}X_{2A}$	X2A $\hat{X}X_{3A}$	$\hat{X}X_{2A}$ $\hat{X}X_{3A}$	$\hat{X}X_{1A}$ — X2A	X2A $\hat{X}X_{3A}$	X1A — $\hat{X}X_{2A}$	X2A $\hat{X}X_{3A}$	$\hat{X}X_{1A}$ — 02A	$\hat{X}X_{2A}$ X3A	$\hat{X}X_{3A}$ X2A				
$\hat{X}\hat{X}_1X_{sc}^s.Y$ Y present	394	338	17	6	..	243	35	3	33	..	1	7	1077	
Y absent	24	19	366	198	2	53	328	25	..	7	17	1	2	1042
Percent with Y	94	95	4	3	..	82	10	11	100	0	0	..	78	51
$\hat{X}\hat{X}_1FM_{4,sc}^s.Y$ Y present	186	118	12	2	..	91	10	..	40	..	..	..	15	474
Y absent	22	13	160	75	..	20	167	1	..	8	23	..	..	489
Percent with Y	89	90	7	3	..	82	6	..	100	0	0	..	100	49

TABLE 5
Frequencies of autosomally balanced and unbalanced eggs produced by attached-X triploid females

Type of triploid	Number of eggs counted	Number of recovered diploid zygotes			Percent of* autosomally balanced eggs recovered as 2N offspring	Percent of eggs with balanced sets of autosomes	Percent of eggs with unbalanced sets of autosomes
		XX2A	XY2A	$\hat{X}\hat{X}Y_{2A}$ Total			
1. $\hat{X}\hat{X}_1X$ Percent of eggs	1960	60	51	80 191	30-36	32-27	68-73
		3.0	2.6	4.0 9.6			
2. $\hat{X}\hat{X}_1FM_4$ Percent of eggs	2809	63	59	87 209	11-24	67-31	33-69
		2.2	2.1	3.1 7.4			

* These percents are derived from the values shown in Table 3.

Analysis of autosomally unbalanced eggs

Proportion of balanced versus unbalanced eggs produced: Autosomally unbalanced eggs produced by triploid females ordinarily lead to lethal aneuploid zygotes. The total proportion of such eggs can be estimated by making egg counts and determining from the diploid offspring produced what the total number of autosomally balanced eggs should be, the remainder being the unbalanced eggs. The ratio of diploid offspring to total number of autosomally balanced eggs can be found by using the frequencies of the different segregation types recorded in Table 3. The results of such egg counts and calculations are shown in Table 5. They indicate that about 70 percent of the eggs from attached-X triploid females with a normal free X chromosome are unbalanced autosomally. A certain proportion of the eggs which would have been recovered as diploid offspring probably die for unknown causes and thus contribute to the total pool of "unbalanced" eggs. Because the number of diploid offspring produced is such a small percentage of the eggs laid, the degree of such unknown mortality would have to be very great to significantly affect the 70 percent. For example, if only 90 percent of the potential diploid offspring survived, the frequency of diploid zygotes recovered would be ten percent too low and the calculated 30 percent of eggs with balanced sets of autosomes would be ten percent too low. This would only lower the proportion of autosomally unbalanced eggs to 67 percent.

Because of the uncertainty regarding the viability of metamales the proportion of unbalanced eggs formed by triploid females with the FM4 chromosome could be anywhere from 33–69 percent. There is good reason to believe that the 33 percent figure is too low. As discussed earlier in connection with Table 3 the viability of regular metamales carrying the FM4 chromosome, on which the 33 percent figure is based, was probably too low an estimate of the viability of the exceptional metamales. A direct test of the frequency of unbalanced eggs is also possible by comparing the two types of triploids shown in Table 5 with respect to the frequency with which nondisjunctional sperm are recovered from the same types of males. Frost's data (1961, Table 2, Experiments I, II and III) are here analyzed in this regard. For such a comparison the number of attached-X diploid females produced can be used as a measure of the size of the experiment. From the FM4 type of triploid, 14 nullo II sperm were recovered out of 11,045 attached-X diploid females produced, from the triploids carrying a normal free X chromosome the numbers were 11 out of 8,752 respectively, the rate for both types of triploids being 0.13 percent. This does not necessarily mean that both types of triploid females form the same proportion of unbalanced eggs since Table 5 shows that per egg laid, triploid females with the FM4 chromosome produced only 77 percent as many attached-X diploid females. Thus for an identical rate of autosomally unbalanced eggs produced per attached-X diploid female, the total proportion of unbalanced eggs produced by the FM4 triploid females need only be 52–56 percent.

For any one class of diploid offspring listed in Table 5 the difference in proportion of diploid zygotes recovered between the two types of triploid females is

not significant at the five percent level. For the total number of diploid offspring produced, however, the difference in proportion is significant at the one percent level.

Different types of autosomally unbalanced eggs formed by triploid females: Autosomally unbalanced eggs can be recovered if they are fertilized by the complementary type of unbalanced sperm, or by sperm where the subsequent loss of a paternal chromosome in the developing zygote mimics fertilization by nullo II or nullo III sperm. The latter effect can be accomplished by X-irradiation of sperm and extensive data of this sort are given by PONTECORVO (1943). The former type of sperm result from autosomal nondisjunction in the male, a phenomenon described by STERN (1934), PONTECORVO (1940), and FROST (1961), and that gives rise to diplo II and diplo III sperm as well as nullo II and nullo III sperm. Thus eggs with a II,III,III constitution have been recovered by being fertilized with diplo II sperm (forming intersexes or triploid females) or with nullo III sperm (forming diploid males or females). Eggs with the II,II,III constitution have been recovered in similar fashion with nullo II and diplo III sperm.

PONTECORVO (1943) pointed out that $\hat{X}\hat{X};II,II;III-X;II,III,III$ segregations were just as frequent as $X;II,II,III-\hat{X}\hat{X};II,III,III$ segregations, i.e. that sex chromosome orientation with respect to the autosomes was random. He also found the same to be true when nondisjunction of the sex chromosomes had occurred. The frequency of nondisjunction was high. Of the 140 exceptions he recovered in which one paternal autosome had been lost, 22 were patroclinous males. However it is not possible from his data to disentangle the various factors leading to sex chromosomal nondisjunction. The triploid females used by PONTECORVO carried attached-X chromosomes and a free X chromosome carrying two inversions, and about 60 percent of the females also carried a Y chromosome.

Table 6 shows how the autosomal exceptions reported by FROST (1961, Table 2) were broken down with respect to the egg types recovered. It pools data obtained from attached-X triploid females with a normal free X chromosome as well as from triploid females with the FM4 chromosome. Since any given line in Table 6 represents just one type of nondisjunctional sperm, the number of different egg types recorded in that line reflects their relative frequency. Allowance must be made, of course, for the lower viability of intersexes or triploid metafemales. Thus data for sperm types no. 2, no. 3, no. 6, and no. 7 accord with PONTECORVO's observation that free X and attached-X chromosomes occur with equal frequency in both types of autosomally unbalanced eggs. The data for sperm types no. 1 and no. 5 show that nondisjunction of the X chromosomes is quite frequent, four out of the 18 eggs (22 percent) recovered were nullo X. The occurrence of triplo X eggs is also demonstrated in the line for sperm type no. 8. Of the 18 eggs recovered with sperm types no. 1 and no. 5, 11 came from triploid females with the normal free X chromosome, two being nullo X, the proportion of nondisjunctional eggs for the FM4 triploid females being two out of seven. Consequently the frequency of sex chromosomal nondisjunction is relatively high for both types of females. It should be noted that the data for sperm type no. 5 of

TABLE 6

*X chromosome constitution of autosomally unbalanced eggs produced by attached-X triploid females**

Constitution of sperm	Constitution of egg II,II;III				Sex types of offspring
	0	X	XX	XX,X	
Nullo II sperm					
1. X bearing	3	8	—	—	diploid male and female
2. Y bearing	—	6	8	—	diploid male and female
Diplo III sperm					
3. X bearing	—	3	3	—	intersex and triploid female
4. Y bearing	—	—	0	0	intersex and triploid female
Nullo III sperm					
II;III,III					
5. X bearing	1	6	—	—	diploid male and female
6. Y bearing	—	5	5	—	diploid male and female
Diplo II sperm					
7. X bearing	—	0	4	0	intersex, triploid female, and triploid metafemale
8. Y bearing	—	—	0	1	intersex and triploid female

* This table represents a further analysis of the information on autosomal nondisjunction in males recorded in Table 2 of FROST 1961.

Table 6 came from an experiment where the possibility of the triploid female parents carrying a Y chromosome had not yet been excluded. However, judging from the frequency of exceptional diploid males, the proportion of such females, if they occurred, must have been very small.

The two types of unbalanced eggs discussed so far are not the only ones produced by triploid females. In crosses with triploid females having three free X chromosomes STERN (1934) found nullo II and nullo III eggs (X;0;III and X;II;0 respectively), and FROST (1961) also reported the occurrence of a nullo II egg (X;0;III) from an attached-X triploid female. These egg types were recovered when fertilized by diplo II or diplo III sperm, thus forming diploid offspring. To gain an estimate of the relative frequency of nullo II eggs we can compare them with II,II;III eggs which also result in diploid offspring if fertilized by nullo II sperm. Assuming that nullo II and diplo II sperm occur equally often, as indicated by the results of FROST (1961), the two different egg types would be recovered with equal frequency if they occurred with equal frequency. The same type of comparison can be made for nullo III eggs and II,III,III eggs. In FROST's experiments only one 0;III egg was recovered compared to 25 II,II;III eggs. In STERN's experiment the ratio was seven to six respectively for chromosome II, and for chromosome III the ratio was five to two. The higher frequency of nullo-type eggs observed in STERN's experiments may reflect the influence of the different structure of the X chromosomes present in the triploid females used.

DISCUSSION

BEADLE (1935) suggested that as a result of the mass and number of chromosomes gathered together at metaphase they would tend to become oriented so that both sides of the metaphase plate would have equal numbers of chromosomes, a tendency termed the crowding effect by SANDLER and NOVITSKI (1956). In this way BEADLE explained why $\hat{X}\hat{X}1A-X2A$ segregations were more common than $X1A-\hat{X}\hat{X}2A$ segregations, and why $\hat{X}\hat{X}\hat{X}1A-02A$ segregations were more common than $01A-\hat{X}\hat{X}\hat{X}2A$ segregations. The present paper shows that the segregation of the Y chromosome follows the same rule, as it is most often recovered at the pole with the fewest chromosomes.

The possibility that chromocentral regions of non-homologous chromosomes might pair and subsequently disjoin was suggested by MULLER (cited by PONTECORVO 1940) as an explanation for the preferential segregations observed in triploid females. This idea did not receive further attention until it was proposed anew in more detail by SANDLER and NOVITSKI in 1956. The latter two authors reviewed evidence for considering heterochromatic regions of the chromosomes to be nonspecific in their pairing affinities, and suggested that if each set of homologues in a triploid formed a bivalent plus a univalent, non-homologous pairs could easily form between the heterochromatic kinetochore regions of the different univalents. They pointed out that pairing between chromosome II and III would result in unbalanced eggs and that pairing between an X chromosome and an autosome would result in the preferential recovery of eggs from the $\hat{X}\hat{X}1A-X2A$ segregation. They suggested that in attached-X triploid females the free X chromosome and attached-X chromosomes would often form what was essentially a single bivalent, thus decreasing opportunities for non-homologous pairing.

About the same time that SANDLER and NOVITSKI suggested the above hypothesis a similar proposal was made with respect to interchromosomal effects on segregation in diploid females. COOPER, ZIMMERING, and KRIVSHENKO (1955) suggested that non-homologous pairing was responsible for the increased frequency of primary nondisjunction of the X chromosomes observed when both the X chromosomes and an autosome were heterozygous for inversions. These authors, however, remained noncommittal about the chromosome regions involved in the non-homologous association.

Much of the new information on triploid segregations presented here can be readily explained by the non-homologous pairing hypothesis of SANDLER and NOVITSKI.

In the discussion which follows it will be assumed that the pairing tendencies of the attached-X chromosomes are never entirely "self-satisfying" as indicated, for example, by the fact that the Y chromosome and attached-X chromosomes in diploid females regularly segregate to opposite poles. Consequently, whenever the attached-X chromosomes and free X chromosome have not paired with each other in triploid females, both chromosomes are considered to be available for non-homologous association. Pairing may not always ensure disjunction but for

the purposes of the present discussion the terms "pairing" and "association" are used in a restricted sense to indicate only those cases in which the pairing does ensure the disjunction of the two chromosomes involved.

That 70 percent of the eggs produced by attached-X triploid females with a normal free X chromosome are unbalanced autosomally can be accounted for by pairing between chromosomes II and III. Such pairing always results in unbalanced eggs. If the "extra" chromosomes II and III segregated randomly with respect to each other one would expect only 50 percent of the segregations to result in unbalanced eggs. The excess over 50 percent could be accounted for if 40 percent of the segregations were preceded by II-III pairing, half of the remaining segregations also producing unbalanced eggs.

There are two ways in which the "extra" chromosomes II and III could be expected to segregate randomly with respect to each other, (1) if all associations were homologous and the two autosomal trivalents oriented randomly at the metaphase plate, and (2) if non-homologous associations formed between an autosome and a sex chromosome, the resulting non-homologous pair orienting randomly with respect to the other chromosomes. This second way suggests that if the sex chromosomes were made more available for non-homologous pairing X-chromosome-autosome pairs should form at the expense of II-III pairs and the total frequency of autosomally unbalanced eggs should drop from 70 percent to a value closer to 50 percent. Such appears to be the case for the triploid females carrying the FM4 chromosome. The multiple inversions present in the free X chromosome would make both the attached-X chromosomes and the free X chromosome available for non-homologous pairing.

Practically all of the X chromosome nondisjunction occurring in the FM4 triploid females can be attributed to X-chromosome-autosome pairing since the frequency of $01A-\hat{X}XX2A$ segregations is negligible compared to the frequency of $\hat{X}XX1A-02A$ segregations. The former type of segregation would never occur as a result of X-chromosome-autosome pairing but should be as frequent as $\hat{X}XX1A-02A$ segregations were the X chromosomes segregating randomly with respect to the autosomes. If we take 54 percent as the best estimate of the frequency of autosomally unbalanced eggs formed by the FM4 triploid females, then autosomally balanced eggs account for 46 percent of the segregations and close to half of these balanced eggs would result from the $\hat{X}XX1A-02A$ segregation.

The introduction of a Y chromosome causes a marked increase in the frequency of $01A-\hat{X}XX2A$ segregations occurring in the FM4 triploid females. Since nondisjunction of the X chromosomes can result from X-Y pairing, and since the latter type of pairing reduces the number of X chromosomes available for non-homologous pairing, a portion of the segregations resulting in X chromosome nondisjunction could be expected to show independent orientation of sex chromosomes and autosomes. It is not contradictory to the above reasoning to realize that pairing of the Y chromosome with an X chromosome also makes the remaining X chromosome available for non-homologous pairing. Thus in examining the

effect of the Y chromosome on segregation in attached-X triploid females with a normal free X chromosome, the increase in nondisjunction frequency is accompanied by the same marked preference for the $\hat{X}\hat{X}\hat{X}1A-02A$ segregation. The $\hat{X}\hat{X}\hat{X}1A-02A$ segregations were 3–6 times more frequent than $01A-\hat{X}\hat{X}\hat{X}2A$ segregations but for every one of the latter segregations there should be one of the former type where no X-autosome association was involved either. Consequently anywhere from 50–29 percent respectively of the segregations resulting in X chromosome nondisjunction involved independent orientation of sex chromosomes and autosomes. One could imagine that the X chromosomes would be least available for non-homologous pairing when the Y pairs with both the free X chromosome and an arm of the attached-X chromosome.

An examination of the consequences of X-Y pairing makes it seem unlikely that the Y chromosome regularly pairs with the X chromosomes. It should be possible for the attached-X chromosomes to pair with the free X chromosome and the Y chromosome. Such pairing would result in regular disjunction of the X chromosomes but cannot be very frequent since there is no indication that the Y chromosome segregates preferentially from the attached-X chromosome. The Y chromosome could pair with both the free X chromosome and an arm of the attached-X chromosomes, or it could pair with either of the X chromosomes separately. The former situation would always result in X chromosome nondisjunction and the latter situation would result in X chromosome nondisjunction 50 percent of the time since the X chromosomes would segregate randomly with respect to each other. For the triploid females with the normal free X chromosome, at least, the nondisjunctional segregations account for only 20–30 percent of the autosomally balanced eggs (see Table 3). Consequently the Y chromosome, at most, would only be paired with the X chromosomes in 40–60 percent of the segregations producing autosomally balanced eggs.

To explain the preferential recovery of the Y chromosome at the 1A pole one must make one or both of the following assumptions, depending on the frequency of X-Y pairing: (1) when the Y chromosome had not paired with an X chromosome, it usually had paired with an autosome, or (2) when the Y chromosome had paired with one of the X chromosomes, the remaining X chromosome usually had paired with an autosome. Since the Y chromosome was recovered at the 2A pole about ten percent of the time, in only 20 percent of the regular X chromosome segregations would it not be necessary to invoke one of the above assumptions.

Attached-X triploid females show striking differences from free X diploid females with respect to frequencies of X chromosome nondisjunction. Primary nondisjunction in diploid females occurs with a very low frequency, less than 0.1 percent (BRIDGES 1916), and is affected very little by the presence of structural heterozygosity in the X chromosomes (STURTEVANT and BEADLE 1936). In attached-X triploid females, however, primary nondisjunction certainly occurs with a frequency greater than 0.1 percent, more probably with a frequency of several percent, and the introduction of the FM4 free X chromosome causes high

frequencies of nondisjunction. Frequencies of secondary nondisjunction parallel the differences observed in primary nondisjunction. In diploid females with normal X chromosomes the frequency of secondary nondisjunction is about eight percent (BRIDGES 1916), but the introduction of a Y chromosome into attached-X triploid females produces frequencies of nondisjunction of 20–30 percent. The results of such comparisons, as well as the different types of preferential segregations shown by triploid females, agree well with the view that in triploid females meiotic pairing is by twos, and that one member of any given set of homologous chromosomes is usually available for non-homologous associations.

Triploid metafemales (4X3A) provide one further test of this view of chromosome segregation in triploids. In triploid metafemales carrying attached-X chromosomes, one normal free X chromosome, and the FM4 chromosome, FROST (1960) reported that most of the attached-X chromosomes recovered came from 2A eggs, and that most of the free X chromosomes recovered came from 1A eggs. This type of preferential segregation is the opposite of that found in attached-X triploid females (see Table 2 and BEADLE 1934, 1935). The attached-X chromosomes and the normal free X chromosome would be expected to pair most of the time but the FM4 chromosome should usually be available for non-homologous associations. Thus the attached-X chromosome and the normal free X chromosome should usually separate from each other randomly with respect to the autosomes, but the FM4 chromosome should be found preferentially at the 1A pole. The resulting segregations would be of two kinds, FM4 $\hat{X}$ X1A-X2A and FM4X1A- $\hat{X}$ X2A. The latter type of segregation produces the observed result. The only offspring recoverable from the former type of segregation would be metamales, intersexes, or diploid metafemales. None were recovered but this is not significant since only one of the 16 2X2A eggs recorded was recovered as an intersex. This explanation would predict that in triploid metafemales carrying two normal free X chromosomes this type of preferential segregation would disappear. If both free X chromosomes paired with the attached-X chromosome, the resulting complex should orient randomly with respect to the autosomes. If the two free X chromosomes paired with each other, the attached-X chromosomes should be available for non-homologous pairing and $\hat{X}$ XX1A-X2A segregations would be more frequent than $\hat{X}$ XX2A-X1A segregations. However the latter type would be recovered preferentially since the former type leads to offspring with very poor viability.

There were two observations which violated the crowding effect rule. The first is compatible with the analysis made up to this point and concerns the apparent high frequency of X chromosome nondisjunction among the autosomally unbalanced eggs formed by attached-X triploid females with the normal free X chromosome. One would expect that frequencies of X chromosome nondisjunction in autosomally unbalanced eggs should be the same, or even less than the frequencies observed in autosomally balanced eggs. For example, if both the attached-X chromosomes and the free X chromosome had formed separate non-homologous pairs with different autosomes, segregation of the two pairs would

result either in autosomally balanced eggs but nondisjunction of the X chromosomes, or in autosomally unbalanced eggs but regular disjunction of the X chromosomes. If only one non-homologous pair formed and the remaining sex chromosome and autosome segregated randomly, nondisjunction of the X chromosomes should occur equally often in both autosomally balanced and unbalanced eggs. Table 3 indicates that the frequency of X chromosome nondisjunction among the autosomally balanced eggs is 1–17 percent. The observation of two nondisjunctional eggs out of 11 autosomally unbalanced eggs suggests that the true frequency of nondisjunction in the former case lies somewhere in the middle or upper part of the range.

The second violation of the crowding effect rule concerns the existence of X;0;III or X;II;0 eggs. At present there appears to be no adequate explanation for the occurrence of such eggs. The non-homologous pairing hypothesis does not provide an adequate explanation since it is difficult to see how so few chromosomes could determine the disjunction of so many other chromosomes to the opposite pole. The presence of undetected autosomal inversions might provide a possible mechanism for the production of such eggs. Four strand double cross-overs within heterozygous X chromosome inversions are known to result in nullo X eggs (STURTEVANT and BEADLE 1936). A similar type of double crossover occurring within a paracentric autosomal inversion might prevent the two chromosomes involved from reaching the future egg nucleus if the chromosome bridges did not break. The remaining chromosome could be expected to segregate to the future egg nucleus pole or to the future polar nucleus pole at random. In order to explain STERN's (1934) results on this basis one would not only have to assume that both chromosome II and III were carrying inversions but that the frequencies of four strand doubles were enormously high. Nullo type eggs were recovered as frequently as II,II;III or II;III,III eggs and it seems unlikely that triploid females produce autosomally unbalanced eggs with a frequency lower than 50 percent.

While the present information on segregations in triploid females provides abundant evidence for interactions between sex chromosomes and autosomes, it does not indicate whether there are preferential associations between particular chromosomes. From a study of autosomally balanced eggs, for example, it is not possible to tell whether the sex chromosomes had paired with chromosome II or III, even if both were "marked" by the use of multiple inversions. If a sex chromosome had paired with the marked chromosome II, then the only eggs that would be recovered would be those in which the marked chromosome III had also proceeded to the pole opposite the sex chromosome. Studies of autosomally unbalanced eggs should provide answers to this question but our present information is rather meagre. PONTECORVO's results would indicate that non-homologous pairs do not form exclusively between only one of the autosomes and the sex chromosomes, but it is difficult to know just how much non-homologous pairing would have been expected in the triploid females he used. The most rewarding studies would be on free X triploid females, or on attached-X triploid females

with the FM4 chromosome where the majority of the segregations probably involve non-homologous pairing between the X chromosomes and autosomes.

SUMMARY

Evidence is presented which confirms BEADLE's (1934, 1935) findings that primary nondisjunction of the X chromosomes occurs with an appreciable frequency in attached-X triploid females, that the segregation of the autosomes is highly non random when X chromosome nondisjunction occurs such that 01A-XXX2A segregations occur with a negligible frequency compared to XXX1A-02A segregations, and that the presence of a Y chromosome produces large increases in X chromosome nondisjunction. Attached-X triploid females have been further studied in relation to the following points:

1. The presence of a free X chromosome carrying multiple inversions causes large increases in the frequency of primary nondisjunction. The increase is accompanied by the same preferential orientation of the autosomes.

2. The presence of a Y chromosome causes substantial increases in the frequency of 01A-XXX2A segregations, but XXX1A-02A segregations are still 3-6 times more frequent.

3. When regular disjunction of the X chromosomes takes place, the Y chromosome is almost always recovered at the pole with one set of autosomes. When nondisjunction of the X chromosomes occurs, the Y chromosome is almost always recovered at the nullo X pole.

4. The number of regular diploid offspring recovered from triploid females with a normal free X chromosome or a free X chromosome carrying multiple inversions, accounts for only 9.6 percent and 7.4 percent respectively of the total number of eggs produced. The corresponding estimates of the total frequency of autosomally balanced eggs produced by the two types of triploids are 27-32 percent and 31-67 percent respectively.

5. Further evidence is given supporting PONTECORVO's (1941) observation that there is no preferential segregation of the X chromosomes with respect to the autosomes among autosomally unbalanced eggs.

6. A coherent explanation of all the above known facts concerning segregation in triploid females can be made on the basis of SANDLER and NOVITSKI's (1956) hypothesis that pairing can frequently occur between non-homologous univalents in their heterochromatic kinetochore regions. However there is no adequate explanation as yet for the occurrence of nullo II and nullo III eggs reported by STERN (1934) and FROST (1961).

7. The sharp reduction in the number of attached-X diploid females produced by triploid females carrying a Y chromosome is probably caused by low viability of attached-X females receiving both the maternal and paternal Y chromosomes.

8. Age of the triploid female, up to 15 days, has no effect on the frequency of X chromosome nondisjunction or other patterns of chromosome segregation.

ACKNOWLEDGMENTS

The author is grateful for DR. CURT STERN's encouragement and interest

throughout these investigations and for his critical advice during the preparation of this manuscript.

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