PREFERENTIAL SEGREGATION OF THE Y CHROMOSOME IN SPERMATOGENESIS OF DROSOPHILA MELANOGASTER

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ABSTRACT

PREFERENTIAL SEGREGATION OF THE Y CHROMOSOME IN SPERMATOGENESIS OF DROSOPHILA MELANOGASTER

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Deviations from expected 1:1 sex ratio in organisms utilizing XY-sex determination are frequently observed. Aberrant sex ratios may be due to factors operating prior to fertilization, causing changes in the primary sex ratio, or subsequent to fertilization, resulting in a change in the secondary sex ratio. It has been established that normally deviant sex ratio shifts noted in *Drosophila melanogaster* are due to factors functioning prior to fertilization.

A relationship between paternal age and sex ratio due to a pre-fertilization phenomenon has been described. With an increase in the age of the male parent a decrease in the frequency of recovery of the Y chromosome is found. The object of this study is to demonstrate preferential segregation of the Y chromosome as a possible explanation for the paternal-age-related sex ratio shift.

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In order to test the hypothesis proposed crosses were made to determine the frequency of recovery of the Y chromosome. From a cross involving an attached-XY male and a normal female, critical evidence to support the hypothesis was obtained. This could be concluded since the Y chromosome is recovered with a decreasing frequency as the age of the male increases even when the Y chromosome is attached to an X chromosome. In all of the crosses carried out in this study this decrease in recoverability of the Y chromosome was realized independently of the chromosome complement of the aging male. Whether this preferential nonrandom segregation is due to the size of the Y chromosome or to some mechanism within the chromosome or its centromere is, as yet, undetermined.

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PREFERENTIAL SEGREGATION OF THE Y CHROMOSOME IN SPERMATOGENESIS OF DROSOPHILA MELANOGASTER

INTRODUCTION

Discernible discrepancies in the theoretical progeny ratios, presumed by the principles of random segregation and random fertilization, have been frequently noted. Various causative mechanisms resulting in aberrant ratios have been proposed. These mechanisms may act during meiosis, gametogenesis or upon the zygote causing a reduction in number or the elimination of a particular class of progeny.

Deviations from the theoretical 1:1 sex ratio in organisms utilizing X-Y sex determination have been studied. Through experimentation with <u>Drosophila</u> it has been possible to obtain clear and precise analysis of sex determination and the hereditary factors influencing it. In <u>Drosophila</u> normal females produce eggs which contain one set of autosomes and one X chromosome and males produce sperm containing one set of autosomes and either an X or a Y chromosome. The principle of random segregation supposes that equal numbers of X and Y bearing sperm will be produced. Assuming that fertilization also occurs at random, a sex ratio of 1♀:1♂ should be achieved. However, many deviations from the expected 1:1 sex ratio have been reported. According to Brehme (1943) aberrant ratios may be a result of factors which affect the production or viability of X and Y sperm, factors affecting the balance between sex-chromosomes and autosomes, lethal factors affecting the survival of one or the other sex and factors causing reversal of the sex of the individual.

One of the first aberrant sex ratios to be studied was described by Morgan (1911). He found that female *Drosophila* carrying the mutant for rudimentary wings are almost sterile when mated to mutant males. However, when these females were mated to normal males their offspring were in the ratio of 2 females to 1 male. He attributed this distortion in sex ratio to a lethal factor contained in the single X-chromosome of the lost males passed on to them by their mothers. Counce (1956) studied the embryology of the rudimentary mutant and found that it affects larval differentiation.

Gershenson (1928) described a sex-ratio abnormally found in a wild population of Drosophila obscura resulting in the production of almost all female progeny. He attributed this abnormality to a sex-linked mutant whose action was analogous to that of a gametic lethal, in that it renders Y-bearing spermatozoa incapable of participating in fertilization. This phenomenon has been referred to as "sex-ratio." Further analysis of the "sex-ratio" condition was carried out by Sturtevant and Dobzhansky (1936) and Wallace (1948) in Drosophila pseudoobscura. It was shown that "sex-ratio" was widely distributed both geographically and taxonomically. Cytological examination of spermatogenesis by Sturtevant and Dobzhansky led them to conclude that in males carrying "sex-ratio" the X chromosome undergoes an equational division at each meiotic division and at the same time the Y chromosome degenerates. Thus, all the products of spermatogenesis would receive an X chromatid. Gershenson suggested that since there was an absence of differential viability the "sex-ratio" factor would increase in the wild population and would be fatal to the race. Wallace (1948) pointed out that such an increase does not actually occur due to seasonal fluctuations in the frequency of "sex-ratio." Therefore, natural selection prevents the X chromosome bearing "sex-ratio" from replacing its normal homolog.

Novitski (1947) described an anomalous sex ratio condition in *Drosophila affinis* which resulted in the production by certain *affinis* males of only male offspring. He referred to this phenomenon as "male sex-ratio." He stated that this condition has as its basis a genetic constitution involving the "sex-ratio" X chromosome found in *Drosophila pseudoobscura* and a recessive gene on chromosome B. Its mode of action may correspond to the "sex-ratio" condition described for the *obscura* group.

Another type of sex ratio abnormality producing unisexual progenies has been extensively studied. The factor, sex ratio (*SR*), which results in an absence of male progeny was first reported by Magni (1953) in *Drosophila bifasciata* and by Cavalcanti and Falcao (1954) in *Drosophila prosaltans* and by Carson (1956) in *Drosophila borealis*. *SR* was found to be transmitted exclusively from mothers to daughters, which led to the conclusion that this abnormality was due to the transmission of a causative agent through the cytoplasm of the egg. This causative agent acted by killing the eggs fertilized by Y-bearing sperm. Magni was able to effect a "cure" for <u>SR</u> by high temperatures and Cavalcanti and Falcao found strains containing nuclear genes which disrupt the transmission of "sex-ratio" particles.

Malogolowkin and Poulson(1957) reported the existence of a maternally inherited "sex-ratio" condition in <u>Drosophila willistoni</u> which could be effectively transmitted by injection of ooplasm from infected into normal females. Poulson and Sakaguchi (1961) were able to establish the presence of treponema-like spirochetes in the hemolymph of adult "sex-ratio" females which are completely correlated with the production of unisexual progenies. They concluded that the "sex-ratio" condition in <u>Drosophila</u> species is the consequence of a stabilized host-parasite relationship which is dependent upon the genotype of the host and of the infectious agent.

Aberrant sex ratios have also been reported in higher animals whose sex determination is dependent upon the heterogametic male. Varied hypotheses and correlations have been proposed in an attempt to decipher the cause of these deviant ratios.

Weir (1958) demonstrated by artificial insemination experiments that an abnormal sex ratio he observed in mice was due to a distortion in the primary sex ratio and not to differential survival of zygotes. He hypothesized that the X- and Y-bearing sperm are already present in disproportionate numbers by the time they reach the ductus deferens. A correlation between blood pH and sex ratio in mice was also investigated (Weir, 1962). The offspring of a high-pH mouse strain resulted in a higher percentage of males, and a low-pH mouse strain was associated with a low percentage of males. This shift in sex ratio was shown by experimentation to be dependent upon the genotype of the male parent. No indication of significant differential mortality of the zygotes was found in the results.

That a positive association exists between mean male sex ratio of children and the longevity of their parents in man has been reported by Lawrence (1940). He also noted that there was no regular increase or decrease in the sex ratio with increasing ages of the parents. Contrary to this finding Novitski (1953), Novitski and Sandler (1956) and Novitski and Kimball (1958) reported a linear relationship between paternal age and secondary sex ratio in man. Analysis demonstrated that there is a decrease in male offspring as the age of the father increases and that this decrease is independent of the age of the mother.

Two cases of age-dependent sex ratio shifts have also been reported in Drosophila. Hannah (1955) found that aging the maternal parent resulted in an increase in the proportion of male offspring. Aging of mature gametes renders them less viable than unaged mature gametes, and certain genotypes proved to be more sensitive to the aging effect than other genotypes. Hannah suggests several mechanisms which may contribute to the modified sex ratio caused by aging: missegregation or loss of a chromosome during meiosis, crossing-over lagging, or nuclear-cytoplasmic interaction. An opposite effect of aging in Drosophila was also described. Yanders (1965) demonstrated that aging of Drosophila males causes a shift in sex ratio toward a greater proportion of female progeny. This paternal-age-related sex ratio shift is analogous to that found in man.

A possible explanation for the observed deviations from the expected 1:1 sex ratio in animals was proposed by Novitski (1951). He found that in Drosophila melanogaster females the physically shorter chromosome of a structurally heteromorphic pair is included in the functional egg nucleus more frequently than the longer chromosome. The effect was demonstrated by the use of ring-X and attached-X chromosomes and confirmed by experiments involving structurally dissimilar rod chromosomes. This preferential recovery was attributed to non-random disjunction of chromosomes at the second meiotic division of oogenesis. Novitski suggested that this phenomenon may be responsible for aberrant sex ratios where sex is determined by a heteromorphic pair of chromosomes.

Deviations from theoretical ratios other than sex ratio have also been studied. In their analysis of segregation in a translocation heterozygote, Novitski and Sandler (1957) postulated that not all of the products of spermatogenesis are functional. They found that in certain translocation-bearing (T (1:4) B^S) males complementary classes of gametes were not recovered in equal numbers. Egg count experiments gave no evidence for differential zygote mortality. Gamete lethality and sperm competition were ruled out as possible causes of the distorted segregation ratios. They proposed that there exists a regular class of nonfunctional gametes and that particular chromosomes segregated preferentially into these non-functional meiotic products.

Using the same Bar-Stone translocation males, Zimmering and Barbour (1961) observed an "age effect" associated with the recovery of aberrant gametic ratios. Observations indicated that the first sperm released by young Bar-Stone males clearly gave aberrant ratios; however, those sperm released later by the same males show almost no distortion. That physiological differences between groups of cells destined to give rise to different sperm batches exist was suggested by Zimmering and Barbour. They also speculated that their results might have some bearing on the finding by Novitski and Sandler (1956) that the secondary sex ratio in humans is dependent upon paternal age. This could be accounted for by a shift in the relative frequencies of X and Y-bearing sperm with an increase in the age of the father.

A cytogenetic study designed to elucidate the SD (segregation-distorter) phenomenon in *Drosophila melanogaster*, which was described by Sandler, Hiraizumi, and Sandler (1959), was undertaken by Peacock and Erickson (1965). The SD locus is proximally located on the right arm of the second chromosome and it functions as an extreme case of meiotic drive. When males are heterozygous for SD, the SD^+-bearing gamete is rendered nonfunctional. This phenomenon requires synapsis in order to function. The SD effect does not occur in females. Sandler *et al*. (1959) presented a formal cytogenetic model based on preliminary evidence in which the SD locus caused a break in its synapsed homologue. This break resulted in the formation of a dicentric bridge at anaphase II due to sister strand reunion, ultimately causing death of the SD^+-bearing cells. However, Peacock and Erickson's (1965) recent cytological observations indicated that meiosis was completely normal and all the products of meiosis proceeded through spermiogenesis to yield motile sperm. Both SD and SD^+-bearing sperm were shown to be capable of entering the sperm storage organs of the female. Counts of stored sperm and of progeny recovered from similarly inseminated females were made in order to determine the possibility of selection occurring between the time of sperm storage and fertilization. These counts showed that only one-half of the sperm stored by a female from a mating with an SD male are capable of fertilizing an egg. Those sperm not able to fertilize an egg presumably carried the SD^+ chromosome. The same situation with regard to half of the sperm being nonfunctional was exhibited by the Oregon-R control group. This led the investigators to the conclusion that one-half of the products of spermatogenesis are regularly nonfunctional in *Drosophila melanogaster*. This possibility had been anticipated by Novitski and Sandler (1957) in their work with the Bar-Stone translocation gametes. They proposed the concept, upon which the above conclusion was based, that a regular class of nonfunctional gametes exists and that particular chromosomes had certain probabilities of being included in the functional sperm. Peacock and Erickson proposed an inequality of the two spindle poles at first anaphase such that one pole ultimately leads to the formation of two functional sperm and the other pole yields nonfunctional sperm. Concerning the SD phenomenon it was concluded that SD influences the orientation of the second chromosome bivalent at metaphase so that the SD-bearing chromosome almost always moves to the pole which produces the two functional gametes.

The finding made by Peacock and Erickson (1965) has provided satisfactory explanations for various sex ratio phenomena. Recent reexamination of the "sex-ratio" phenomenon found in the obscura group was carried out by Novitski, Peacock and Engel (1965). Cytological investigations revealed that, contrary to the earlier reports of Gershenson (1928), Sturtevant and Dobzhansky (1936) and Wallace (1948), there is no replication of the X chromosome during meiosis. Meiosis, however, was found to be quite abnormal in spermatogenesis of "sex-ratio" males. During the second meiotic division the Y chromosome loses its characteristic appearance and forms a chromatin mass devoid of a centromere and lacking centromeric activity at anaphase II. This degeneration of the Y chromosome was also noted by the earlier investigators. Half of the meiotic products resulting from this abnormal meiosis lack a sex chromosome. Since the "sex-ratio" effect results in the production of all female progeny the hypothesis that one-half of the products of spermatogenesis are regularly nonfunctional afforded a plausible explanation for this phenomenon. Simply stated, at anaphase I the X chromosome preferentially moves to the functional pole leading to the production of two functional X-bearing gametes.

Hanks (1965) suggests that the deviant sex ratios he noted in the wild-type strains of Drosophila melanogaster, Oregon-R and Canton-S could possibly be explained on the basis of the hypothesis that functional and nonfunctional poles exist at anaphase I. An excess of one sex could be the result of the orientation of the X-Y bivalent at metaphase 1 so that the chromosome recovered more frequently proceeds to the functional pole more often than its homologue. Hanks further suggests that this explanation may account for normal deviant sex ratios in general.

Yanders (1965) also adopted Peacock and Erickson's hypothesis to explain the paternal-age-related sex ratio shift he found in Drosophila melanogaster. He suggested that this shift was due to abnormal segregation of the X and Y chromosomes to the "functional" and "nonfunctional" poles with an increasing tendency for the Y chromosome to go to the nonfunctional pole as the male ages. Yanders also proposed that this type of nonrandom disjunction, which produces a change in the primary sex ratio, may be responsible for the relationship between paternal age and sex ratio in humans reported by Novitski (1953), Novitski and Sandler (1956), and Novitski and Kimball (1958).

The study presented here is based upon the assumption that the mechanism proposed by Peacock and Erickson (1965), in which chromosomes segregate preferentially to functional and nonfunctional poles, does exist. Two alternative explanations can be offered for the shift in sex ratio related to paternal age noted by Yanders (1965). These explanations or hypotheses involve the preferential movement of the X and Y chromosomes to the functional and nonfunctional poles. The first hypothesis suggests that the X chromosome shows a preference to move to the functional pole more frequently than it does to the nonfunctional pole. The second hypothesis assumes that it is not the X chromosome that demonstrates preferential movement but it is the Y chromosome that chooses to segregate nonrandomly. Yanders (1965) proposed that the shift in sex ratio is due to the second alternative explanation.

The experiments undertaken in this study were conducted to analyze the plausibility of the second hypothesis. This hypothesis is testable by experimentation if carried out in the following manner:

1. Wild-type males were mated to wild-type females to establish the activity of X and Y chromosomes in a normal situation.

2. To test between recovery of gametes and differential survival of zygotes wild-type males were crossed to attached-X females carrying a free Y chromosome.

3. The third cross utilizing attached-XY males and wild-type females contributed the critical evidence needed by demonstrating the movement of the Y chromosome when it is attached to an X chromosome.

MATERIALS AND METHODS

Three stocks of *Drosophila melanogaster* were used; Oregon-R (OR), wild-type strain, attached-X (y m f/y w f:=) and attached-XY $(Y^{S}\ w\ y \cdot Y^{L}\ y^{+}/\underline{yw}/0)$. The wild-type stock was obtained from the University of Chicago and the attached-X and attached-XY stocks were obtained from Oak Ridge National Laboratores. Cultures of these stocks were maintained in the laboratory by mass transfers in half pint milk bottles on nutrient medium (modified after Carpenter, 1950). The cultures used for the crosses were transferred every other day and kept in a constant temperature room at 23°C.

All the males and females used in the crosses were collected as virgins. Collections were made within a 12 hour period thereby minimizing variation in age. Virgin females were aged on nutrient medium at 23°C for six days after eclosion. Virgin males were aged for a maximum of 24 hours after eclosion on nutrient medium at 23°C.

The crosses were designed to show a paternal-age-related sex ratio shift. Therefore, the male's age increased while the female's age was kept constant at the time of mating.

In the Day 1 mating each 24 hour aged virgin male was introduced into a vial containing 2 virgin females (aged 6 days). The mating vials were then placed in the constant temperature room for 24 hours. At the end of this 24 hour period (mating period) the males were removed from the mating vials and transferred to a new vial containing 2 more 6 day aged virgin females (Day 2 mating). At this time the male was 48 hours old. The females from the Day 1 mating were removed from the mating vials and placed in separate vials where they were permitted to lay eggs for nine days. This process, transferring the males to two new females at 24 hour intervals, was carried out for 21 consecutive days. All the matings and transfers were made without etherization.

Progeny counts were made on all the vials, i.e., the mating vials and the vials into which the females had been separated. The parental females were removed from the vials and discarded after they were permitted to lay eggs for nine days. This prevented the inclusion of the parental females in the progeny counts. The number and sex of the offspring were recorded according to the parental male and his age in days at the time of mating. Counts of the progeny were made every other day for ten days. Any fly remaining stuck in the medium after a collection was removed, examined, and recorded also.

Three crosses were run concurrently. The three crosses were as follows:

Cross I	OR♂♂	X	OR♀♀
Cross II	OR♂♂	X	$\widehat{XX}Y$♀♀
Cross III	$\widehat{XY}$♂♂	X	OR♀♀

Six males were used for each of the crosses carried out. Crosses II and III were repeated at which time ten males were used for each cross. The crosses were repeated in order to confirm the results obtained from the first experiments. Weighted linear regression analyses were used to obtain the slopes for the above crosses.

RESULTS

The results of the progeny counts and the sex ratio, i.e., the proportion of female progeny, for the crosses carried out are summarized in Tables I, II, and III. Curves obtained from weighted linear regression analyses of the results of the separate crosses are presented in Figure 1. The slope values of the curves are also given in Figure 1.

The slope obtained from the data relating sex ratio to paternal age in Cross 1 (OR♂♂ X OR♀♀) was positive, i.e., the proportion of female offspring increased with the increasing age of the male parent. The value for the slope was .0020, which is in agreement with the value of .0034 obtained by Yanders (1965). In this cross the male progeny derived their Y chromosome from the male parent whose sex chromosomes were of the normal XY composition. This increase in sex ratio could be explained equally well by either of the alternative hypotheses proposed; i.e., the X chromosome may be demonstrating a preference for the functional pole or the Y chromosome may be preferentially choosing the nonfunctional pole more often as the male ages.

The combined data of Crosses II and IIa (OR♂♂ X $\widehat{XX}$Y♀♀) resulted in a negative slope (-.0048). This indicated that the relative frequency of female progeny decreased with the increase in paternal age. In the OR♂♂ X $\widehat{XX}$Y♀♀ cross there was a free Y in the chromosome complement of the female parent. The male offspring are the recipients of the free Y from the female parent and an X chromosome from the male parent. The female offspring received a Y chromosome from the male parent and an attached-X chromosome from the female parent. As one would expect from the data obtained from Cross I, the number of progeny receiving the Y chromosome from the parental male decreased as the male aged. However, in the OR♂♂ X $\widehat{XX}$Y♀♀ cross the female progeny received a Y chromosome from the parental male and therefore, the female class of progeny decreased. Essentially the same effect was recognized in this cross, i.e., the recoverability of the paternal Y chromosome decreases with an increase in the age of the male, as was seen in Cross I. The decrease in sex ratio observed in Cross II and Cross IIa therefore may be interpreted by either of the proposed hypotheses.

The sex ratio for the progeny in the OR♂♂ X $\widehat{XX}$Y♀♀ crosses began considerably lower than that of the OR♂♂ X OR♀♀ cross. Although a lowered sex ratio is expected, due to the nature of the cross, this does not fully explain the .4209 sex ratio observed for the Day 1 matings. Upon checking the stock cultures from which the attached-X females were obtained a sex ratio of .4549 was observed. The lower ratio was therefore attributed to a reduced viability of the $\widehat{XX}Y$ zygotes. This factor did not change the interpretation of the results since the expected decrease in sex ratio as the age of the male increased was realized.

The slope obtained from the third Cross ($\widehat{XY}$♂♂ X OR♀♀) was also negative. The value for the combined slopes of the crosses was -.0024, which points out that the relative frequency of female progeny decreased as the age of the male parent increased. In this cross the X and Y chromosomes of the male parent are attached and move during meiosis as a single univalent chromosome. The female progeny receive this univalent and also an X chromosome from the female parent resulting in the following chromosome complement: $X\widehat{XY}$. The male progeny are of the chromosome complement XO, since they received an X chromosome from the female parent and a nullo gamete from the parental male.

The negative slope for the sex ratio in the progeny of Cross III indicated that the attached-XY chromosome preferentially segregates to the nonfunctional pole. This cross constitutes the critical test for support of the second alternative hypothesis since the Y demonstrates a preference for the nonfunctional pole during anaphase I of meiosis even when it is attached to an X chromosome. If the X chromosome was the dominant factor in this particular case of nonrandom segregation one would expect a higher recovery of the univalent-XY chromosome, since the X chromosome is normally recovered more frequently.

TABLE I.

SUMMARY OF THE RESULTS OF THE PROGENY COUNTS AND THE SEX RATIO FOR THE OR♂♂ X OR♀♀ CROSS. (CROSS I)

	CROSS I	
Mating Period	Number	Frequency of ♀♀
1	814	.5418
2	943	.5398
3	919	.5147
4	1225	.5069
5	1409	.5089
6	1296	.5355
7	886	.5034
8	964	.5394
9	1314	.5381
10	1087	.5317
11	1090	.5266
12	1040	.5260
13	869	.5374
14	441	.5533
15	696	.5805
16	618	.5485
17	718	.5794
18	200	.5900
19	311	.5595
20	183	.5191
21	494	.5162

TABLE II.

SUMMARY OF THE RESULTS OF THE PROGENY COUNTS AND THE SEX RATIO FOR BOTH THE OR♂♂ X $\hat{X}XY$♀♀ CROSSES (CROSS II AND CROSS IIa) AND THE COMBINED DATA FOR THESE CROSSES.

	Cross II		Cross IIa		Combined Data of Crosses II & IIa	
Mating Period	Number	Frequency of ♀♀	Number	Frequency of ♀♀	Number	Frequency of ♀♀
1	424	.3939	906	.4040	1330	.4008
2	578	.4204	1372	.4125	1950	.4149
3	742	.4259	853	.4174	1595	.4213
4	698	.4226	1265	.4119	1963	.4157
5	442	.4457	1319	.4321	1761	.4355
6	660	.4515	775	.3265	1435	.3840
7	621	.3961	1219	.3519	1840	.3668
8	404	.4530	1183	.3297	1587	.3611
9	219	.3379	733	.3820	952	.3718
10	234	.4316	660	.3970	894	.4060
11	303	.3498	548	.4179	851	.3937
12	268	.3843	453	.4305	721	.4133
13	254	.3937	424	.4222	678	.4115
14	76	.3289	349	.3095	425	.3129
15	79	.3671	368	.3694	447	.3624
16	96	.3854	151	.3046	247	.3360
17	98	.3571	209	.2153	307	.2606
18	---	-----	169	.2426	169	.2426
19	7	.2857	100	.4100	107	.4019
20	65	.4615	127	.4016	192	.4219
21	20	.3000	3	.3333	23	.3043

TABLE III.

SUMMARY OF THE RESULTS OF THE PROGENY COUNTS AND THE SEX RATIO FOR BOTH THE X̂Y♂♂ X OR♀♀ CROSSES (CROSS III AND CROSS IIIa) AND THE COMBINED DATA FOR THESE CROSSES.

	Cross III		Cross IIIa		Combined Data of Crosses III & IIIa	
Mating Period	Number	Frequency of ♀♀	Number	Frequency of ♀♀	Number	Frequency of ♀♀
1	8	.3750	188	.4787	196	.4745
2	234	.4744	1279	.5035	1513	.4990
3	293	.5734	901	.4639	1194	.4908
4	701	.4964	1196	.5426	1897	.5256
5	856	.4872	1389	.5061	2245	.4989
6	417	.5252	1274	.5000	1691	.5062
7	356	.4522	1269	.4728	1625	.4683
8	280	.4393	1183	.4531	1463	.4505
9	419	.4654	1097	.5114	1516	.4987
10	364	.5440	1426	.5007	1790	.5095
11	580	.5362	653	.5314	1233	.5337
12	477	.4654	564	.5018	1041	.4851
13	720	.4306	850	.5318	1570	.4853
14	296	.4966	898	.4554	1194	.4657
15	181	.4033	643	.4883	824	.4697
16	274	.4781	784	.4949	1058	.4905
17	390	.4538	1044	.4550	1434	.4547
18	---	-----	586	.4249	586	.4249
19	231	.5498	243	.4691	474	.5084
20	12	.2500	65	.4462	77	.4156
21	372	.4597	86	.4070	458	.4498

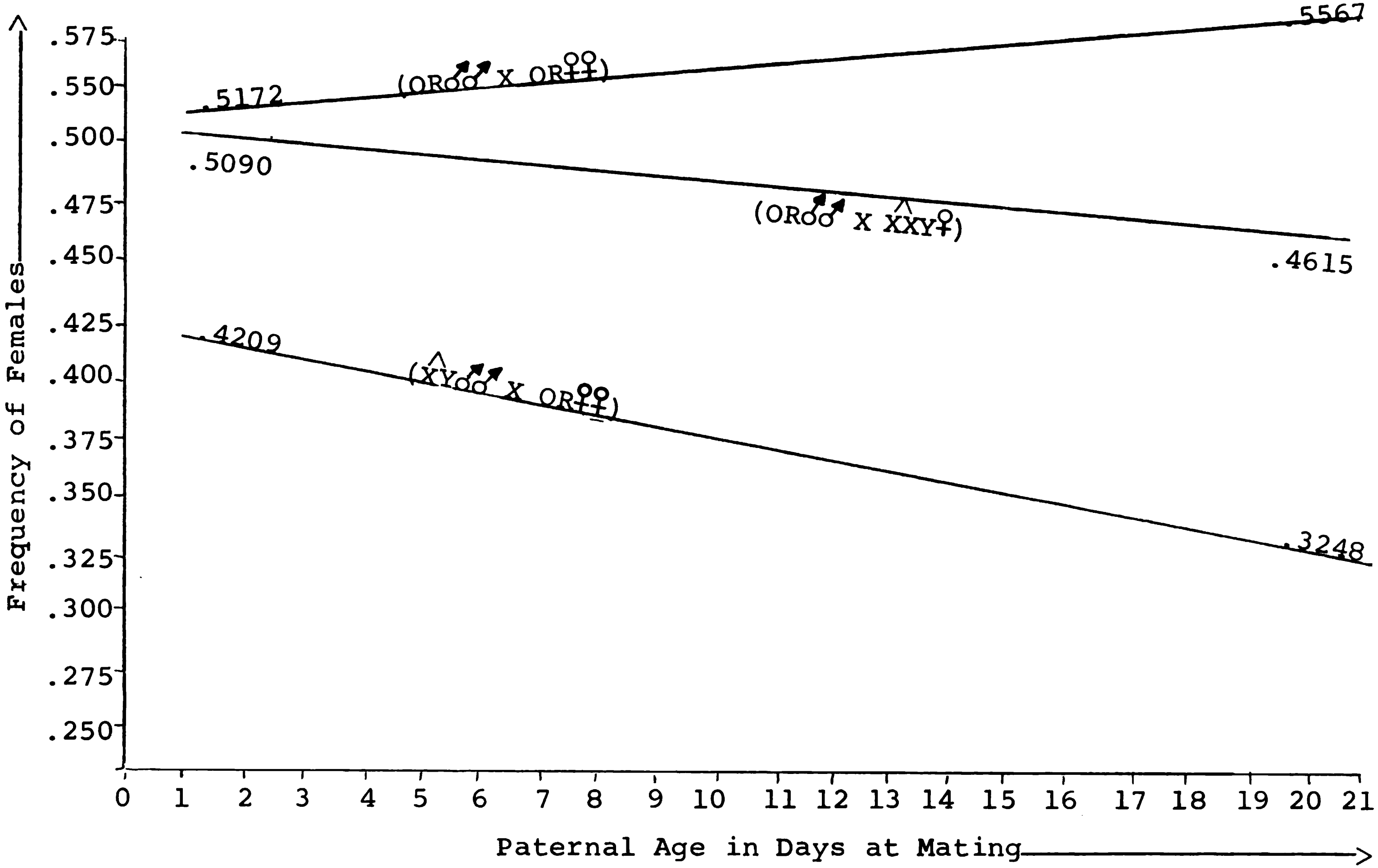

Figure 1.--Weighted linear regression lines for the combined data of the three crosses. The slope values for these lines are as follows:

OR♂♂ x OR♀♀ ----- .0020

OR♂♂ x $\hat{X}$XY♀♀ ---- -.0048

$\hat{X}$Y♂♂ x OR♀♀ ----- -.0024

DISCUSSION

An attempt was made to demonstrate the relationship of preferential segregation of the X and Y chromosomes to the age of the male parent by the use of attached-X and attached -XY stocks. The experiments carried out indicated that recovery of the Y chromosome is age dependent regardless of the chromosome complement of the aging male. In all the crosses an inverse relationship was observed between increasing paternal age and the frequency of recovery of the Y chromosome. The results of the experimental cross involving an attached-XY male and a normal female indicated that the Y chromosome is recoverable less frequently as the male ages even when it is attached to an X chromosome. This leads us to believe that the Y chromosome plays the dominant role in the meiotic behavior of the XY bivalent.

It has already been established that normally aberrant sex ratios are dependent upon the male parent (Yanders, 1965; Hanks, 1965). Such deviations from a 1:1 ratio may be due to factors operating before fertilization, causing a change in the primary sex ratio, or after fertilization, affecting the secondary sex ratio. That the deviations noted are due to a prezygotic phenomenon rather than zygotic lethality was concluded by both Yanders (1965) and Hanks (1965). Additional evidence supporting this conclusion was obtained from two of the crosses carried out in this work, in which wild-type Oregon-R males were mated to both free X and attached-X females. If the aberrant sex ratios were dependent upon differential lethality of one of the sexes, the effect of paternal age on sex ratio should be the same in both crosses, and the regression lines calculated from the data should be in the same direction. That is to say, if an increase in the number of female progeny is noted in one cross it should also be noted in the second cross. This, however, is not the case. The free X cross resulted in an age-dependent increase in the proportion of female progeny and the attached-X cross resulted in an age-dependent decrease in the proportion of female progeny. This strongly indicates that the shift in sex ratio is due to a prezygotic phenomenon rather than preferential lethality of one of the sexes during embryonic development.

A number of theories have been developed which might be utilized to explain the decrease in recovery of the Y chromosome related to paternal age. Novitski (1951) described a type of nonrandom disjunction in <u>Drosophila</u> females which is dependent upon the size of homologues. When two were of unequal size, the smaller homologue was recovered more frequently. Novitski suggested that this meiotic phenomenon may be the cause of off-sex ratios in animals whose sex is determined by a heteromorphic pair of chromosomes.

An age effect was observed in the recovery of abnormal gametic ratios resulting from a Bar-Stone translocation [T(1:4) B^S] in <u>Drosophila</u> males by Zimmering and Barbour (1965) who ascribed it to distorted segregation ratios. They found that the shorter homologue was recovered more frequently in younger males; however, in older males, the distorted ratios tended to disappear, indicating that both homologues were recovered with almost equal frequencies. They proposed that this may be the result of physiological differences between the groups of cells destined to give rise to the different sperm batches. They speculated that the change in the secondary sex ratio in humans correlated with the increasing age of the father (Novitski and Sandler, 1956) might be interpreted on the same basis. They conjectured that the relative frequencies of X- and Y-bearing sperm shift with the increase in age of the father so that younger fathers have a greater probability of having sons than do older fathers. The Y chromosome in man is the shorter homologue and tends to be recovered more frequently from younger males than the longer X chromosome, but both homologues tend to be recovered with nearly equal frequencies from older males.

The shift in sex ratio related to paternal age in *Drosophila* is in the same direction as that found in humans; i.e., as the parental male ages the sex ratio shifts toward a greater proportion of females. Though the shift is in the same direction the explanation suggested by Zimmering and Barbour (1961) cannot be applied to *Drosophila*. In *Drosophila* the Y chromosome is the larger of the heteromorphic sex chromosomes; and, in accordance with Zimmering and Barbour's hypothesis, the larger homologue is recovered less frequently in the progeny of young males. But, contrary to the effect noted by them, the frequency of recovery of the larger homologue decreases linearly as the male ages. This linear decrease was noted in the results of all the crosses.

Novitski and Sandler's (1957) concept of a regular class of non-functional gametes proposed to explain the unequal recovery of gametes from certain translocation-bearing

[T(1:4) B^S] males was the basis for Peacock and Erickson's (1965) conclusion in their study on the nature of the segregation distortion phenomenon. From their investigation they were able to conclude that *Drosophila melanogaster* regularly forms two functional and two nonfunctional sperm from each primary spermatocyte. They attributed this to the inequality of the spindle poles at anaphase in Meiosis I, whereby one pole ultimately produces two nonfunctional sperm and the other pole two functional sperm. Hanks (1965) suggested that the above hypothesis might be used to explain the normally deviant sex ratios he observed in two wild-type strains of *Drosophila melanogaster*. He suggested that the homologue recovered in excess, in this case the X chromosome, has a slightly greater probability of segregating to the functional pole during anaphase 1 than the other homologue.

Yanders' (1965) observations on the paternal-age-related sex ratio shift in *Drosophila melanogaster* were also explained on the basis of Peacock and Erickson's hypothesis. He interpreted the shift as an increase in the probability that the Y chromosome will go to the nonfunctional pole at anaphase 1 as the male ages. The results obtained in the present work are consistent with his interpretation.

The results of the crosses performed strongly indicate that the Y chromosome "preferentially" segregates to the nonfunctional pole more often as the age of the male increases rather than the X chromosome "preferentially" segregating to the functional pole more often. Critical evidence supporting this conclusion was obtained from the cross involving the attached-XY male. The decrease in female progeny of the chromosome complement $X\hat{X}Y$ with the increase in the age of the male is evident even when the Y chromosome is attached to an X chromosome. An acceptable hypothesis for this result would be that the Y chromosome demonstrates a preference for the nonfunctional pole.

Since the above conclusion was arrived at by the use of an attached-XY male, it is feasible to exclude the possibility that this effect on segregation is due to the use of a univalent as opposed to a bivalent? That univalents do not show random segregation was suggested by Peacock (1965). By use of attached-XY males he found that the frequency of recoverable nullo gametes is slightly higher than that of univalent attached-XY gametes. It appeared that the unpaired chromosomes showed polarized movement in the primary spermatocycte. This oriented segregation of univalents could be explained by preferential movement of the univalent to the functional pole. Peacock inferred that this nonrandom segregation of univalents might be a function of chromosome composition and structure, as well as total genotype. His findings therefore do not exclude the proposition of preferential segregation of the Y chromosome.

In all of the data presented, the shorter chromosomes, and in the univalent the nullo gametes, were recovered with a higher frequency. Chromosome size, therefore, may be the important factor in the phenomenon of nonrandom segregation. It would be more interesting, however, to assume that the preferential segregation of the Y chromosome is due to some mechanism which influences the alignment or movement of the chromosomes at anaphase I, for this is subject, in principle, to experimental test. This factor could be contained within the entire Y chromosome or it may be limited to a specific portion of it, such as the centromere. The results presented here support either hypothesis. In all the crosses the Y chromosome showed a preference for the nonfunctional pole as the male aged indicating that the mechanism or factor may be in the entire chromosome. Since the attached-XY stock used was composed of both the X and Y chromosomes but presumably only a Y centromere (Lindsley and Novitski, 1958) it is possible that the Y centromere itself, rather than the entire chromosome, may be the factor governing the preferential movement of the Y chromosome to the nonfunctional pole. Experiments utilizing Y chromosomes with centromeres of different origins may provide sufficient evidence to solve the above problem.

SUMMARY

Preferential segregation of the Y chromosome in spermatogenesis of Drosophila melanogaster is proposed as a possible explanation for the observed change in sex ratio related to paternal age. In order to test this hypothesis three different crosses were carried out:

1) OR♂♂ X OR♀♀
2) OR♂♂ X $\widehat{XX}Y$♀♀
3) $\widehat{XY}$♂♂ X OR♀♀

In all of the crosses performed the recoverability of the Y chromosome decreased as the age of the male parent increased. These results were interpreted on the basis of Peacock and Erickson's (1965) hypothesis that "functional" and "nonfunctional" poles exist at anaphase 1 of meiosis and upon Yanders' (1965) observations that the paternal-age-related sex ratio shift he noted is due to abnormal segregation of the sex chromosomes. The decrease in recovery of the Y chromosome in the progeny of aging males was therefore attributed to preferential segregation of the Y to the nonfunctional pole.

The critical test of the above hypothesis was obtained from the cross involving attached-XY males and normal females. The Y chromosome tended to segregate to the nonfunctional pole more frequently as the parental male aged even when it was attached to an X chromosome. This observation supports the contention that the Y chromosome is the dominant member of the XY-bivalent in producing the nonrandom segregation which occurs in spermatogenesis.

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