ARE DEVIANT SEX RATIOS IN NORMAL STRAINS OF DROSOPHILA CAUSED BY ABERRANT SEGREGATION?[1]

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Received February 11, 1965

THE term "aberrant segregation" is used here to include any prezygotic phenomenon which causes production of uneven numbers of either of the two sexes or the two classes of progeny from a testcross. Such phenomena have been referred to as "drive" and if a meiotic mechanism is the cause, "meiotic drive" (SANDLER and NOVITSKI 1957; NOVITSKI and HANKS 1961). DUNN (1953) originally discussed the implications of such phenomena. The deviant sex ratios referred to are those characteristic of either fully diploid species or fully diploid strains within a species.

Different wild strains of *Drosophila melanogaster* show characteristic sex ratios. WARREN (1918) conducted a rather extensive study on three wild stocks of *D. melanogaster* collected in Indiana and concluded that the overall percentage of females was 51.3. The sex ratio of *D. melanogaster* is given as 54% females in the *Handbook of Biological Data*, p. 519, 1956. Evidence will be given suggesting that the genotype of the male parent is the main cause of the sex ratio difference between two wild strains of *D. melanogaster*.

Deviant sex ratios are often found in various species of mammals, and occasionally there is evidence relating the sex ratios to the genotype of the male parent. WEIR, (1953, 1955, 1960) has derived a high pH mouse strain associated with a high percentage of males, and a low pH strain associated with a low percentage of males. He finds that the sex ratio is associated with the genotype of the male parent under the experimental conditions and there is no evidence of a significant influence of prenatal mortality on the overall results. MCWHIRTER (1956) reports that the major component determining the sex ratio in cattle is related to the male parent. In addition to these cases, a number of the strains of mammals listed in the *Handbook of Biological Data*, p. 519, 1956, have secondary sex ratios significantly different from 1:1.

NOVITSKI and I. SANDLER (1957) provide evidence which suggests that not all the products of spermatogenesis are functional. PEACOCK and ERICKSON (1965) provide further evidence that only approximately half of the sperm produced are functional and suggest a "functional" and a "nonfunctional" pole at first anaphase. A new application of their hypothesis is suggested as a general explanation of normal deviant sex ratios.

[1] This work was supported by a grant (GB-456) from the National Science Foundation.

MATERIALS AND METHODS

Strains of Drosophila, Oregon-R and Canton-S, were brought from E. NOVITSKI's laboratory at the University of Oregon in 1961, and kept by mass transfer since that time. With the exception of Table 2 (where the total progeny are given), each percentage value is based on a minimum of 200 progeny. All flies were kept at 23° to 25°C and cultured on yeast, cornmeal, sugar, agar, and propionic acid food seeded with live baker's yeast, except that some of the time the molasses was replaced by sugar and part of the propionic acid was replaced by phosphoric acid. Stock *y*, yellow, *f*, forked, attached-X and *al*, aristaless; *ru*, roughoid, females were brought from NOVITSKI's laboratory in 1961. Extensive testing of males was done by mating to three to five *al*; *ru* or *y f* attached-X females, except for the reciprocal crosses recorded in Table 2. In Table 2, one Oregon-R female was used for one type of cross and four Canton-S females were used for the other. The unequal number of females was due to an oversight, but it was considered unlikely to have an effect on the overall result. When three to five females were used, bottles were subcultured two to three times allowing 2 to 3 days per culture bottle, and then each culture was counted every 2 to 3 days to completion. In order to avoid any possible effect of the age of the male in the regression experiment recorded in Table 3, half of the males were mated first to free X and second to attached-X females, and the order was reversed for the other half.

RESULTS

The nature of the inheritance of the sex ratio: Do deviations from a 1:1 sex ratio have a genetic basis, and, if so, is the genotype of the male or female parent more important in determining this deviation? A significant amount of evidence was gathered over a period of several years which suggested that a mass inbred strain of Oregon-R had a characteristically higher percentage of females

TABLE 1

Characteristic sex ratios of Canton-S and Oregon-R males

Canton-S male × 5 *al*, *ru* females		Oregon-R male × 5 *al*, *ru* females	
Total progeny	Percent females	Total progeny	Percent females
469	52.7	499	51.7
489	50.1	284	50.7
317	51.1	570	54.2
550	50.7	372	51.4
536	54.3	796	52.1
380	50.5	650	48.0
234	47.9	651	57.5
352	52.3	924	52.8
492	49.0	829	51.3
348	51.2	876	56.3
1120	50.5	571	55.0
571	55.3	492	56.3
334	50.3	731	56.4
635	52.3	392	55.9
709	51.8	594	52.5
	$\bar{X} = 51.3, n = 15$		$\bar{X} = 53.5, n = 15$
	$P < 0.01$		

Representative data are presented. A test of significance using MANN-WHITNEY U test was performed. At least 200 progeny were obtained per value.

than a mass inbred strain of Canton-S. Oregon-R males from the strain were tested extensively by mating them to several tester females and results showed a mean of 55.4% females. The mean number of progeny from the Oregon-R males was 965.

This test was repeated using Canton-S males as a control. The results are shown in Table 1. Oregon-R males again gave a high percentage of females (53.5) while Canton-S males gave a lower one (51.3). In order to avoid making any assumptions about the parameters of the populations, the MANN-WHITNEY U test (SIEGEL 1956) was used instead of the t-test. The P value rejecting the hypothesis of equal means was $<.01$. A consistently high percentage of females in Oregon-R male progenies under similar environmental conditions suggests that the trait is a stable genetic characteristic.

Reciprocal matings between the two strains were made and the results are given in Table 2. When Canton-S males are mated to Oregon-R females the mean percentage of females is 51.4, but when Oregon-R males are mated to Canton-S females the mean percentage of females is 53.6. Tests for homogeniety of data were made between Table 1 and Table 2, and P was $>.85$. The largest component determining the higher percentage of females is apparently determined by the

TABLE 2

Reciprocal matings between the two strains, Canton-S and Oregon-R

Canton-S males × Oregon-R female		Oregon R male × Canton-S females	
Total progeny	Percent females	Total progeny	Percent females
90	53.3	302	55.0
223	51.1	263	54.8
168	49.4	298	59.7
177	49.7	236	59.8
177	51.4	317	52.7
197	45.2	190	53.7
208	52.4	152	52.0
162	54.3	296	50.7
209	51.2	362	57.7
89	55.7	342	50.3
185	49.2	287	57.8
123	53.7	231	46.3
198	53.5	140	49.3
189	55.0	298	51.7
226	46.5	224	59.4
	$\bar{X} = 51.4, n = 15$	285	50.9
		270	52.6
		234	50.4
		291	54.0
		190	56.3
		339	54.4
		290	53.4
		303	48.8
			$\bar{X} = 53.6, n = 23$

genotype of the male since the sex ratio was unchanged for the two types of males regardless of the strain of their mates.

The fact that the main effect on the sex ratio is apparently determined by the genotype of the male parent eliminates the possibility that the high percentage of females is caused by a simple sex-linked semilethal gene. This gene would have to come from the female, and the evidence shows that the main effect is not caused by the female parent (Tables 1 and 2). Since the progeny produced from the reciprocal matings all have the same autosomal background, insofar as half of the autosomes came from Canton-S and half from Oregon-R, then a simple autosomal effect on mortality can be ruled out. The results from reciprocal matings do not rule out the possibility of a semilethal gene on the Y chromosome of the Oregon-R males.

Zygotic mortality versus a prezygotic phenomenon: The most direct way of obtaining information on this point is to count the eggs laid and then record the number of adults finally produced after development is complete. This was done in conjunction with another experiment in which a mating of Oregon-R males to yellow females served as a control. This mating resulted in 94.9% eclosion from 1,373 total eggs, and the sex ratio was 54.4% females. PEACOCK and ERICKSON (1965) report a similar experiment using similar stocks giving 90% eclosion. This evidence does not rule out the possibility that zygotic mortality could account for the off sex ratio.

Other evidence on this point is as follows: It was thought that if in fact the sex ratio in the progeny of Oregon-R males was determined by the genotype of the male parent and not by the genotype of the progeny, then if single males are mated both to free X females and attached-X females there should be a correlation of percent females in the first cross with percent males in the second cross. In order to cancel any age effect on the sex ratio, two runs were made and the results pooled. In the first run, the males were mated to attached-X females first and free X females second, and in the second run the order was reversed. The results (Table 3) show a mean percentage of females of 54.1 in the free X crosses and 60.4% males in the attached-X crosses. The amount of regression was determined for the two measurements, an analysis of variance method was used for a test of significance, and a summary of results is presented in Table 4. $P<0.01$ for the pooled results, and the inference is that there is a high degree of correlation between the percentage of females in the free X mating and the percentage of males in the attached-X mating when single males are used for both crosses. These results support the contention that the high percentage of females is a prezygotic phenomenon. It would be a violation of the law of parsimony to propose a mechanism dependent only on the male which would kill males selectively in one type of cross (free X) and kill females selectively in another type of cross (attached-X).

DISCUSSION

The results indicate that a normally deviant sex ratio is determined mainly by the male parent and is prezygotic in nature. Additional evidence concerning

TABLE 3

Matings of single Oregon-R males to both free X and attached-X females

Total progeny	x % ♀ Free X mating	Total progeny	y % ♂ Attached-X mating	Total progeny	x % ♀ Free X mating	Total progeny	y % ♂ Attached-X mating
1096	57.7	513	62.2	425	54.6	279	64.5
1215	52.8	435	55.2	903	50.5	655	63.2
681	60.8	604	65.2	711	56.1	507	66.9
692	53.6	372	61.3	1018	53.6	437	58.1
342	57.0	417	56.6	655	56.5	310	61.3
499	55.1	549	61.8	1095	55.0	552	56.9
1026	55.0	469	59.9	661	58.7	483	57.1
1151	52.2	549	57.4	657	51.8	368	59.8
386	47.4	334	55.7	684	53.5	528	55.3
1179	53.0	464	59.7	697	52.5	400	59.8
808	56.6	553	65.3	1215	50.0	384	64.1
552	54.4	455	56.3	919	54.0	372	57.5
846	50.0	548	58.6	693	47.8	373	52.8
707	54.2	494	55.5	485	55.3	324	67.3
1209	51.5	264	59.1	606	54.0	253	53.4
1153	53.3	411	56.9	278	60.1	235	68.1
785	52.2	600	61.5	656	61.3	355	63.4
533	50.7	428	62.8	829	58.4	406	67.5
788	50.5	561	57.8	605	52.3	214	63.6
1071	52.5	416	58.6	748	54.3	338	71.0
668	59.4	625	63.0	813	53.9	239	56.5
333	48.3	564	60.6	592	52.7	283	60.8
1085	55.2	547	60.7	875	57.8	230	63.9
1070	53.6	558	62.7	770	53.8	326	56.4
1197	53.4	692	57.7				
468	58.8	481	61.5				
842	52.3	472	60.2				
	$\overline{X} = 53.8$	$n = 27$	$\overline{X} = 59.8$		$\overline{X} = 54.5$	$n = 24$	$\overline{X} = 61.2$

Pooled results: $n = 51$; $\overline{X} = 60.4$ (attached-X crosses), $\overline{X} = 54.1$ (free X crosses).

Each percentage value represents a minimum of 200 progeny. Unweighted values were used to obtain the grand means of each experiment.

the sex ratio in Drosophila is given by YANDERS (1965), who found that the sex ratio showed a trend toward a greater proportion of females, and this proportion was dependent upon the age of the male parent at the time of mating. This find-

TABLE 4

Regression analysis and the test of significance using analysis of variance

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	50	874.55	
Regression	1	139.59	139.59 $P < .01$
Error	48	734.96	15.31

$b_{yx} = 0.53$; $b_{xy} = 0.30$, where x = percentage of females, and y = percentage of males.

ing tends to support the contention that the sex ratio is determined by the male parent and is a prezygotic phenomenon.

WEIR (1953, 1955, 1960) showed that high blood pH, low blood pH, and even standard mouse strains may have deviant sex ratios. WEIR has selected for high and low venous blood pH mouse strains and has found the high pH associated with 52.8% males and the low pH with 41.8% males at weaning. The latest results were obtained after 30 generations of brother-sister matings. By making reciprocal matings between the two different sex-ratio lines in repeated experiments, he showed in a remarkable fashion that the sex ratios are dependent on the male parent. He finds no evidence for a significant influence of prenatal mortality on the overall results. In addition, WEIR (1960) shows that mouse strains C3H (52.2% males) and C57B1/6 (52.3% males) have sex ratios that depart significantly from equality.

It is well known that in the white population of the United States the secondary sex ratio (51.4% males) is significantly different from a 1:1 (*Handbook of Biological Data*, p. 519, 1956). Furthermore, NOVITSKI (1953) has produced evidence of a change in the secondary sex ratio in humans that is associated with the age of the father. He remarkably foresaw that a meiotic interpretation was probable.

Many of the strains and species of Mammalia have significantly deviant sex ratios and perhaps there is a better explanation for these than zygotic mortality. MCWHIRTER (1956) reported on the sex ratio from records in Scotland of artificial insemination, in which there are a total of 37,515 calves. Only 32 bulls were used and the proportion of females was 50.1%; however, the total heterogeniety x^2 is 123.4 ($P \ll 0.001$). In 12 bulls the sex ratios deviated significantly from the 1:1 ratio and in seven of them $P < 0.01$. The major component determining the sex ratio in these data is due to the male parent. In addition, of the various species and strains of mammals for which secondary sex ratios are given in the *Handbook of Biological Data*, p. 519, 1956, ten out of 45 have means in which the 95% confidence limits (some estimated) do not overlap 50, and an additional 12 are borderline in this respect. Of the ten that seem clearly significant, all but one give an excess of males. Since the male is the heterogametic sex in mammals, there should be a better explanation for at least some of the deviant sex ratios than zygotic mortality because, for example, in humans the heterogametic and not the homogametic sex is thought to be the weaker one.

A possible explanation for the deviant sex ratios: NOVITSKI (1951) suggested that nonrandom disjunction of structurally different chromatids at the second meiotic division could be responsible to some extent for deviations from the expected 1:1 sex ratio. This was suggested for animals where sex is determined by a heteromorphic pair of chromosomes. NOVITSKI and I. SANDLER (1957), working with the translocation, T(1;4)B^s, first suggested that certain gametes are regularly nonfunctional and that particular chromosomes are included in the functional sperm with a predictable probability. They clearly foresaw and predicted the possible generality of the phenomenon. PEACOCK and ERICKSON (1965) found the remarkable fact that in SD (Segregation Distorter) and Oregon-R

strains only about one half of the sperm are functional. In SD the conclusion was that, since SD always separated reductionally from SD^+ at first anaphase and since almost all the functional gametes carry SD, one pole at the first division must produce two nonfunctional sperm. They proposed for SD that the definitive event is an orientation of the chromosome-2 bivalent at metaphase I such that the SD bearing chromosome proceeds toward the functional pole at anaphase in most cases.

Normal deviant sex ratios may be explained in the following way: It is suggested that the X Y bivalent at metaphase I is oriented so that the chromosome recovered in excess proceeds toward the functional pole with a slightly greater probability than its homologue. This explanation could not only account for the deviant sex ratios cited here, but may also account for normal deviant sex ratios generally. Perhaps meiotic-drive mechanisms allow for the adjustment of the mean sex ratio to near optimum for a given strain or a given species.

I am grateful to PROFESSOR E. NOVITSKI for his continued interest and advice. I wish to thank DRS. E. NOVITSKI and W. JAMES PEACOCK for reading the manuscript, and MRS. HELEN WEBB for excellent technical assistance.

SUMMARY

The sex ratios of two wild-type strains of *D. melanogaster* were analyzed. One (Oregon-R) consistently produces a greater percentage of females than the other (Canton-S). The sex ratio difference between the strains is apparently determined by the genotype of the male parent and not the genotype of the female or progeny; furthermore, it is not caused by zygotic mortality. Cases of deviant sex ratios in Mammalia where the effect is mainly determined by the male parent are discussed. The following explanation (made possible by the discovery by E. NOVITSKI and I. SANDLER that not all products of spermatogenesis are functional) is suggested for deviant sex ratios in normal strains of *D. melanogaster*, in the other strains described, and perhaps quite generally: The excess of one sex results from a slightly greater probability that the corresponding sex chromosome will segregate to the "functional" pole during anaphase I and thus be present in functional sperm. The homologue, on the other hand, segregates to the "functional" pole less often.

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