A RELATIONSHIP BETWEEN SEX RATIO AND PATERNAL AGE IN *DROSOPHILA*¹

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THE sex ratio in genetically normal organisms utilizing X-Y sex determination should in theory be 1:1, but this expectation is seldom—if ever—realized. Most populations which have been studied in detail exhibit some significant deviation from this expectation, either toward a slight excess of males, as in man, or an excess of females, as in *Drosophila*. These deviations could be due to factors affecting the primary sex ratio or the secondary sex ratio, or both.

A relationship between paternal age and secondary sex ratio has been reported in man by NOVITSKI (1953), NOVITSKI and SANDLER (1956) and NOVITSKI and KIMBALL (1958). A decrease in the proportion of males is evident in the offspring of older fathers, but the sex ratio is independent of maternal age. Maternal age can play an important role in *Drosophila*, however. HANNAH (1955) found that aged females produced an increased proportion of males in their progeny. No effects of paternal aging have been reported in *Drosophila*.

Evidence will be presented indicating that the aging of nonirradiated *Drosophila* males produces a shift in sex ratio in the same direction as that noted in man (i.e., towards a greater proportion of females), and opposite to the effect noted when *Drosophila* females are aged. If the males are irradiated, the proportion of females is reduced, but the effects of paternal age are still recognizable.

MATERIALS AND METHODS

Stock: A strain of Oregon-R *D. melanogaster* was used exclusively. This was obtained from the University of Chicago (courtesy of W. K. BAKER) in 1959, and has been maintained since by mass transfers in this laboratory. All flies were reared, mated, and stored at 23°C on modified CARPENTER's (1950) medium.

Irradiation: Males to be irradiated were placed in plastic tubes in groups of 25 to 30 and exposed to X rays from a General Electric Maximar-250-III, operating at 250 kv, 15 ma, with .5 mm copper filter. The total dose of 4500r was given at rates ranging between 325 and 375 r/min.

All males were virgin at the time of treatment, and were 1 day (12 to 24 hours), 7 days, or 14 days old. All females were 4 to 6 days old at the time of mating.

Mating: All matings and transfers were made without etherization. Each male was placed in a separate vial with two females within 1 hour of treatment, and transferred to a new vial with new females at 24-hour intervals for a total of eight mating periods, after which he was discarded.

Within 4 hours after the males were removed, one female was taken from each vial for use in another study (YANDERS, in preparation); the other female was left in the vial and permitted to continue laying eggs for nine more days. A few females were chosen at random when removed

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at this time, and their sperm storage organs were excised and examined. No sperm were seen in the ventral receptacles or spermathecae in either controls or treated groups, so sperm utilization must have been nearly complete.

Progeny counts: The progeny from each vial were removed and counted daily until the culture was exhausted. After each collection, each vial was inspected carefully and any fly remaining stuck was removed and examined.

RESULTS

The results are tabulated in Table 1, and presented graphically in Figure 1. A general upward trend in the proportion of females over the eight mating periods is noted in each of the control groups. The relationship between paternal

FIGURE 1.—Sex ratio (females/total) of progeny produced in eight sequential mating periods following X-irradiation of males of three different ages with 4500r (broken line) and their unirradiated control (solid line). Mean values: 0 = control, X = treated.

TABLE 1

Proportion of female progeny (♀♀) recovered after eight sequential 24-hour mating periods following X-irradiation of males of three ages with 4500r (treated) and their untreated controls. N = total number of progeny

Group		Mating period								Mean
		1	2	3	4	5	6	7	8	
1-day males	Control ♀♀	.508	.518	.503	.517	.515	.527	.530	.527	.518
	N	4,519	5,638	5,330	5,659	5,029	5,350	5,752	3,601	40,878
	Treated ♀♀	.493	.491	.493	.463	.509	.379	.515	.508	.488
	N	1,699	3,286	3,202	1,575	404	169	165	130	10,630
7-day males	Control ♀♀	.518	.542	.533	.533	.537	.558	.585	.592	.542
	N	9,314	6,411	5,501	4,988	5,110	3,163	3,620	2,638	40,745
	Treated ♀♀	.471	.500	.526	.510	.497	.481	.478	.125	.502
	N	2,381	2,369	2,960	2,394	758	260	67	8	11,197
14-day males	Control ♀♀	.533	.545	.544	.571	.579	.572	.579	.572	.555
	N	7,059	7,161	5,457	3,771	3,728	2,844	1,929	2,056	34,005
	Treated ♀♀	.502	.509	.552	.517	.551	.527	.523	.449	.523
	N	2,117	2,251	2,053	1,414	959	239	44	98	9,175

age and daily sex ratio within age groups, as tested by the Spearman rank correlation (SIEGEL 1956), was significant in each of the control groups ($P < 0.05$), but did not reach this level of significance in any of the three treated groups, although their early trends are similar to the controls. It is possible that irradiation damaged whatever mechanism is responsible for the paternal age effect, but it seems more probable that random deviations in sex ratio due to the small numbers of offspring in the later mating periods in treated groups are responsible for the failure of these differences to be statistically significant. (The Spearman test does not take relative size of groups into consideration.)

Not only does the proportion of females tend to increase over the mating periods within an age group, but the results suggest that the increase is related to the total age of the father, possibly influenced by his previous mating history. The mean proportion of females in both treated and control groups is lowest for the youngest males, intermediate for the 7-day males, and highest for the oldest males, and the differences in means are highly significant when tested by chi-square. When the sex-ratio data from control groups were ranked according to total age of male (using only the first seven days of mating to eliminate overlaps on Days 8 and 15) and tested by the Spearman rank correlation, a value of $P < .01$ was obtained. This tends to support the idea that the paternal age effect was continuous and additive throughout the three-week period which was studied.

The results also demonstrate a highly significant reduction in the mean proportion of female progeny following irradiation of males, a phenomenon which is not always detected (LINDSLEY, EDINGTON, and VON HALLE 1963). Daily values for treated groups are lower, in all but one instance, and tend to parallel those of their controls. The only major discrepancies occur in the later mating periods when the numbers of offspring in treated groups were exceedingly small.

Curves obtained from weighted linear regression analyses are presented in Figure 2. Separate slopes were calculated for each of the three age groups within a treatment, as well as for the summed data of each treatment. There is a reasonable degree of correspondence among slopes, the single exception being the negative slope obtained for the youngest treated males. The similarity between slopes calculated for the summed data of control and treated groups is striking, and supports the notion that the influence of paternal age on sex ratio is not appreciably changed by radiation.

DISCUSSION

Deviations from a 1:1 sex ratio may be produced by factors which operate prior to fertilization, thereby causing changes in the primary sex ratio, or subsequent to fertilization, thereby causing changes in the secondary sex ratio. For the most part, changes in the secondary sex ratio can be attributed to preferential lethality of one or the other sex sometime during development (Hannah 1955). The overall reduction in the proportion of females sired by X-rayed males noted in my results is most probably due to such preferential lethality, and reflects a

FIGURE 2.—Regression slopes showing dependence of sex ratio on paternal age in males of three ages X-rayed with 4500r (broken lines), and their unirradiated controls (solid lines). Slopes based on summed data are given for control (heavy solid line) and treated (heavy broken line). The slope values are:

<i>Age of male</i>	<i>Control</i>	<i>Treated</i>
0-7 days	.0031	— .0037
7-14 days	.0087	.0061
14-21 days	.0074	.0060
Summed data	.0034	.0025

higher rate of induced dominant lethals produced in X-bearing sperm (see discussion in LEA 1955).

The sex-ratio shift associated with paternal age can hardly be due to preferential lethality, however, for the conditions of the experiment are such that the shift takes place in the direction opposite to that which would be expected. For example, abnormal sex ratios can be due to sex-linked recessive lethals; but in order for these to reduce the proportion of male progeny, the mutations must occur or be carried in the maternal sex chromosomes, and would not show the observed relationship to paternal age. Of course, sex-linked lethal mutations undoubtedly occurred even in the nonirradiated male parents, and the frequency of these might be expected to increase slightly as the males aged. But such lethals would act to reduce, rather than increase, the proportion of female progeny if they exerted even the slight detrimental action in heterozygotes which has been reported (e.g., STERN, CARSON, KINST, NOVITSKI, and UPHOFF 1952), and furthermore, the magnitude of this effect would be exceedingly small.

The most reasonable hypothesis is based on the suggestion of PEACOCK and ERICKSON (1965) that functional and nonfunctional poles exist in spermatocytes at anaphase I. They believe that the two spermatozoa which ultimately develop from the nonfunctional pole, while viable and motile, do not perform successfully in fertilization. If there exists a mechanism which segregates chromosomes preferentially to the functional and nonfunctional poles, such chromosomes will occur in disproportionate ratios in the progeny, and can result in one type of

"meiotic drive." This hypothesis, which is similar to the scheme suggested by NOVITSKI and I. SANDLER (1957), is not only adequate to explain PEACOCK and ERICKSON's results with the segregation distortion (SD) phenomenon, but has been adopted by HANKS (1964) to explain differences in the mean sex ratio between the Canton-S and Oregon-R strains of *D. melanogaster*.

I suggest that the paternal-age related sex-ratio shift in *Drosophila* is also due to abnormal segregation; and furthermore, that the tendency of the Y chromosome to go to the nonfunctional pole at anaphase I increases as the male ages. This type of nonrandom disjunction, which produces a change in the primary sex ratio, could also be responsible for the relationship between paternal age and sex ratio in human populations reported by NOVITSKI (1953), NOVITSKI and SANDLER (1956) and NOVITSKI and KIMBALL (1958). In fact, such a mechanism was implied by NOVITSKI (1953). If this hypothesis is correct, the employment of paternal age as an easily controlled experimental variable in studying abnormal segregation offers new possibilities for further investigation of this basic and apparently widely occurring phenomenon.

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SUMMARY

Drosophila melanogaster males aged 1, 7, or 14 days were mated at 24-hour intervals to pairs of virgin females and transferred to new females at 24-hour intervals for eight mating periods. The sex ratio of their progeny showed a trend toward a greater proportion of females which was related to the age of the parent male at the time of mating. Irradiation of the males reduced the proportion of their female progeny, but the shift in sex ratio showed the same relationship to paternal age as in the untreated groups. An age-related, nonrandom meiotic segregation of sex chromosomes into functional and nonfunctional sperm provides a satisfactory explanation for these results.

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