THE EFFECTS OF AGING IRRADIATED DROSOPHILA SPERM ON SEX-LINKED LETHAL MUTATION RATE AND SEX RATIO

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

James E. Treska

1962

ABSTRACT

THE EFFECTS OF AGING IRRADIATED DROSOPHILA SPERM ON SEX-LINKED LETHAL MUTATION RATE AND SEX RATIO

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Since it has long been assumed that the survival and behavior of irradiated Drosophila sperm is the same as that of non-irradiated sperm up to the point of fertilization, the object of this study was to investigate the possibility of any deviation from this assumption. Chronological changes in sex-linked lethal mutation rate and sex ratio were utilized to serve as detectors of any gametic lethals, delayed damage or differential selection mechanisms induced in the irradiated sperm.

Muller-5 tests were utilized to detect sex-linked lethals of irradiated non-aged or aged mature Drosophila sperm. Changes in the sex ratio were detected in the F_1 progeny of females inseminated by irradiated or non-irradiated males.

The results seem to indicate that the sex-linked lethal mutation rate is not sensitive enough to detect gametic lethality. Studies of the sex-linked lethal mutations suggest an enhancement of the irradiation damage. Studies

of the sex ratio seem to indicate that there is a differential selection for gametes after storage, correlated with the type of chromosomes contained in the sperm. However, in neither study was a statistically significant difference found between non-aged and aged irradiated sperm.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Zoology

1962

ACKNOWLEDGMENTS

I wish to thank Dr. Armon F. Yanders for the helpful guidance afforded to me. I sincerely acknowledge the fact that my intellectual growth was greatly enhanced by his experienced discussions and skilled criticisms.

To Dr. Philip Clark, I am grateful for the professional advice on the statistical analysis of my data and for the helpful criticisms of my application of the statistical results.

I want to thank, also, Dr. U. V. Mostosky of the Department of Surgery and Medicine for the X-ray treatments. Without his concern and care for accurate treatment, these experiments would have been impossible.

This study was conducted during the tenure of a National Defense Education Act Predoctoral Fellowship, and partially supported by a grant to Dr. A. F. Yanders from the U. S. Atomic Energy Commission (Contract AT [11-1]-1033).

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I. INTRODUCTION

Ever since the principle of random fertilization was postulated, exceptions which disturbed the theoretical ratios have been noted. Occurrence of lethal gene mutations, lethal chromosomal aberrations and selective gametogenesis are all possible causal mechanisms in producing aberrant ratios (Novitski, 1957). Aberrant ratios due to zygote lethality include a spectrum of specific and non-specific mechanisms. Gene mutations and chromosomal aberrations incurred in the gamete can inflict lethal results on the subsequent zygote formed from such a gamete, depending on the nature of the mutation or aberration. The selective gametogenetic mechanism occurs in certain cases in Drosophila in which some males produce only X-bearing sperm by an errant meiotic cycle (Sturtevant and Dobzhansky, 1936).

These postulates of ratio-disturbing mechanisms seem to explain many cases. The question raised at this point is, are there any other types of causal mechanisms? In other words, could other mechanisms come into play after gametogenesis and before zygote mortality?

One can test to eliminate zygote mortality and selective gametogenesis by simple methods. Inspection of the classes of progeny and cytological investigation of gametogenesis should indicate whether selective gametogenesis is the factor. To test and eliminate zygote mortality, one need only count eggs laid and adults produced from the observed number of eggs. If the differences in egg hatch are insufficient to account for the disproportion in the expected adult classes, zygote mortality can be ruled out.

To study the problem of aberrant ratios, the mature gamete is a particularly good object of investigation. The use of mature sperm has the technical advantage that it can be experimentally treated and studied in many ways, with reference to the genetic content of individual cells.

At this point, it should be noted that an important assumption has been made; all sperm, regardless of the condition of their genetic constituents, are capable of initiating fertilization. Muller and Settles (1927) specifically tried to test the hypothesis that the gene content within a spermatozoan affected its viability. They concluded that with the use of sex-ratio studies and a particular chromosome deletion, the genes distinctive of

the X and Y chromosomes and a particular chromosome segment are non-functional in the maintenance of the life of the sperm. In the latter case, sperm containing aneuploid chromosome sets were shown to accomplish fertilization. Evidence supporting the assumption is based on the fact that gametes containing gross chromosomal damage and even whole chromosomal losses still initiate fertilization. Also, gametocytes containing such genetic damage are capable of forming functional gametes. Muller showed in a particular instance that a deletion in a chromosome had no effect on the sperm carrying it, yet if the deletion was not covered by the translocated portion carried by another chromosome in the egg, the resulting zygote would die.

Recently Sandler and Novitski (1957) have noted another genetic phenomenon in Drosophila, meiotic drive, which leads to aberrant ratios of offspring. This is exemplified by the gene Segregation-Distorter (SD), which, when heterozygous in the male, renders the SD^{+}-bearing gametes non-functional. They experimentally eliminated the possibility of zygote lethality, and explained the distorted ratio as being the result of a break which produces a chromatid bridge. Cells resulting from nuclei containing bridges may be incapable of proceeding normally through spermatogenesis,

but cytological evidence has not yet been found. They have analyzed the distortion as being a selective gametogenic mechanism.

How can those aberrant ratios, in which selective gametogenesis and zygote mortality have been ruled out as possible explanations, be explained in terms of known genetic mechanisms? Caspari and Stern (1947) irradiated Drosophila sperm stored in the spermathecae of females over a period of twenty-one days. At the end of this period, they found the induced mutation rate slightly below expectation, but not statistically different. Novitski (1947), assuming that this result might be the consequence of a "recovery" effect, designed an experiment which he thought would test it. Comparing mutation rates of irradiated Drosophila sperm, those unaged and those aged in the female's spermathecae for twenty-one days, he found no statistically significant difference in the mutation rate. However, it was noted that in each experimental group, the non-significant deviations were all of the same direction, that being slightly lower than the expected lethal mutation rates.

These latter works are mentioned to introduce the possibility of gametic lethality as another mechanism

which would distort expected ratios. Differential gametic lethality correlated with genetic content of the sperm might explain the apparent changes in the forementioned work of Caspari, Stern, and Novitski. However, they disregard the possibility of gamete lethality because they believe male gametes are unaffected by their chromosomal content.

Another possible explanation of the results of Caspari, Stern and Novitski is restitution of induced mutations. This recovery mechanism, given time to operate, should reduce the number of induced mutations in sperm aged after treatment when compared to non-aged treated sperm.

It then becomes a problem to establish the actual existence of restitution or gametic lethality, and also to distinguish between them if it is found that they both exist. If it could be demonstrated that gametes are eliminated before or during their storage in the female, one might have an observable clue to gamete lethality. Yanders (1959) has noted that, in fact, treated sperm stored in sterile females were eliminated in an unexpected, yet predictable manner. If one assumes all sperm live approximately the same length of time, or are all eliminated in the same manner, an abrupt drop in a survival

curve should be noted. However, the curve shows a more gradual drop in the survival of the sperm. This observation might then be interpreted as being a manifestation of gamete lethality.

The second approach in the investigation of irradiation damage on mature sperm was the study of any possible shift in the sex ratio of stored sperm. This line of investigation was undertaken with the premise that the sex-ratio method would be more sensitive in detecting gametic lethals than the sex-linked lethal method. Sex ratios have long been used by geneticists as an indicator of natural and experimental factors which might influence any one of the multiple processes involved in the ultimate determination of sex. Any modification of an intrinsic or extrinsic factor, which can influence some step from the differentiation of a primordial germ cell to a sexually-determined zygote, will usually reflect its influence by causing a modification in the sex ratio. For a more thorough review of aberrant sex-ratio mechanisms, Hannah (1955), Novitski (1957), Zimmering (1960) and Paulson and Sakaguchi (1961) provide a detailed study and analysis of some of the points I have mentioned.

A majority of sex-ratio diverters seem to manifest themselves by causing the occurrence of abnormal cytogenetic mechanisms during gametogenesis and very early cleavage. Genetic mechanisms such as frequent nondisjunction will cause a shift in the sex ratio. Other specific cytogenetic mechanisms, based on chromosomal aberrations, can also cause a sex-ratio shift. It has been stated that, of chromosomal changes, translocations, inversions, deletions, etc., single breaks in the sex chromosomes were thought to form the principle contribution to the sex distortion. Cytogenetic mechanisms of selective segregation or elimination of sex chromosomes during meiosis, resulting in a deficiency of certain types of gametes or change in the ratio of sex chromosomes to autosomes giving aneuploid types, can also change the sex ratio (Hannah, 1955).

Aging effect of the eggs in the females, another sex-ratio modifier, might be a mechanism which makes an egg differentially susceptible or less compatible with the multiple factors of sperm type and environmental conditions (Hannah, 1955). In other words, aged mature gametes become "over-ripe," and consequently are less compatible with the fertilizing partner. Hence fertilization and subsequent early cleavage processes, resulting from the union of

certain fertilizing partners, are made more sensitive in terms of nuclear-cytoplasmic relationships that may exist.

Aside from aging mature gametes, there seems to be a change in sex ratio among offspring of older parents (Lawrence, 1940; Novitski and Sandler, 1956; Hannah, 1955; Zimmering, 1960). Zimmering (1960) states that some aging effects, which manifest themselves as sex-ratio distorters, might be explained as the consequence of physiological differences due to aging on different groups of cells destined to give rise to different sperm batches. The question raised here is, how does this "physiological difference" actually select preferentially one sex type over the other in meiotic or fertilization processes? Zimmering (1960) suggests that the non-function of certain sperm types is associated with some peculiarity of the particular combination of chromosomes involved, such that some oddity in the mechanics of meiosis results in the nonfunctioning of certain meiotic products. The concept of differential survival encompasses a multitude of actual possible sensitive stages, from the primordial differentiation, through the actual fertilization processes, to the initiation of zygote determination, which may be affected.

Still another general mechanism of sex-ratio diversion might be attributable to differential sperm competition between sperm of different genotype. There may be specific ways which this mechanism may manifest itself. For instance, there may exist differential compatibility of X- or Y-bearing sperm with regard to the male's own storage organ, transmitting fluid, female's storage organ or genital tract and possibly, with regard to penetration of the egg membrane. Since there is evidence that differentiation exists between X- and Y-bearing sperm, in terms of electrophoretic response (Gordon, 1956), it seems reasonable to assume there might be other biochemical differences possessed by the X- or Y-bearing sperm. The problem concerning the existence of such a distinction is to unravel the mechanisms and the period which are responsible for the gametic differentiation.

The proposed research was initiated to test the hypothesis that the phenomenon of gametic lethality exists, and that the sperm is vulnerable to certain abnormal genotypic constituents which it may contain. X rays were utilized as a tool, as irradiation yields a spectrum of sperm containing induced aberrations. After irradiation, the affected sperm will contain both detectable lethals and gametic lethals, if they exist. Most work on irradiated sperm assumes that

the irradiated sperm, which may exhibit a spectrum of physiological and genetic damage, will function precisely as do non-irradiated sperm, up to and including fertilization. It is generally held that only after the onset of fertilization and during ensuing development can the sperm manifest this spectrum of irradiation damage.

The investigation proposed here centered around the effect of irradiation on the mature sperm, and how this effect, if any, influences the final measurement of the mutation frequencies and sex ratio. Mature irradiated sperm were stored for a period of time. During storage, some gametes will not survive. The cause of death may be the result of incompatibility with the female genital tract, loss of motile activity, or other intrinsic physiological breakdown. All of these events may be dependent on a few active genes in the mature sperm. To attribute sperm death to genetic damage alone, one must assume that irradiation damage to the cytoplasmic portion of the sperm will be more or less uniform, both in the distribution among the gametes and in the type of damage inflicted. This assumption would follow from knowledge of the random nature of radiation damage, but may not be warranted if there is a correlation between genetic and cytological damage which is

not testable with present techniques. Gametic lethals might be the result of specific gene mutations. Hence, some gametes will survive regardless of gross chromosomal and gene damage, if these specific gene mutations have not occurred. If gametic lethality due to specific mutations does occur, it would support the hypothesis that a mutation is a change in a preexisting gene.

II. MATERIALS AND METHODS

Stocks of Drosophila melanogaster Oregon-R (OR), wild-type strain, and Basc X-chromosome tester strain (Muller-5) were used exclusively. For the sex-linked mutation experiment, two groups of OR males, aged exactly three days on nutrient medium (modified after Carpenter, 1950), were exposed to 0 (control) or 2760 r units of X rays. This procedure was repeated five times. Within minutes after exposure, the irradiated males were mass-mated to virgin Muller-5 females, aged seven days on Offerman's (1936) medium. After twenty-four hours, the females were separated from the males, and divided into two groups. One group was stored at 10.5°C on Offerman's medium. This temperature was chosen because preliminary experiments indicated maximum survival and minimal egg laying during the duration of storage. The other group was placed on nutrient medium and allowed to lay eggs immediately at a constant temperature of 22°C.

After twenty-one days, the stored females were transferred to 22°C temperature and placed en masse on heavily yeasted food in order that they might resume egg laying as fast as possible. These females were transferred every two days. Each F_1 female was mated with several stock

Muller-5 males in an individual vial. Vials containing no normal red-eyed males, seven or more Muller-5 males, and a total of twenty flies were scored as lethal. If a vial could not meet this requirement of progeny number or distribution, the heterozygous females were mated with Muller-5 males for a retest.

Exactly the same irradiation procedure was used for the sex-ratio experiments. Two identical experiments composed of two groups of OR males, aged three days on nutrient medium, were exposed to 0 (control) or 2760 r of X rays. In these experiments, the males were mass-mated for twenty-four hours to virgin OR females, which had been aged three days on Offerman's medium. The males were removed and the females were separated into groups. The females of the first group, comprising one-fourth of the total number, were placed individually in vials containing nutrient medium. These females were transferred to a second vial at the end of six days of egg laying, and discarded at the end of the second six-day period. Individual records were kept for each female. The second group of females was stored on Offerman's medium for two weeks at 10.5°C., after which time they were treated exactly like the unaged females. All F_1 progeny of the

aged and unaged females were classified as to sex. Every adult fly in each vial was scored, even those which were dead or stuck to the medium.

The source of X rays was a General Electric Maximar-250-111, operating at 250 kv, 15 ma, with a 50 mm copper filter. The dose rate at 32 centimeters target distance was calibrated at 144 r/minute prior to the first experiment and at 120 r/minute after the last experiment.

III. RESULTS

Sex-linked Lethals

The values for sex-linked lethals of treated and control groups are shown in Table I. The percentage of sex-linked lethals in groups I, II and III were the result of an irradiation exposure of 144 r/minute for twenty-three minutes, whereas groups IV and V represented an exposure of 120 r/minute for twenty-three minutes. The stored groups I, II and III were unfortunately exposed to an unexpected drop in temperature from 10.5°C to 7°C for approximately three days. Group I was exposed to this temperature during the last week of storage, whereas groups II and III were affected during the second and first week respectively. No such problem occurred during storage of groups IV and V. Numbers of Muller-5 females, which were in the constant temperature room, were killed by the drop in temperature. Exact figures were not obtained on the percentage survival of the Muller-5 females during storage, but approximately 5 to 25% of groups I, II and III survived storage. This is compared to approximately 40% for groups IV and V. In each stored group, the number of females stored was approximately six hundred to eight hundred.

Table I. Sex-linked lethal data

Group	Unaged				Aged			
	Normal	Sterile	Lethals	% Lethal	Normal	Sterile	Lethals	% Lethal
I	1,427	170	145	9.22	240	36	29	10.78
II	362	155	37	9.27	63	18	12	16.00
III	1,062	331	115	9.77	43	36	3	6.52
IV	1,008	110	91	8.28	284	16	37	11.52
V	1,036	105	94	8.32	519	32	64	10.98
Con-trol	1,533	350	4	0.26	1,395	69	4	0.29

Table II. The t test on sex-linked lethal data

Σd	$\bar{d}$	Σd^2	$(\Sigma d/5)^2$	SSd	$s^2_{\bar{d}}$	$s_{\bar{d}}$	t
10.94	2.188	75.86	23.937	51.926	2.60	1.611	1.36

The number of stored Muller-5 females which laid fertile eggs is small. Examination of several females' ventral receptacles at the end of the storage period showed a moderate number of motile sperm. Also it was observed, at the end of the storage period, that the females were quite emaciated, and it took approximately seven days on heavily yeasted food for them to resume egg laying. At the end of the time, most of the females were very healthy looking, and the ovaries were seemingly well developed. However, at this time there were only a very few fertile eggs laid. Possibly during that transition period of re-building the germ tissues in the females, the sperm were eliminated. How this may have affected the final lethal expression of the stored groups is highly speculative. Another difference to be noted between groups I, II and III and groups IV and V is that, in the former groups, the Muller-5 females were allowed to lay their eggs on individual vials, whereas in the latter groups, the Muller-5 females were allowed to lay their eggs in culture bottle *en masse*.

The percentage of lethals, resulting from unaged females, corresponded well with those values in the literature. Assuming 2.89% lethals/1000 r to be the correct rate of sex-linked lethals using X rays (Lea, 1955), then the expected

values for groups I, II and III should have been 9.57%, whereas for groups IV and V, 7.9% is the expected value. However, since the intensity was only calibrated at the initial irradiation and one week after the final, the value given as the dosage rate possibly fluctuated between the extremes of 144 r/minute and 120 r/minute. The difference in dose rate may be the result of a technical breakdown of the X-ray machine in a period between groups III and IV. If the values of the unaged sex-linked lethals are correct, it would seem that the OR males received more than 120 r/minute in groups IV and V, but less than 144 r/minute.

Another category in Table I is the sterile group. This was a general group which included any vial in which no offspring were observed, whether it was the result of sterility on the part of either male or female, or whether either or both flies died before egg laying proceeded.

In all groups of the aged irradiated sperm, except group II, the percentage of lethals is greater than that in the corresponding unaged sperm. A test for significance of aged lethal values over that of the unaged values is given in Table II. The t value, calculated to be 1.36, falls far short of showing any difference between the unaged and aged lethal rate. Four of the five experimental

groups show an apparent increase in the sex-linked mutation rate, while only one group (that having the smallest number of Muller-5 tests) showed a decrease. This consistency suggests that this was not a random occurrence, but it is important to note that Novitski (1947) reported exactly the opposite tendency for the aged groups in four experimental runs.

Sex Ratios

Results of the sex-ratio studies are shown in Tables III and IV and in Figure 1. In both control groups, there was an apparent increase of females as the age of both female and sperm increased. The experimental group showed exactly the opposite results. The corresponding points of the control and irradiated groups seemed to show the same magnitude of response, but the slopes are in different directions.

Points A and B in Figure 1 represent the lumped sex-ratio data of the progeny of the same unaged females for different egg-laying periods. Points C and D represent the lumped sex ratio of the progeny of the aged females at two later egg-laying periods. The notations, A', B', C' and D', refer to the lumped sex ratios of females containing irradiated sperm. To represent lumped sex ratios graphically,

Table III. Results of experiment one on sex ratios

Table III. Results of experiment one on sex ratios

	Control				Irradiated			
	Unaged		Aged		Unaged		Aged	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1st 6 days	8,276	8,080	8,316	7,950	2,737	2,799	2,465	2,688
2nd 6 days	2,484	2,326	1,787	1,537	1,503	1,618	831	965
Total	10,760	10,406	10,103	9,487	4,240	4,417	3,296	3,653
% of total	50.84	49.16	51.57	48.43	48.98	51.02	47.43	52.57
Lumped	A_1 = .5060		C_1 = .5113		A_1' = .4944		C_1' = .4784	
♀/total progeny	B_1 = .5164		D_1 = .5376		B_1' = .4816		D_1' = .4627	
Sterile P_1 ♀♀	1		26		9		28	
Fertile P_1 ♀♀	116		223		141		426	
Offspring/♀	182.5		87.9		61.4		16.3	

Table IV. Results of experiment two on sex ratios

	Control				Irradiated			
	Unaged		Aged		Unaged		Aged	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1st 6 days	7,111	6,650	9,038	8,571	1,958	2,076	2,749	2,903
2nd 6 days	4,345	4,177	1,198	1,108	1,885	1,964	600	694
Total	11,456	10,827	10,236	9,679	3,843	4,140	3,349	3,587
% of total	51.41	48.59	51.40	48.60	48.14	51.86	48.21	51.79
Lumped	$A_2 = .5168$		$C_2 = .5133$		$A_2' = .4854$		$C_2' = .4864$	
♀/total progeny	$B_2 = .5099$		$D_2 = .5195$		$B_2' = .4897$		$D_2' = .4637$	
Sterile ♀♀	4		16		5		52	
Fertile ♀♀	111		234		145		398	
Offspring/♀	200.8		85.1		55.1		17.5	

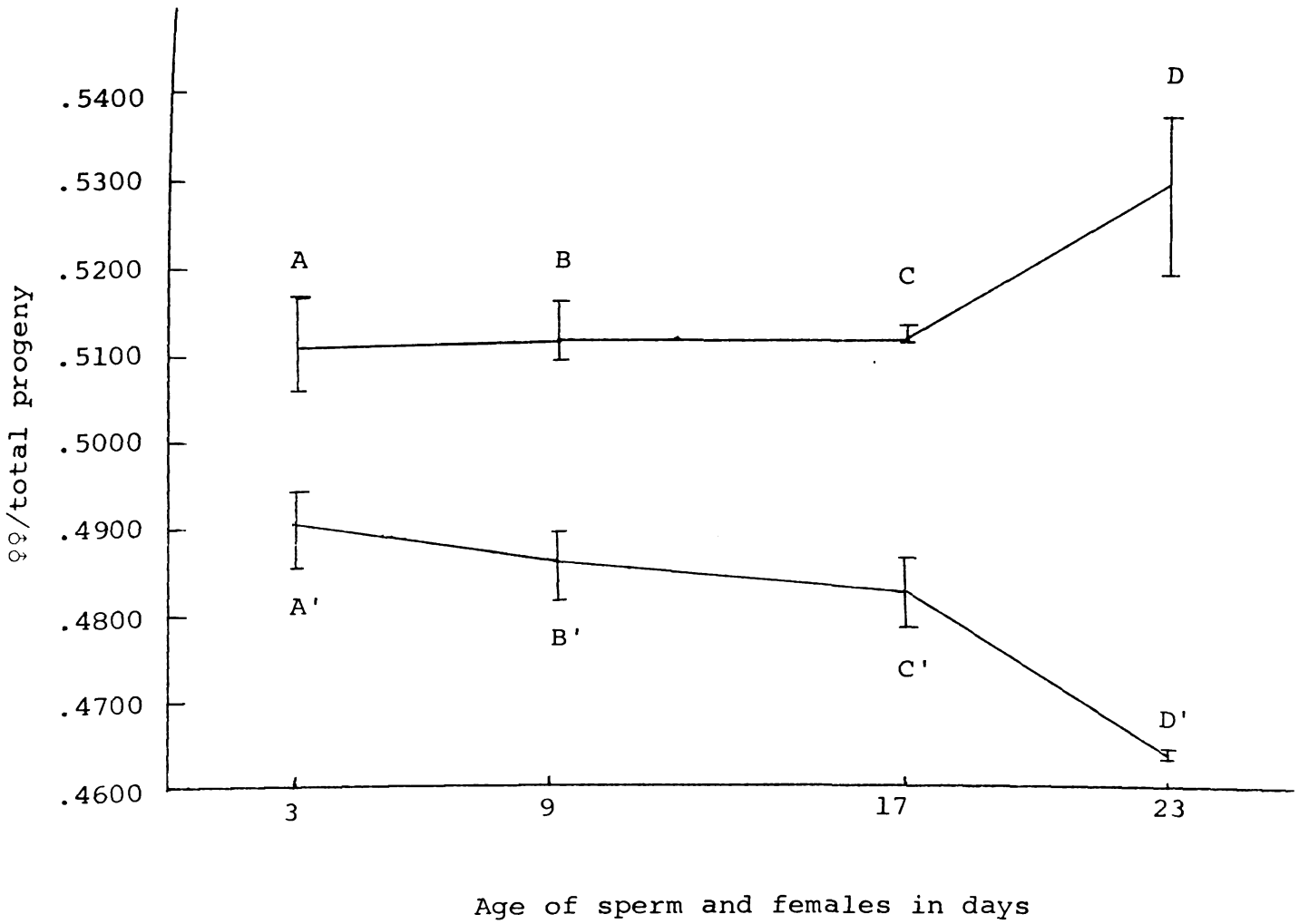

Figure 1. Chronological lumped female/total progeny ratios*

*Results of sex-ratio experiments one and two.

the midpoint of the egg-laying period was used. The 2 X 2 Chi square contingency tests were made for all the identical points in order to determine if the two experiments might be different from each other based on the assumption that the individual females were not heterogeneous within a group. Results are in Table VII. It is apparent that all identical points are not significantly different from each other. Hence, for the remainder of the statistical tests, the corresponding points of the two experiments were combined.

The 2 X N Chi-square contingency tests were made for each of the eight points in order to test for heterogeneity of the individual females with regard to sex ratio of their F_1 progeny. Females were categorized with regard to total progeny size for a given egg-laying period. Twenty-five of these females were picked randomly from the most frequent category and used in the 2 X N Chi-square tests. These tests, results of which are in Table V, showed that there was no significant heterogeneity with regard to sex ratio in the females having the same progeny size.

Since sex ratio may vary with the progeny size, it was decided to categorize the females arbitrarily with regard to total progeny number and to select twenty females of the

most frequent category at random from each given egg-laying period. Since it was not possible to get enough females of the most frequent category of the first egg-laying period who were also in the most frequent category of the second egg-laying period, it was decided that females from the egg-laying periods with equal progeny numbers would be used. Results of the latter test, which were made with a Mann-Whitney-U test (Siegal, 1956), are given in Table VI. It was concluded that the sex ratio of females with the same progeny size did not differ with respect to either the first or second egg-laying period.

The combined progeny of corresponding points of the two experiments were then subjected to 2 X N Chi-square contingency tests in order that any difference in the lumped sex ratio of any two points might be detected. The latter was based on the assumption that no heterogeneity existed in females in a given period, and that no heterogeneity between experiments existed. Results of these tests are in Table VIII, and indicate that detectable differences do not exist between A, B and A', B'. However, in the control group at point C, which represents the sex ratio of the progeny produced from two-week aged females and sperm, we can see that it differs significantly from point D at the

2.5 percent level. The same trend is evident for points C' and D' in the irradiation experiment at the 10 percent level. Fully aware of the implications involved, Chi-square tests were made between points D and the lumped points, A, B and C, for both the control and irradiation experiments. Table VIII shows statistical significance at the 1 and 2.5 percent levels for the control and irradiation experiments respectively. These later tests were made a posteriori.

In summary, the observations were: first, an apparent increase in the rate of induced sex-linked lethal mutation rate after storage of irradiated sperm; and second, an apparent effect of irradiation on the differential survival of X- versus Y-bearing sperm.

IV. DISCUSSION

Sex-linked Lethals

There are several factors which must be discussed with regard to the apparent increase of lethals on storage of irradiated sperm. First, it might be suggested that the cold temperature of storage, not the actual aging of the sperm, is the factor responsible for the apparent increase of lethals. Novitski (1961) reported an experiment, wherein inseminated females, which were subjected to the cold immediately after irradiation, showed an increase in sex-linked lethals. However, because of the small numbers involved (11/94), and because he could never repeat the experiment, the results were never thoroughly analyzed.

Novitski (1948) has shown that cold temperatures, much colder than used in my experiments, deseminated females. Since the stored females always gave fewer progeny, and since examination of dissected ventral receptacles seemed to show fewer sperm in the aged females than in the unaged, it might be suggested that the colder temperature had an effect of decreasing the number of sperm in the female without complete desemination. This is only tenable if aging is not a factor, however it is also recognized that

this reduction may be due to aging alone. Whether or not the reduction of sperm was random with regard to sex-linked lethal-bearing sperm is the important point to note. This will be discussed later in connection with the sex-ratio results.

Another aspect of the influence of temperature on the apparent increase of sex-linked lethals may be its enhancement of the irradiation damage. This could possibly be demonstrated if not all sex-linked lethals are produced by direct primary irradiation events. However, most radicals formed by irradiation have extremely short half-lives. Also, if some of the lethals are the result of secondary events, these secondary mechanisms would have to have a long period of effectiveness, because the irradiated sperm were held for twenty-four hours at room temperature after irradiation before they were subjected to the cold temperature. It has been suggested (Baker, 1953) that genetic restitution may exist, hence the effect of the cold temperature may be that of inhibiting a recovery mechanism. Maybe by delaying the recovery process for a certain time, such as by storage, the irradiation damage is irreparable, hence the apparent increase of sex-linked lethals.

The actual aging of the sperm may be all important with the temperature factor being negligible. Again, the inference would be that either increased damage occurred in the aged irradiated sperm, or that there was selection for pre-existing lethal-containing sperm. The latter does not seem reasonable unless the selection is not for the sex-linked lethal-bearing sperm, but that of the X-bearing sperm. This point is the basis for the second phase of my research.

Sex Ratio

Observations have been made (Lea, 1955) that irradiated rod-X chromosome males have more males than females among their offspring. This phenomenon has been explained by the assumption that fewer dominant lethals appear in the Y-bearing sperm than in the X-bearing sperm. In my experiment, unaged irradiated sperm yielded a sex ratio comparable to that reported for the dosage I used (2760 r). Furthermore, in testing whether there might be delayed effects, such as an alteration of the dominant lethal rate or selection against the irradiated X- or Y-bearing sperm, an apparent shift in the sex ratio of progeny of aged females was noted.

In order to analyze this decrease in female progeny of aged irradiated sperm, work on the effect of aging the

maternal parent on the sex ratio was referred to (Hannah, 1955). She ran experiments using a series of differently aged virgin Drosophila females, and she mated them to different types of male flies. Her studies included both single and mass-mating results. In the experiment using these aged females, she noted a linear decrease of females with increasing age of the maternal parent. Her control consisted of unaged females mated to the same type of males used in the experimental groups, and it yielded a sex ratio not significantly different than a one to one ratio, with a slight excess of females. Also, she noted that in offspring, produced from eggs laid during the first twenty-four hours after mating, there were fewer females than in offspring from subsequent egg laying from the same female. An explanation for the latter observation is that these first laid eggs were "overripe" eggs, and an incompatibility between the aged egg cytoplasm and the X chromosome of the sperm occurred. She aged females at 18°C and 26°C and noted that those flies stored at 26°C showed a much more rapid reduction of female progeny than those at 18°C. This can be explained by assuming that aged eggs in the virgins stored at 18°C were less "ripe" than those stored at 26°C.

A change in the sex ratio occurred in all offspring of older females, not attributable to any aged eggs. In other words, after the aged eggs were laid, the subsequent sex ratio of the unaged eggs of these aged females showed a declining number of females. Whether this is due to differential viability of the sexes or to some other mechanism has not been shown.

Hannah also noted that the change of the sex ratio of offspring of aged females resembled results similar to results of irradiation and suggested that radiation damage on the chromosome and aging through the intermediary of the cytoplasm might have common factors in the production of lethal and non-lethal losses. This does not imply that the aging processes induce gross structural changes as do X rays, but that there might exist a common process in both. If irradiation could induce a premature aging process, besides inducing gross chromosomal damage, then possibly an aberrant sex ratio might result from a radiation induced aging effect, and not due to any radiation induced chromosomal damage. In other words, aging and irradiation processes are not dissimilar mechanisms, but instead irradiation induces an aging process no different than that of natural aging.

With reported evidence showing a linear decrease in females in the progeny of aged control flies, how do I explain my seemingly contradictory results? My data on the controls seem to show a definite increase of females in both aged and unaged groups. The first group of female progeny of the unaged control females is not significantly different in proportion from the first group of aged control females which are fourteen days older. The similarity in the two groups, points A and C (Figure 1), might be explained in terms of lower temperature at which the aging took place. Hence, even though the aged females are fourteen days older, they may be almost physiologically equivalent to the unaged females, in terms of the mechanisms of maternal aging. Both the aged and unaged control second broods, points B and D (Figure 1), show an increase of females. Superficially this looks as though it may represent a non-continuous function; the last two points, C and D, represent a retarded aging due to cooler temperature of storage.

Before I can elaborate on this latter point, I should try to explain the observed increase of females in the progeny of progressively aging females when theoretically a maternal influence should be affecting the sex ratio in the

direction of more males. The big difference between my experiment and that of Hannah's is that mine is a reflection of a probable multivariant mechanism in sex-ratio determination. Hannah was able to separate the effects of temperature and aging of the females, while I have not made any attempt to control aging of the female, and most important, I have the added variant of aging the sperm. Hannah held this variant constant by aging only virgin females, while I aged impregnated females. Also, I aged females on a minimal medium, while Hannah aged her flies on nutrient medium. Physiological differences might explain the apparently different results. Hence, in my results (Figure 1), each sex-ratio point represents the combined effects of aging of the females, aging eggs and aging of the sperm. These together may explain superficially an increase in females rather than a decrease in the controls in both aged and unaged groups.

Generally then, it seems that even though aging of my control females should decrease females in the progeny, an opposing factor of concurrent aging of the sperm overcomes the latter process and tips the balance in favor of an increase of females. I infer this increase of females to be the result of a selective advantage in favor of the

X-bearing sperm, which is dependent on the aging of the sperm. In time, the Y-bearing sperm, which does not have this advantage, will be selected against. This advantage, then, would have to overcome the maternal aging effect which seems to make the zygote of the X-bearing sperm nucleus and the aged egg nucleus less viable than the zygote of the Y-bearing sperm nucleus and aged egg. What this advantage may be, and when and how this differentiation between X- and Y-bearing sperm is determined is pure speculation. The X- and Y-bearing sperm might actually be differentiated by cytochemical properties, which when aged, might have differential survival benefits. This implies that somewhere in spermatogenesis the gene(s) on the X chromosome is(are) responsible for the differentiation of the X-bearing sperm from the Y-bearing sperm. Here, one can raise a powerful question. Assuming that the X chromosome is responsible for the differentiation, when do these X chromosomal genes act? Do they exert their influence continuously, and only because of their absence in the Y-bearing sperm are they detected? Possibly these genes are only activated during spermatogenesis. These are just facets of the old question, do all the genes act the same in all cells?

After observing Drosophila spermatogenesis, C. W. Metz (1923) reported that the Y chromosome was condensed, hence presumably inert, while the X chromosome was not, indicating that it might be actively playing a part in spermatogenesis. This by no means proves the forementioned assumption of an active set of genes on the X chromosome; however if the X chromosome is thought to be active during spermatogenesis, then at least the observation can be said to be suggestive.

There might be a mechanism, such as has been shown in certain marine animals (Tyler, 1957), whereby a "fertilizin-antifertilizin" system exists. It would be baseless to assume that exactly this mechanism might exist, since it has not been shown to be anywhere near universal, and since, in the case of Drosophila, a different fertilization environment would not seem to utilize this exact system. However, this same fertilizen principle might be used to explain the sperm's ability to enter the micropyle and penetrate the egg membrane. This system, if it did exist, would have to be quite radio-sensitive, as perhaps suggested by my experiments.

Let us look at the results of the irradiated sperm, and possibly we can get more ideas as to the selective mechanism that seems to exist. Here again, it was noted that the older the irradiated sperm (both aged and unaged)

became, the more males appeared in the F_1 progeny. Superficially, this curve corresponds to Hannah's maternal aging effect curve. However, my curve is a composite of at least four sex-ratio influencing variables: aging of the egg; aging of the sperm; aging of the female; and irradiation damage to the sperm. An important point to note is that, at the level of irradiation used here, the apparently active factor of aging the sperm is drastically altered by the irradiation so that it can not overcome the constant maternal effect. Hence, in effect, the irradiation eliminates the possible selective advantage of the X-bearing sperm. Therefore, the irradiation sex-ratio results might essentially represent a maternal effect curve.

General

With the two experimental results in mind, the apparent increase of lethals and apparent decrease of female progeny after aging of irradiated sperm, an analysis will be made in order that clarification can be made of the seemingly contradictory results. A model based on radiation-induced chromosome breaks might be used to explain the observed results of both sex-linked lethals and sex-ratio studies. Baker (1958) suggested that there may be differential

consequences due to the length of time after breakage and before reuniting of the broken end of the chromosome. Also there may be differences in the genetic effect in the way the X or Y chromosome reunited after a delay in the restitution process. Baker suggested that both the X- and Y-bearing sperm are equally likely to have chromosome breaks induced in them by irradiation. Sister fusion would lead to dominant lethality by the formation of breakage-fusion bridges. The longer the length of time between initial breakage and bridge formation, the more resistant the bridge is to breakage. This would lead to an increase of X/O males, if the X chromosome was involved in the bridge. However, X/O male frequency could not explain the large shift in the sex ratio.

Considering the sex-linked lethal studies again, it is known that the X chromosomes tested for lethals did not contain a dominant lethal. Had they contained a dominant lethal, zygotes containing them would have died in the F_1. The X chromosomes tested might be one of these three types: first, those not having any induced lethal mutations; secondly, those originally having induced chromosomal breaks, but later restituted to a normal condition; and thirdly, those which contain sex-linked lethals. Restitution

would permit some sperm to survive instead of being siphoned off as dominant lethals. If this were indeed a possibility, whereby some restitutions "saved" a recessive lethal, then it might be used as an explanation for the apparent increase of lethals on storage. One interpretation of Baker's (1953) is that the longer the period after irradiation, the greater the chance of restitution of the chromosomes in the sperm. This was indicated by a drop in the dominant lethal rate in the second batch of irradiated sperm. It is hard to apply this model to explain the sex-ratio data. Possibly, this is because too many variables were involved in the experiment; not all of these variables have known rates or slopes of effect. If increased restitution occurred on aging, a decrease in dominant lethals would have occurred. Hence, an increase in the F_1 females should have resulted. However, it may be that sex-ratio studies are not sensitive enough to detect this amount of restitution, yet it might be detected by sex-linked lethal studies.

One might also explain the aberrant results by suggesting sperm competition on the assumption that sperm are utilized in a manner reflecting their genetic damage. Since it is not known how a sperm is released from the female's storage receptacle for fertilization, it might be postulated

that only the "healthy" sperm are used when the sperm number is great in the female, and only after squandering all of the healthy sperm on earlier fertilizations, the affected sperm are used for later fertilizations. In other words, competition might be greater when there are more sperm, due to polyspermy; and, as the sperm are depleted, aberrant sperm are more liable to participate in fertilization. This model could be used to explain both the apparent increase of sex-linked lethals and the change in sex ratio after aging. Also, it may explain the threshold-like results of the sex-ratio studies in Figure 1.

V. SUMMARY

The research was designed to test whether or not mature irradiated sperm were differentially affected after aging. The use of sex-linked lethals and chronological sex ratios were used as possible indices of any divergence from expected behavior of the irradiated sperm. In both tests, females were divided into two groups after twenty-four hours of mating. One group was allowed to lay eggs immediately, the other was stored for two to three weeks at 10°C. After this storage, they too were allowed to lay eggs.

In the case of the sex-linked lethal mutation studies, the resultant mutation rate of the aged sperm was compared to that of the unaged sperm. In four of five runs of this experiment, it was shown that there was an apparent increase in the mutation rate, although it was not statistically significant.

With the use of chronological sex ratios, the studies seemed to indicate that the older the irradiated sperm became, the less chance the X-bearing sperm had in survival ability. The opposite trend was noted for the control aged X-bearing sperm. Statistically the observed differences, due to age of sperm, were not significant. However, because

of the relative consistency of the results, it may be said that the observed results are highly suggestive of differential survival.

Factors possibly contributing to the observed results, such as aging females, temperature effects, restitution, enhancement of irradiation damage, and sperm competition, were discussed. If the observed differences are real, the causal mechanism is still in doubt.

APPENDIX

Table V. Results of 2 X N Chi square contingency tests for heterogeneity of individual females of a given egg laying period

Period	Progeny size	N	df	χ^2 value	P value
A	50-120	26	25	25.72	.50 > P > .25
B	50-120	25	24	16.89	.90 > P > .75
C	30-60	26	25	13.19	.975> P > .950
D	30-60	26	25	17.85	.90 > P > .75
A'	25-35	26	25	40.56	.05 > P > .025
B'	25-35	26	25	25.54	.50 > P > .25
C'	10-15	26	25	18.92	.90 > P > .75
D'	10-15	26	25	37.58	.10 > P > .05

Table VI. Results of Mann-Whitney-U tests for females having same progeny sizes in two consecutive egg laying periods

Periods	Progeny size	P value
Control A vs. B	50-120	.1660
Control C vs. C	30-60	.2148
Irradiated A' vs. B'	25-35	.2709
Irradiated C' vs. D'	10-15	.2578

Table VII. Results of 2 X 2 Chi-square contingency tests for heterogeneity of corresponding periods of the two sex-ratio experiments*

Periods	χ^2 value	P value
A_1 vs. A_2	3.42	.10 > P > .05
B_1 vs. B_2	0.52	.50 > P > .25
C_1 vs. C_2	0.13	.75 > P > .50
D_1 vs. D_2	1.72	.25 > P > .50
A_1' vs. A_2'	0.73	.50 > P > .25
B_1' vs. B_2'	0.43	.75 > P > .50
C_1' vs. C_2'	0.67	.50 > P > .25
D_1' vs. D_2'	0.0003	.975 > P > .950

*Based on the assumption of no heterogeneity of the individual females comprising each period.

Table VIII. Results of 2X 2 Chi-square contingency tests for heterogeneity of the lumped sex ratio at different egg-laying periods**

Periods	χ^2 value	P value
A vs. B	0.06	.90 > P > .75
C vs. D	6.13	.025 > P > .010
A' vs. B'	0.31	.75 > P > .50
C' vs. D'	3.56	.10 > P > .050
A+B+C vs. D	7.08	.010 > P > .005
A'+B'+C' vs. D'	6.25	.025 > P > .010

**Based on the assumptions that heterogeneity between females of a given period did not exist, and that there was no heterogeneity between the corresponding points of the two experiments.

Table IX. Results of 2 X 2 Chi-square contingency tests for heterogeneity in the lumped sex ratio of aged sperm compared to unaged sperm

Experiment	χ^2 value	df	P value
Control (A+B vs. C+D)	1.02	1	.50 > P > .25
Irradiated (A'+B' vs. C'+D')	1.69	1	.25 > P > .10

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