THE EFFECTS OF STORAGE UPON THE DIFFERENTIAL SURVIVAL AMONG SPERMATOZOA OF DROSOPHILA MELANOGASTER

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Michael E. Myszewski

1962

ABSTRACT

THE EFFECTS OF STORAGE UPON THE DIFFERENTIAL SURVIVAL AMONG SPERMATOZOA OF DROSOPHILA MELANOGASTER

by Michael E. Myszewski

Interpretation of some recent research opened the question as to whether selection might act on the haploid level. The object of this study was to determine whether differential survival of mature spermatozoa could be detected among progeny recovered from females stored after insemination.

Virgin OR females were mass-mated to heterozygous males of two different genotypes (Cy/Pm, Cy/lethal). After mating, the inseminated females were divided randomly into equal groups. One group was allowed to lay eggs immediately, the other after being stored one or two weeks. Progeny were scored as to sex and genotype. Two runs were made for each of the male genotypes tested. Chi-square analysis of the data indicated statistical differences among the progeny. These differences were attributed to differential recovery of the Curly, Plum and lethal chromosomes. Preferential recovery of females over males was noted.

Sperm competition has been postulated as a possible causal mechanism producing these differences. Types of sperm competition are discussed.

THE EFFECTS OF STORAGE UPON THE DIFFERENTIAL SURVIVAL AMONG SPERMATOZOA OF DROSOPHILA MELANOGASTER

By

Michael E. Myszewski

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 would like to express my thanks to Dr. Armon F. Yanders for proposing this study and for the most generous and interested encouragement he has given me throughout its progress.

I am grateful to Dr. Philip Clark for suggestions pertaining to the statistical treatment of the data and for the discussions which have helped me to formulate attitudes towards research.

I would also like to thank Gloria Stanich for isolating some of the lethal stocks used in this study.

This study was supported in part by a grant to Dr. A. F. Yanders from the U.S. Atomic Energy Commission (Contract A T[11-1] - 1033).

TABLE OF CONTENTS

LIST OF TABLES

I. INTRODUCTION

Random segregation and random fertilization presume that the appearance of equal numbers and kinds of progeny should occur. Occasional exceptions to these principles have been noted, and result in discernible variations. Various factors may act to cause aberrant ratios to appear, namely, abnormal meiosis, zygote mortality and gametic lethality. These factors, which may act at different times during stages of development, result in the non-appearance or reduction in number of a particular class of offspring.

Abnormal meiosis

During the process of meiosis, various phenomena act in different ways to favor, or conversely, to hinder the developmental process of a genotype.

It has been shown (Frost, 1961) that segregation of autosomes in attached - X triploid females is highly non-random when X chromosome non-disjunction occurs. In addition, the presence of a Y chromosome will produce large increases in X-chromosome nondisjunctions. These results are explained by Frost on the basis of Sandler and Novitski's (1956) hypothesis that pairing can frequently occur between non-homologous univalents in their heterochromatin kinetochore regions.

Besides this occurrence of preferential segregation among autosomes, a case has been found where the size of the

homologous chromosomes influences the recovery of these homologs (Novitski, 1951). When the homologs are of unequal size, the smaller of the two homologs is recovered about twice as frequently as the larger. This was termed non-random disjunction and was further studied by Novitski and Sandler (1956). Using tandem metacentric and tandem acrocentric compound X chromosomes, with or without a homolog, it was found that in the absence of a homolog there was a deficiency in the number of newly generated single chromosomes formed by crossing over. In the absance of a homolog, some fraction of these single chromosomes proved lethal which resulted in recovery of fewer individuals in this class.

The segregation mechanism may be altered in other ways as evidenced by certain wild populations of the house mouse (Dunn and Suckling, 1956). An allele, at the T locus ($\underline{t^w}$), has been found to be transmitted with a higher frequency than either the wild type allele or the T allele (Brachyury) in the heterozygous males. The higher frequency of transmission has been attributed to abnormalities in the segregation mechanism. The specific reason for aberrancy at the $\underline{t^w}$ locus, other than abnormal segregation, has not been determined. However, recent findings for another allele of the T locus ($\underline{t^e}$) indicate that, for this case, selective fertilization may be the causative agent for aberrant ratios such as were described for the $\underline{t^w}$ locus (Bateman, 1960, a,b.).

In addition to these agents, another force has been described, in which the gametes derived from a heterozygote

vary from the expected 1 : 1 ratio. This force, termed "meiotic drive" (Sandler and Novitski, 1957), may be the cause of the abnormal segregation mechanism in the mouse, described previously, but this has not yet been proven cytologically.

The first case of meiotic drive was reported by Gershenson (1928) in Drosophila obscura males. These individuals produced many more female than male progeny and the deviation could not be attributed to zygote mortality. Sturtevant and Dobzhansky (1936), after a detailed cytogenetical study using Drosophila pseudoobscura, concluded that the sex ratio difference was due to abnormal spermatogenesis resulting in all of the spermatozoa being X - bearing, the Y chromosome having degenerated. A case of meiotic drive has recently been found in a natural population of Drosophila melanogaster (Sandler, Hiraizuma, and Sandler, 1959). This particular example results from a second chromosome locus in or near the proximal heterochromatin. The chromosome containing this factor, termed Segregation - distorter (SD), is recovered more frequently than is the normal chromosome among the progeny of male heterozygotes. Similar to Dunn and Suckling's (1956) abnormal segregation mechanism, ratio distortion due to SD is never found to occur in females. In addition, the ratio distortion due to SD apparently is effective only when synapsis occurs, and comes about as a failure of the sperm carrying the normal allele to form or to function normally. The failure of sperm formation has been attributed

by Sandler, Hiraizuma and Sandler to the SD locus which causes a break at the SD+ locus at synapsis, and results in a chromatin bridge at anaphase II. This bridge is subsequently lethal to resultant cells, causing the variation in recovered progeny. It must be realized that no unequivocal cytological evidence has yet been presented to support this interpretation of SD.

There is evidence that some products of spermatogenesis in mammals will undergo degeneration. These include both spermatocytes (Roosen-Runge in Novitski and Sandler, 1957) and spermatogonia (Oakberg, 1956). So far as Drosophila is concerned, a similar degeneration of meiotic products has not been found (Cooper in Demerec, et al, 1950).

Zygote mortality

Although all of the developing gametes may have escaped elimination by the various mechanisms listed above, and have been produced in equal numbers, after fertilization, another category of causes may act to produce reduction or eradication of some classes of progeny. Any number of gene mutations produce lethal effects in the zygote (see list in Bridges and Brehme, 1944). These will act at any time from fertilization to the onset of eclosion. Besides gene mutation, chromosomes carrying aberrant portions such as, translocations, inversions, deletions, or duplications may be discriminated against. Polyploid or polysomic conditions may also result in ratio distortion.

Gametic Lethality

After gametogenesis and prior to the formation of a zygote, there is the probability that some mechanism will act to cause differential survival among the gametes. The case for gamete lethality as a cause of differential recovery of progeny in Drosophila has been disputed by Muller and Settles (1927). Their experiments indicated that fertilization can occur regardless of the gene content of the spermatozoa and that spermatozoa are not differentially viable due to gene content. Various aspects of the problem which were tested in Drosophila supported their hypothesis. The relative numbers of X and Y - bearing spermatozoa surviving after a lapse of time were observed by comparisons of sex ratio, but did not differ significantly. Gametes deficient for portions of the second chromosome which had become translocated to the third chromosome also were tested. Those gametes which carried the deficiency should have showed lower survival rates than normal gametes had the genes been functioning at this time. Instead, Muller and Settles found that the deficient eggs, when fertilized by uncompensated spermatozoa, resulted in non-viable zygotes. Fertilization had been accomplished by these aberrant gametes.

Self-sterility as described for plants has been found to account for discrepant ratios. Sperm competition has been postulated to account for a deviation in the primary sex ratio in humans. The possibility of sperm competition in Drosophila is usually discounted because of the short distance

which must be covered by the spermatozoa from the storage organs of the female to the point of entry into the egg. However, Novitski and Sandler (1957) have described a case which indicates not all products of spermatogenesis are functional. The mutant that they studied was Stone's Bar $\left[\underline{T}\ (1;4)\ B^{S}\right]$, a translocation between the X chromosome and the chromosome IV. Males would carry the translocated X - IV chromosome and in addition a normal IV and a Y. The four types of gametes were normally recovered in equal numbers but departures from the expected numbers were found. Zygote mortality was ruled out by egg count experiments although this was not tested. The authors felt that gamete lethality as commonly conceived would not explain the variant ratios. Their explanation for this phenomenon was that some products of spermatogenesis are not regularly functional and that these were the longer components of the translocation. If some of the meiotic products could be shown to be non-functional (or to have degenerated, as previously mentioned for mammals), then to postulate gametic lethality, the supposition would be that the time of the non-functioning was restricted to the spermatozoan stage. In that the non-functioning was predetermined in the meiotic stages, it could not be considered to be ordinary gamete lethality. Other factors may also function at the gametic level. Certain "t - alleles" have been recovered in male mice far more frequently than expected (Dunn and Suckling, 1956). Ratios indicate that a particular class of sperm is recovered in 80 percent to 90

percent of the progeny. Dunn and Suckling attribute these aberrant ratios to abnormalities in the segregation mechanism. Working with different t - alleles, Bateman (1960, a,b) has recovered similar ratios. Evidence was found here (1960, b) however, that one of the tested t - alleles, (tailless - Edinburgh, t^e) when present in spermatozoa, caused a non-random conjugation between gametes. When a choice of eggs was presented by heterozygous (T/+ and T/t^e) females, tailless - Edinburgh spermatozoa united more frequently with normal (+) than with brachyury (T), and more frequently with brachyury than tailless - Edinburgh eggs. Bateman felt that this was evidence for selective fertilization in the mouse and indicated that the spermatozoa would be more readily able to enter the egg carrying the favored genotype. In Drosophila, Lobashov (1939), has reported that if a female is allowed to copulate with each of two males having different genetic constitutions, the sperm of lower viability will be used to a lesser extent by the female after the double copulation than had she been mated only to the male with the less viable sperm. This was interpreted as selective fertilization favoring the more viable sperm.

It is possible for other factors to cause discrepant ratios to appear in progeny counts. Unisexual ratios in Drosophila ("sex-ratio", SR) have been attributed to maternally transmitted spirochetes (Poulson and Sakaguchi, 1961). This condition would be manifested by disturbances in the development of the male zygote leading to loss of this class of offspring. Specific gene mutations which will effect ratios re-

covered have also been reported. Bell (1954) reports a mutation (daughterless, da) which will produce only male progeny. Sturtevant (1945) described one whose presence will transform females into males (transformer, tra).

Present Work

The present work attempts to determine if there is preferential survival exhibited by spermatozoa of various genotypes. If the genotype of a spermatozoon has an effect on the survival of that gamete, then the effect should be more pronounced after a period of storage. Some studies (Novitski and Sandler, 1957; Dunn and Suckling, 1956; Bateman, 1960, a, b) may be interpreted as evidence that selection may take place on the haploid level. Effects on sex ratios have also been noted after ageing the maternal parent (Hannah, 1955) and after ageing the spermatozoa in the inseminated female (Trosko, 1962). These too, may be interpreted as evidence for gamete lethality. Gamete lethality should be demonstrated by the preferential survival of one genotype in the progeny of a heterozygote. By observing progeny ratios recovered from such individuals from stored stocks, and comparing them to non-stored stocks, it was hoped to demonstrate that the survival of spermatozoa is correlated with genotype.

II. MATERIALS AND METHODS

One wild type strain (Oregon - R) and six mutant stocks of *Drosophila melanogaster* were utilized. The mutant stocks are as follows:

Curly, Plum (*Cy/Pm*) is a balanced lethal tester stock. *Curly* is a second chromosome mutant usually lethal when homozygous. The wings curl up strongly; the wing texture is slightly thinner than wild type with wrinkles in the upper surface. Inversions are found in the left and right arms [*In (2L) Cy, Cy (included), In (2R) Cy, cn*^{2} (*included*)] which strongly suppress crossing over in the second chromosome. *Plum* has been described as an eye color similar to *brown* (*bw*) or *purple* (*pr*), mottled with darker sploches or speckles which deepen with age to a reddish color. An inseparable inversion accompanies *Plum* [*In (2LR) Pm*], which acts to suppress crossing over on the second chromosome. *Plum* is also lethal when homozygous. Both *Curly* and *Plum* are recessive lethals which will exhibit a dominant phenotypic effect when heterozygous.

Curly, Plum, Hairless, Inversion 3R (*Cy/Pm; H/In3R*) is also a balanced lethal tester stock. The *Curly* and *Plum* mutants are the same as described above for the *Cy/Pm* stock. *Hairless* (*H*) is a third chromosome mutant which acts mainly to remove various bristles, especially the post-verticals and abdominals. Like *Curly* and *Plum*, it is homozygous lethal. *Hairless* is balanced with an inversion,

In (3R) Mo (Inversion (3R) from Missouri wild stock). It is not lethal when homozygous but females are often sterile. Crossing over is suppressed along the third chromosome.

The five chromosome II recessive lethals (Curly/lethal) tested were induced by exposure to approximately 2000 r units of X-rays. The source of the X-rays was a General Electric Maximar - 250 - 111 which was operated at 250 kv, 15 ma, with a 50 mm copper filter. The dose rate was approximately 136 r/minute. Second-chromosome lethals were detected by standard tests and maintained in the balanced lethal stock.

All stocks were maintained on a semi-synthetic nutrient medium (modified after Carpenter, 1950). Stocks were mated and stored on modified Offerman's (1936) medium, to prevent the newly inseminated females from laying eggs. Also, this medium was not conducive to the growth of yeast which in two weeks would often overgrow the surface of the nutrient medium.

Two runs were made for each group tested with variations in procedure as indicated.

Adults

From the stocks listed above, Cy/Pm, Cy/Pm; H/In3R and Cy/lethal males were collected prior to mating. These males were not necessarily the same age but varied from one to seven days. Oregon-R females were collected as virgins. They were stored from the time of collection in a constant temperature room at 10° C. for a period of seven days. After the indicated storage time, groups of the OR females were

removed from the constant temperature room and mass-mated to the various mutant males on Offerman's medium for a 24 hour period.

For each group mated, an excess of males was used. After the 24 hour mating period, the males were separated from the females and discarded. Females were divided at random into two groups which were equal in size. The Group A females were the control group. These females were placed in individual shell vials containing the modified Carpenter's medium and allowed to lay eggs immediately. Group B, the experimental group was placed en masse on fresh Offerman's medium and returned to the 10° C. constant temperature room for seven days in the case of the Cy/Pm; H/In3R and the first run of Cy/Pm inseminated females, fifteen days for the Cy/lethal and second run of Cy/Pm inseminated females. Group A females were handled in two ways. The first run of Cy/Pm and Cy/Pm; H/In3R inseminated females were allowed to lay all of their eggs in the same shell vial. These females were discarded at the end of twelve days. The females inseminated on the second run by the Cy/Pm males, and all of the Cy/lethal inseminated females, were transferred to new vials containing modified Carpenter's medium at the end of six days. After six days in the fresh vial, a total of twelve days in all, these females were discarded. Group B females were removed from the constant temperature room at the end of seven days in the case of those inseminated by the first run of Cy/Pm males and by Cy/Pm; H/In3R males and at the end of fifteen days for those

inseminated by the second run of Cy/Pm males and by Cy/lethal males. In a pattern similar to the Group A females, the Group B females which were inseminated by the first run of Cy/Pm males and by Cy/Pm; H/In3R males were placed in individual shell vials on modified Carpenter's medium and allowed to lay all of their eggs therein. After twelve days, they were discarded. Group B females inseminated by the second run of Cy/Pm males, or by Cy/lethal males, were placed in individual shell vials and transferred at the end of six days to fresh vials. At the end of twelve days they were discarded. All of the stocks described were kept at a constant temperature of 22° C. except when stored at 10° C. as was indicated.

Progeny

Progeny from the various crosses began to emerge eleven days after the females were allowed to lay eggs. Most of the progeny were counted at two day intervals. However, circumstances dictated at times that some counting take place at three day intervals. Similarly, it was sometimes possible to count some groups every day. All progeny, including flies which were stuck to the medium, were scored when it was possible. The number which could not be identified was small and would not influence the final results. The data were tabulated in such a way as to compare the non-stored group to the stored group for four categories. The F_1 progeny were genotypically Curly/+ or lethal/+ (or Pm/+). Phenotypically, the progeny were scored as Curly, Plum or in the case of the lethal-bearing

group, wild type. Within each genotypic group (Curly/+, lethal/+, or Plum/+) a record was kept of the number of individuals of each sex. In the final analysis, four groups of progeny were scored: Curly males, Curly females, lethal (Plum) males, lethal (Plum) females.

Two changes were made between the first and second runs of the females inseminated by Cy/Pm males. Females of the second run were transferred to new vials at the end of six days. This was done to prevent crowding of the progeny and also facilitated counting the progeny in the event that any were stuck to the medium. There was also less chance that the parent female would become stuck to the medium in the event that yeast would overgrow the surface. The storage time of the females was also changed, because studies by Trosko (1962) indicated that changes due to storage may not occur until after fourteen days.

After the first run had been made, involving Cy/Pm and Cy/Pm; H/In3R stocks, it was decided to narrow the study to possible differences involving only the second chromosome. For this reason a second run involving the Cy/Pm; H/In3R stock was not made and the results of this first run were not included in the analysis of the data.

III. RESULTS

In that each lethal being considered is assumed to be different, patterns which were obtained for each lethal shall be discussed. Each of the six lethal stocks was analyzed in various ways by means of 2 X 4 and 2 X 2 contingency Chi-square tests (See Appendix for details). Two of the lethals (lethal - 2, lethal - 22) tested showed no heterogeneity for any of the tests; another lethal (lethal - 4) showed significance for only one of the thirty Chi-square tests applied to it. The other three lethals are the ones which will be discussed here (lethal - 3, lethal - 9, Cy/Pm).

lethal - 3 (1-3)

Within the lethal-bearing group of progeny storage of sperm seemed to favor the recovery of X-bearing sperm, as increases in the number of females were noted. This was most evident in the data obtained for Run II (Table VI) and the composite data for Runs I and II (Table VII). For both sets of data, storage seemed to change the pattern established among the lethal-bearing non-stored individuals (males appearing more frequently than females) to a case where the stored lethal females were more abundant than the stored lethal males. The largest deviation manifested itself in the non-stored group.

Within the females, it appeared as though Curly-bearing chromosomes were favored over lethal-bearing in the non-stored class. This situation changed upon storage when more lethal than Cy chromosomes were recovered. The total

number of Cy and lethal individuals for both stored and non-stored classes was approximately equal. Again the largest deviation was found in the non-stored group.

While Cy and lethal chromosomes were recovered in approximately equal numbers, it was found in the stored group that more females were recovered than males. It was noted, particularly in the lethal classes, that males were less frequent than females. Cy males were produced either as frequently or more frequently than Cy females.

In general, for the 1 - 3 group, several effects were noted. Storage tended to discriminate against the male, particularly those carrying the lethal chromosome. Storage tended to reverse a trend in which more males than females were produced for non-stored classes. Deviations, when they occurred, tended to be greater in the non-stored groups than the stored groups.

lethal - 9 (1-9)

Results obtained from Run I (Table I) for lethal - 9 represent a small sized sample when compared with Run II (Table II). Results obtained in Run I are therefore considered to be unique for that set of data and are not noted in either Run II (Table VI) or the composite data (Table VII). Within the stored lethal chromosomes, more males were recovered than females (163 to 119). This occurrence was regarded as influencing the combined Cy, lethal data for Run I where significance was once more achieved. In that these values for the stored group were obtained from only four females, these discrepant results were attributed to

random fluctuation.

For Run II and subsequently the composite data, there was a tendency for the Cy chromosome to be recovered more frequently than the lethal chromosome. This peculiarity was found in both the stored and the non-stored groups with one exception. The exception occurred in the female group in which genotype was compared to stored and non-stored individuals (Table VI and Table VII). Within the stored groups, although the Cy chromosome was recovered in only slightly higher numbers than the 1-9 chromosome a more marked shift would have occurred had expected figures been realized. A similar pattern, although not as pronounced, was noted in males.

The 1-9 group seemed to be characterized by the recovery of more Cy than lethal chromosomes. This tendency, though still evident, was moderated upon storage.

Cy/Pm

Females which were not stored produced more Cy than Pm offspring. This situation was reversed on storage. A larger deviation was found between the Cy and Pm individuals in the stored group than the non-stored group. More Pm than Cy females were found.

In considering the Pm offspring, females were more frequent than males in both the stored and non-stored groups. This deviation was more pronounced in the stored group than in the non-stored group. Although females were more abundant, males were produced within the non-stored group in greater.

numbers than expected, while in the stored groups they were less abundant than expected.

Within the stored group of the Cy/Pm individuals, females of both genotypes appeared more frequently than males. This tendency was more pronounced among the Pm genotype than the Cy. While females were more abundant in both classes, more males were produced than expected in the Cy class and fewer males than expected in the Pm class.

The Cy/Pm stock showed larger deviations in the stored groups than the non-stored. Storage tended to produce fewer males than would be expected. The Plum genotype was recovered more frequently than was the Curly, but not to the point of being significant.

General

Non-stored

The Cy chromosome was recovered more frequently than the lethal-bearing chromosome, but less frequently than the Pm chromosome in the same situation. Cy and Pm individuals were recovered in approximately equal numbers.

Stored

For the Cy/lethal stocks, a moderating influence on deviations was seen among the stored groups. In Cy/Pm stocks, the deviation was more pronounced. The ratio of male to female progeny declined.

IV. DISCUSSION

The experiments indicate a differential recovery of genotypes among the progeny of male heterozygotes. The differences which appear indicate that rather than being obvious, the pursued phenomenon is not universal to all data and is manifested by subtle rather than extreme variation. Indicative of this, are the several tests which show heterogeneity when certain portions of the data are considered (for example, analyzing one genotype at a time, or considering each sex separately). If only the 2 X 4 Chi-square tests had been utilized, these inequalities might have passed unnoticed.

Similarities appear between lethals when the tests indicating heterogeneity are examined. Two items among the data are of particular interest. First, a differential recovery of classes of progeny may reflect differential survival of the gametes carrying the various genotypes. Second, the differential survival is influenced by storage. These two factors seem to function independently of one another. Within the Cy/lethal runs, the variations are more pronounced in the non-stored groups. In the stored groups this variation is lower. Such is not the case in the Cy/Pm stocks, for the greater variation is found in the stored groups. These variations for both the Cy/lethal and the Cy/Pm groups are manifested as preferential recovery of a particular genotype, either one type of mutant chromosome or a particular sex.

The Bar of Stone translocation used by Novitski and Sandler (1957) gave reproducible recovery of abnormal ratios of progeny. In their study, males carrying the translocation were mated with attached - X females. The ratios of progeny recovered were considered to be a reflection of the number and types of gametes produced during spermatogenesis. All sperm types should have been produced in equal numbers but apparently were not. This situation was considered to be one in which all of the products of spermatogenesis did not participate in fertilization. This would be an attractive proposal to explain the present research, but upon closer consideration, several differences are apparent. The stock used by Novitski and Sandler was carrying a translocation which led to inequality in the size of homologs. But all of the homologs used in the present work are of equal size. Also, aberrant forms which would tend to be eliminated because of such structural abnormalities, should, by the design of the experiment, be lost in equal frequency in both the stored and non-stored groups. Any effect between the stored and non-stored groups because of such an abnormality will be cancelled out. The mechanism postulated by Novitski and Sandler also seems to be unable to account for selection of both a mutant allele and a particular sex when applied to the present experiment.

Selective fertilization, postulated by Bateman (1960b) for the house mouse at the T locus, might also be considered here. His work indicated that the high rate of recovery

of the t^e allele might result from unequal ratios of spermatozoa being produced. On the other hand, these ratios might result from the t^e bearing spermatozoa being better able to enter eggs of a favored genotype. If this is the case, even if equal numbers of spermatozoa are produced, those carrying the t^e allele will have a decided advantage. That this type of action has produced the aberrant ratios in the present work is unlikely. The manner of fertilization in the mouse necessitates a relatively long migration of the spermatozoa from the site of deposition to the place of fertilization, and differs from Drosophila, where the spermatozoa need travel only a short distance from the storage organs to the uterus. Furthermore, in the mouse, fertilization will take place shortly after insemination, but Drosophila will store the spermatozoa, utilizing them as needed over a period of days. Also, while Bateman used females of different genotypes, only the OR females were made use of for this work. Finally, storage, not only of the spermatozoa within the female, but also of the females themselves, will be an additional difference.

In that these models do not adequately explain the results obtained, what mechanism can be postulated which will explain them? This mechanism should be able to explain why the Curly chromosome should be favored over the lethal but not the Plum chromosomes; why females should be favored over males; and why storage of these genotypes should result in differences when compared to the non-stored classes.

To suggest that one type of spermatozoa is produced in greater numbers than its alternate type does not seem feasible. This presumes that the favored genotype in the non-stored group (which will also be produced in greater numbers) will be discriminated against more readily than the alternate genotype after storage. Such fluctuation between favor and misfortune implies a fickleness and inconsistancy. in the causal mechanism which does not seem warrented.

It is reasonable then to consider that two mutant characters (Curly and lethal) on homologous chromosomes will result in two types of sperm which differ only in regards to which mutant each is carrying and that the survival is correlated with the presence or absence of a given genotype. Assuming that these two types of sperm are produced in equal numbers during spermatogenesis, they should also be transferred to the female in equal numbers. The deviations which appear more markedly in the non-stored groups of Cy/lethal individuals may reflect an initial advantage of the Curly spermatozoa over the lethal-bearing spermatozoa. This advantage, which may take a variety of forms, is discussed below. This advantage can be lost by the Cy spermatozoa upon storage or the lethal-bearing spermatozoa can better their chances of survival so neither type will have an advantage over the other at the end of the storage period. The Pm chromosome may have a similar advantage over the Cy when both are present in the population of sperm. The difference in this case will not be evident until after a period of storage, for there need be an

initial advantage of the Plum over the Curly genotype. The shift toward a higher proportion of females in the stored group does not appear to be correlated with the shifts in genotype which have been discussed. The lower frequency of males may also be explained by the proposed model, in that the X - bearing spermatozoa will have an advantage over the Y - bearing spermatozoa resulting in selection against the latter gamete.

It now seems appropriate to discuss the various mechanisms in which one type of spermatozoa may be favored over another type. The method of storage was to keep the inseminated females at 10° C. for either one or two weeks. Temperature shocks somewhat lower than this (-5° C., -10° C.) have been shown to completely deseminate fertilized Drosophila females (Novitski and Rush, 1948). Drosophila females are known to lose spermatozoa more rapidly during non-storage as a consequence of egg-laying then when stored at the lower temperatures. In this experiment, egg-laying was prevented by storage of the females at 10° C. In young Drosophila females, the ovaries will not have developed by the time of insemination and will not develop upon storage at the low temperatures. Even at the low temperatures used for storage, some of the spermatozoa undoubtedly are lost and this loss may have been selective. One type of selective loss has been described. Irradiated sperm have been shown to be eliminated more rapidly than non-irradiated from the females in which they were stored (Yanders, 1959). The present work indicates that the elimina-

tion which occurs upon storage is selective for a particular genotype.

A possible mechanism for selective loss has been suggested by Lefevre and Jonnson (1962). They have reported spermatozoa circulating from the storage organs to the uterus and other portions of the genital tract. They indicate that once having left the storage organ, sperm may reenter it. Sperm have been classified as a consequence by them as "inbound" and "outbound" on this basis. While definite evidence has not been obtained in this regard for the present work, one may conjecture how a similar mechanism may explain some of the results. Non-stored sperm may circulate in such a way that the less viable spermatozoa would not be a liable to gain reentry into the storage organs, and gradually be eliminated. The genotype of the individual will influence the viability. Upon storage the initial advantage will either be lowered (as in the case of Cy/lethal stocks) or enhanced (I - Cy/Pm stocks.)

It will be noted that the actual observed values obtained for the Curly and Plum chromosomes do not vary to a great extent, yet there is heterogeneity which can be discerned when the observed values are compared to the expected values. Although the Plum chromosome is not recovered at frequencies much higher than the Curly, it is recovered at a higher rate than may be anticipated from examination of the expected values. The differences discussed with regard to the Cy/Pm stocks refer to the data from Run I and the

composite data. Run II by itself fails to exhibit these differences. The longer period of storage in Run II may explain this on the basis of the model proposed for the differences seen in Run I and the composite data. If the storage period will exert an enhancing influence on the recovery of the Pm chromosome, then Run II, which was stored for two weeks, would show this more markedly than Run I, which was stored for only one week. This implies a fluctuating advantage in which the Pm may be said to have no initial advantage but after storage for one week an advantage over the Cy chromosome may develop. By the end of two weeks of storage again no advantage will exist between the different chromosomes.

The variation noted in the sex ratio is similar to that described by Trosko, (1962) for his aged control group. Upon ageing this group, he found a greater number of females than males. He attributed this to a selective advantage of the X - bearing spermatozoa. This advantage was dependent on the age of the spermatozoa and might have been due to physiological differences intrinsic to these gametes. Physiological differences were suggested by Zimmering and Barbour (1961) as a possible means of explaining the effect of ageing on gametic ratios. Variation in the recovery segregation mechanism of Drosophila males carrying the Bar translocation of Stone appeared to be caused by Y chromosomes and major autosomes designated as "A" (abnormal ratios). In the presence of "E" (equality) Y autosome combinations, the previous variation tended to

disappear, (Zimmering, 1959. 1960). Sperm released by young A - type males caused the characteristic distorted ratios. Sperm released 4 - 6 days later by the same male showed virtually no distortion. Zimmering conjectured that physiological differences between groups of cells destined to give rise to different sperm batches caused the distortion. Similar physiological differences in my experiments could act to distinguish between X - and Y - bearing spermatozoa, rather than between groups of homologs. This distortion could be expressed as a difference in survival rates as shown both by the present work and by Trosko.

The possibility that physiologically different types of gonadal cells exist is supported by Tihen (1946) who reports that two types of spermatogonia, "primary" and "secondary", may be present in Drosophila. The "primary" spermatogonia are characterized by not occurring in groups and also by undergoing asynchronous mitoses. The second type is the "secondary" spermatogonia which occur in well-defined cysts and attain mitotic synchrony. The functions of these two types of cells are different. The primary spermatogonia have as one of their functions the production of the secondary spermatogonia. These latter cells act only to produce primary spermatocytes. Tihen further suggests that the mitotic division of the primary spermatogonia results in the production of one primary spermatogonium plus one secondary spermatogonium. If such differences exist on the spermatogonial level, then they may also be found at the gametic level, and will reflect

the genotype of the sperm itself. This difference may be expressed as differential survival among the gametes.

The data obtained from these experiments indicate preferential survival between the gametes produced by male heterozygotes and stored in the female prior to use. The differential recovery is attributed to an advantage that one genotype holds over the other. The mechanism causing this advantage is unknown although several possibilities are discussed.

V. SUMMARY

The research was designed to detect selection at the haploid level in Drosophila melanogaster. Attempts were made to determine whether storage would produce a differential rate of survival among mature spermatozoa of various genotypes.

Drosophila males of the various genotypes (Cy/Pm; Cy/lethal) were mass-mated to virgin females. The females were divided randomly into two groups, one of which was allowed to lay eggs immediately, the other stored for one or two weeks at 10° C. After the storage period, this group also was allowed to lay eggs. Progeny were scored with regards to their sex and genotype.

The Curly chromosome was found to be recovered with greater frequency than the lethal but less frequently than the Plum. A lower proportion of males were recovered than females, especially in the stored groups. In the Cy/lethal matings, storage exerted a moderating influence while in Cy/Pm matings, it acted to enhance the deviations.

Possible factors which could produce these changes were discussed, including abnormal segregation mechanisms, selective fertilization, differential viability, selective desemination, sperm competition and physiological differences intrinsic to the gamete. The causal mechanism was not known although sperm competition of the type discussed would explain many of the phenomena observed.

APPENDIX

Two separate experimental runs were made for each genotype. The results from each run were analyzed as well as the total data for both runs. The data were tabulated in such a way as to be able to compare the non-stored group to the stored group for four categories. The F_1 progeny were phenotypically curly-winged, plum-eyed or wild type for those respectively carrying the Curly, Plum or lethal alleles. Within each group, Cy, Pm, or lethal, a record was kept of the number of individuals of each sex. In the final analysis, four categories of offspring were scored, Cy males, Cy females, lethal or Pm males, and lethal or Pm females.

The totals which were obtained for the first run of the various matings were tabulated in Table I. Groups Cy/lethal and Cy/Pm were run at different times. Different procedures, as indicated, were used for the Cy/Pm group. The totals obtained varied greatly due to widely varying numbers of surviving females. This number ranged from 4 (stored lethal - 9) to 392 (stored Cy/Pm).

In the second run, (Table II) the Cy/lethal and Cy/Pm groups were run concurrently, using similar procedures as modified from the initial run. The average number of progeny varied considerably from one lethal to another and from stored to non-stored. Higher progeny averages tended to reflect less crowded culture conditions due to transferring the female parent to new vials after six days.

The data obtained for Run I and Run II were lumped together and considered as a composite (Table III) to increase the sample size. Procedures followed were the same except for the Cy/Pm groups which differed in treatment in that females of the second run were stored for a longer period of time before being allowed to lay eggs. These females were also transferred to new vials of food after six days of egg laying. Either run of the Cy/Pm stocks would have been large enough to analyze by itself if it was thought differences between the groups existed. The larger sample size would enable the researcher to detect differences should they occur. Differences which occurred in both runs would be amplified in the composite data. Effects of any fluctuation which might have occurred in only one run would be lessened. With the exception of the Cy/Pm stock, the conditions and procedures for each run were, for all intents and purposes, identical. Large differences in sample size appeared evident between the non-stored and stored groups and were primarily due to the numbers of parent females which were used for each group. Also, the average number of progeny produced by the females for each group, stored and non-stored, was a factor.

Analysis of both runs and the composite data was made by 2 X 4 contingency Chi-square tests and the results tabulated (Table IV). These Chi-square tests involved a comparison of the non-stored group to the stored group for all four genotypic classifications of the progeny (Cy males, Cy females, lethal (or Pm) males, lethal (or Pm) females).

A significant difference was interpreted as a differential survival of one (or more) class (es) of progeny. Subsequent Chi-square tests were used to determine the specific reason (s) for the difference.

With the exception of I - L - 9 (the first run of lethal - 9), all of the groups achieving significance for 2 X 4 Chi-square tests showed a change in the lethal (or Pm) category between the stored and non-stored females. Fewer females than expected were found in the non-stored groups while more were found in the stored group. Only lethal - 9 achieved significance for more than one run. In this regard, the same phenomenon did not produce the deviation for both runs of the lethal - 9 group. Deviation in Run I was due to a sex ratio shift, while Run II demonstrated differential recovery of Curly and lethal chromosomes.

Heterogeneity had been indicated by use of the 2 X 4 Chi-square tests. These were not too specific in that eight categories were being considered with each test. It was thought that more specific information might be found if additional tests were made. The influences of the genotype, sex, and storage could be separated, one from another, and in this way a more exact estimate could be made of the agents causing the deviation. The 2 X 2 contingency Chi-square tests were used since the data did not lend themselves to other statistical applications.

Nine different types of 2 X 2 Chi-square tests were made, manipulating the data in various ways so as to consider

some of the variables while excluding others. These nine different tests were applied to the five lethal and the Cy/Pm stocks for each of the two runs as well as the composite data.

	Curly ♂♂	Curly ♀♀	lethal (Plum) ♂♂	lethal (Plum) ♀♀	Totals
non-stored					
stored					
totals					

The 2 X 2 Chi-square tests were derived from this pattern in the following way. The genotypes Cy and lethal (or Pm) were compared with the stored and non-stored categories for both males and females. The males and females were compared with regard to the genotypes Cy and lethal (or Pm) for both the stored and non-stored groups. All of the males and females, regardless of whether or not they had been stored or non-stored, were compared with the genotypic categories Cy and lethal (or Pm), All of the Cy and lethal (or Pm) genotypes, regardless of sex, were compared to the stored and non-stored categories.

As was noted for the 2 X 4 Chi-square tests, three of the lethals in Run I did not show any heterogeneity (Table V). The three remaining lethals showed varying response to the tests.

Lethal - 3 showed significance only when the stored groups were considered which compared genotype to sex. The number of lethal males was significantly lower than the

lethal-bearing females. Slightly more Cy males were scored than Cy females although the difference was not significant.

For two of the nine tests, lethal - 9 showed significance at the 1 percent level. The lethal category, comparing sex with stored and non-stored, showed a pronounced difference in the sex ratio of the stored group, as more males were produced than females. Also showing significance were males and females of both Cy and lethal genotype which were compared with stored and non-stored groups. In that the lethal group was shown to be varying with regards to the sex ratio, it is not unexpected that this group which represented the lethal plus the Cy group also varied in a similar fashion.

The Cy/Pm stock showed significance for three tests, two at the 2.5 percent level and one at the 1 percent level. The females, comparing genotypes to stored and non-stored, indicated significance at the 2.5 percent level. Non-stored Cy females were produced in excess of non-stored Pm females. This trend was reversed upon storage and more Pm than Cy females were recovered. Showing significance at the 1 percent level was the Pm category, comparing sex with stored and non-stored groups. In both the stored and non-stored groups more females than males were produced. With regard to the expected values, more males than females were produced in the non-stored group but fewer males than females for the stored group. The largest deviation was found in the stored group. Significant at the 2.5 percent level was the stored category, comparing

sex with genotype. More females than males were produced, especially in the Pm group, and more Pm individuals were were produced than Cy.

Three of the groups tested in the second run showed no heterogeneity for the 2 X 2 Chi-square tests (Table VI). The Cy/Pm stock which did show significance in Run I did not do so for this run. Lethal - 4 gave an indication of significance for this run, the only such indication in both runs.

The lethal - 3 test which showed significance was the lethal group comparing sex to stored and non-stored group where males were more numerous than females. The reverse was true among the stored group where more females than males were present. When both groups were considered, more females were produced than males.

Lethal - 4 showed significance at the 5 percent level for one test. In the non-stored group comparing sex with genotype, a higher number of females than males was found for the Cy genotype. While more females than males were found in the lethal group, the difference could not be said to be significant.

Three tests showed significance at the 1 percent level for lethal - 9. Both the male and female groups, comparing the genotypes with the stored and non-stored groups, showed significance. In both cases, the Curly - bearing individuals were expected in greater numbers than were the lethal-bearing individuals. This expectation was realized

for the males. The female Cy stored group was not produced in sufficient number to meet expectations and as a result approximated rather than exceeded the lethal stored group. In that this situation prevailed for both the male and the female groups, it appeared intrinsic to both sexes and a causal mechanism seemed to lie in the genotype of the individual. Among both the males and females, the deviation between the genotypes was more pronounced in the non-stored group than the stored group. Significance was also found in the Chi-square test in which the data for the sexes were lumped and genotype was compared to stored and non-stored groups. This test was essentially the lumping together of the two classifications discussed above for this lethal. The significance achieved here appeared due to the same factors causing the deviation in the above groups. The Cy genotype was more numerous than the lethal and the deviation more pronounced in the non-stored category than the stored.

For the composite data (Run I plus Run II), three of the lethals tested by the 2 X 2 contingency Chi-square tests did not show heterogeneity (Table VII).

Lethal - 3 had three tests showing significance. The female group, comparing genotype with stored and non-stored groups showed significance at the 1 percent level. Although Cy and lethal-bearing individuals were produced in almost equal numbers (5615 versus 5574), the non-stored group had more Cy than lethal individuals and the reverse was true of the stored group where more lethal individuals

appeared. Larger deviations were found in the non-stored group than the stored group. This particular test did not produce significant results for either Run I or Run II. The lethal group, comparing sex to stored and non-stored groups, showed significance at the 1 percent level. Within the non-stored group, males were more numerous than females. This situation reversed itself in the stored group where females were more numerous. Overall, more females were produced than males. The stored group, comparing sex to genotype, showed significance at the 2.5 percent level. While approximately equal numbers of males and females were produced in the non-stored group, more females were expected. The lethal group followed expectations and females exceeded males. With regard to the composite data, more females were produced than males. But, in Drosophila it is a normal occurrence to have more females than males produced.

Lethal - 9 had three tests showing significance, all at the 1 percent level. Run I, being considerably smaller, constituted only a rather small portion of the composite data. The bulk of the data came from Run II which, as noted above, produced significant results for the same tests. As was noted for Run II, the primary reason for the deviation appeared to be a larger proportion of Curly-bearing individuals being produced than lethal - bearing. The deviation between genotypes was more pronounced in the non-stored group than the stored group.

Two tests in the composite data showed significance

for Cy/Pm data. Both of these tests had produced significant results in Run I. While the significance achieved in Run I was at the 1 and 2.5 percent levels, the composite data showed significance at 2.5 and 5 percent levels respectively. The Pm category, comparing sex with stored and non-stored individuals, showed significance at the 2.5 percent level. In both the stored and non-stored groups more females than males were produced. The largest deviation took place in the stored group. Showing significance at the 5 percent level was the stored group, comparing sex with genotype. The major cause of heterogeneity was that more females than males were produced for both Cy and Pm genotypes. While Cy and Pm individuals were produced in approximately equal numbers, a larger deviation took place among the Pm group than the Cy group. More males than expected were found in the Cy group; fewer males than expected in the Pm group.

TABLE I. Progeny in Run I Recovered for Each Lethal Classified As to Genotype and Sex.

LETHAL		CURLY males	CURLY females	LETHAL males	LETHAL females	TOTAL	AVERAGE PROGENY/ FEMALE	NUMBER of FEMALES
L - 2	non-stored	1978	1897	1978	1987	7840	224	35
	stored	1721	1694	1704	1765	6884	121	57
	TOTAL	3699	3591	3682	3752	14724		
L - 3	non-stored	1456	1537	1419	1462	5874	178	33
	stored	1946	1920	1836	2007	7709	151	51
	TOTAL	3402	3457	3255	3469	13583		
L - 4	non-stored	464	464	454	475	1859	232	9
	stored	210	244	231	217	902	90	10
	TOTAL	675	709	685	692	2761		
L - 9	non-stored	358	406	380	440	1584	226	7
	stored	142	139	163	119	563	141	4
	TOTAL	500	545	543	559	2147		
L - 22	non-stored	1639	1678	1583	1598	6498	171	38
	stored	2011	2097	1934	2110	8152	148	55
	TOTAL	3650	3775	3517	3708	14650		

		CURLY males	CURLY females	PLUM males	PLUM females	TOTAL	AVERAGE PROGENY/ FEMALE	NUMBER of FEMALES
Cy/Pm	non-stored	7767	8491	7897	8411	32566	110	295
	stored	8526	9223	8435	9583	35767	91	392
	TOTAL	16293	17714	16332	17994	68333		

TABLE II. Progeny in Run II Recovered for Each Lethal Classified As to Genotype and Sex.

LETHAL		CURLY males	CURLY females	LETHAL males	LETHAL females	TOTAL	AVERAGE PROGENY/ FEMALE	NUMBER of FEMALES
L - 2	non-stored	976	1025	963	1060	4024	212	19
	stored	1739	1818	1720	1814	7091	197	36
	TOTAL	2715	2843	2683	2874	11115		
L - 3	non-stored	981	1004	1001	925	3911	130	30
	stored	1129	1154	1082	1180	4545	130	35
	TOTAL	2110	2158	2083	2105	8456		
L - 4	non-stored	3394	3675	3415	3435	13919	202	69
	stored	2125	2193	2095	2186	8599	169	51
	TOTAL	5519	5868	5510	5621	22518		
L - 9	non-stored	1958	2100	1600	1716	7374	123	60
	stored	2073	2146	1958	2161	8338	141	59
	TOTAL	4031	4246	3558	3877	15712		
L - 22	non-stored	1922	2029	1826	1924	7701	248	31
	stored	1426	1468	1334	1446	5674	158	36
	TOTAL	3348	3497	3160	3370	13375		

		CURLY males	CURLY females	PLUM males	PLUM females	TOTAL	AVERAGE PROGENY/ FEMALE	NUMBER of FEMALES
Cy/Pm	non-stored	3084	3298	2989	3337	12708	161	79
	stored	2815	3116	2787	3080	11798	166	71
	TOTAL	5899	6414	5776	6417	24506		

TABLE III. Summary of Runs I and II (composite)

LETHAL		CURLY males	CURLY females	LETHAL males	LETHAL females	TOTAL
L - 2	non-stored	2954	2992	2941	3047	11864
	stored	3460	3512	3424	3579	13975
	TOTAL	6414	6434	6365	6626	25839
L - 3	non-stored	2437	2541	2420	2387	9785
	stored	3075	3074	2918	3187	12254
	TOTAL	5512	5615	5338	5574	22039
L - 4	non-stored	3859	4140	3869	3910	15778
	stored	2335	2437	2326	2403	9501
	TOTAL	6194	6577	6195	6313	25279
L - 9	non-stored	2316	2506	1980	2156	8958
	stored	2215	2285	2121	2280	8901
	TOTAL	4531	4791	4101	4436	17859
L - 22	non-stored	3561	3707	3409	3522	14199
	stored	3437	3565	3268	3556	13826
	TOTAL	6998	7272	6677	7078	28025

		CURLY males	CURLY females	PLUM males	PLUM females	TOTAL
Cy/Pm	non-stored	10851	11789	10886	11748	45274
	stored	11341	12339	11222	12663	47565
	TOTAL	22192	24128	22108	24411	92839

TABLE IV. Chi-square Values from 2 X 4 Contingency Tables Testing Whether the Differences in Survival Between Stored and Non-stored Sperm Differs Among the Four Combinations of Sex and Genotype for Run I, Run II and Composite Data of I and II.

RUN	L - 2	L - 3	L - 4	L - 9	L - 22	Cy/Pm
I	0.789	4.230	2.613	12.450# 1%	3.452	9.250 5%
II	0.804	7.489	3.853	31.892 1%	0.714	1.455
Sum of I + II (composite)	1.821	9.234 5%	1.634	20.573 1%	3.143	6.221

Red shading indicates significance at the level noted.

TABLE V. Chi-square Values for 2 X 2 Contingency Tables Testing Whether the Difference in Survival of Sperm Differs With Regard to Storage or Non-storage, Sex or Genotype for Run I Data. Those Tests Which Showed Significance for Other Runs are Indicated.

		L - 2	L - 3	L - 4	L - 9	L - 22	Cy/Pm
♂♂	Cy - L vs. NS - S	0.543	0.413	1.084	0.295 II# I+II##	0.007	1.538
♀♀	Cy - L vs. NS - S	0.008	3.762 I+II	1.563	2.912 II I+II	1.356	### 5.048 2.5%
Curly	♂♂ - ♀♀ vs. NS - S	0.316	1.992	1.586	1.246	0.137	0.227
Lethal	♂♂ - ♀♀ vs. NS - S	0.421	1.398 II I+II	0.845	10.979 1%	2.753	8.918 I+II 1%
Stored	♂♂ - ♀♀ vs. Cy - L	1.123	4.983 I+II 5%	2.553	2.860	1.038	5.328 2.5%
Non-stored	♂♂ - ♀♀ vs. Cy - L	1.078	0.220	0.214 II	0.039	0.086	1.380
	♂♂ - ♀♀ vs. NS - S	0.735	0.018	0.057	9.260 1%	2.045	3.161
	♂♂ - ♀♀ vs. Cy - L	2.199	1.883	0.282	0.475	0.352	0.682
	Cy - L vs. NS - S	0.048	0.862	0.022	1.006 II I+II	0.618	0.610

\# II indicates significance was achieved for this test in Run II
\## I+II indicates significance was achieved for composite data
Red shading indicates significance at the level noted for Run I

TABLE VI. Chi-square Values for 2 X 2 Contingency Tables Testing Whether the Difference in Survival of Sperm Differs With Regard to Storage or Non-storage, Sex or Genotype for Run II Data. Those Tests Which Showed Significance for Other Runs are Indicated.

		L - 2	L - 3	L - 4	L - 9	L - 22	Cy/Pm
♂♂	Cy - L vs. NS - S	0.003	0.978	0.258	9.823 I+II## 1% ###	0.080	0.350
♀♀	Cy - L vs. NS - S	0.432	2.968 I+II	2.857	21.839 I+II 1%	0.609	0.448 I #
Curly	♂♂ - ♀♀ vs. NS - S	0.003	0.000	1.528	0.624	0.237	0.879
Lethal	♂♂ - ♀♀ vs. NS - S	0.708	7.109 I+II 1%	0.872	0.366 I	0.301	0.083 I I+II
Stored	♂♂ - ♀♀ vs. Cy - L	0.034	1.139 I I+II	0.065	2.089	0.916	0.000 I I+II
Non-stored	♂♂ - ♀♀ vs. Cy - L	0.571	2.556	4.710 5%	0.000	0.000	1.456
	♂♂ - ♀♀ vs. NS - S	0.349	3.517	0.047	0.014 I	0.000	0.235
	♂♂ - ♀♀ vs. Cy - L	0.366	0.067	2.391	1.112	0.344	0.710
	Cy - L vs. NS - S	0.128	0.228	0.674	30.679 I+II 1%	0.121	0.004

\# I indicates significance was achieved for this test in Run I
\## I+II indicates significance was achieved for composite data
\### Red shading indicates significance at the level noted for Run II

TABLE VII. Chi-square Values for 2 X 2 Contingency Tables Testing Whether the Difference in Survival of Sperm Differs With Regard to Storage or Non-storage, Sex or Genotype for the Composite Data of Runs I and II. Those Tests Which Showed Significance for Other Runs are Indicated.

		L - 2	L - 3	L - 4	L - 9	L - 22	Cy/Pm
♂♂	Cy - L vs. NS - S	0.030	1.340	0.032	6.913 II## 1% ###	0.040	0.520
♀♀	Cy - L vs. NS - S	0.444	6.705 1%	1.440	12.547 II 1%	2.157	2.612 I #
Curly	♂♂ - ♀♀ vs. NS - S	0.551	1.222	0.589	1.346	0.008	0.004
Lethal	♂♂ - ♀♀ vs. NS - S	0.059	6.878 1%	0.347	0.090 I	2.355	5.740 I 2.5%
Stored	♂♂ - ♀♀ vs. Cy - L	0.772	6.038 I 2.5%	0.058	0.950	1.946	3.933 I 5%
Non-stored	♂♂ - ♀♀ vs. Cy - L	1.557	1.889	3.530 II	0.027	0.053	0.126
	♂♂ - ♀♀ vs. NS - S	0.487	1.176	0.016	1.034 I	0.959	3.102
	♂♂ - ♀♀ vs. Cy - L	2.218	0.838	2.674	0.539	0.697	1.366
	Cy - L vs. NS - S	0.328	1.061	0.527	19.133 II 1%	0.823	0.464

\# I indicates significance was achieved for this test in Run I
II indicates significance was achieved for this test in Run II
Red shading indicates significance at the level noted for the composite data of Runs I and II

BIBLIOGRAPHY

Bateman, Nigel, 1960a "High frequency of a lethal gene (t^e) in a laboratory stock of mice." Genetical Research 1 (2): 214-225.

_____, 1960b "Selective fertilization at the T locus of the mouse." Genetical Research 1 (2): 226-238.

Bell, A. E., 1954 "A gene in Drosophila melanogaster that produces all male progeny." Genetics 39: 958-959.

Bridges, C. B. and K. S. Brehme, 1944 The Mutants of Drosophila melanogaster. Carnegie Inst. Wash. Publ. 552, Washington, D. C.

Carpenter, John M., 1950 "A new semi-synthetic food medium for Drosophila." Dros. Info. Serv. 24:96.

Demerec, M. et. al., 1950 Biology of Drosophila. John Wiley and Sons, Inc., New York. pp. 1-61.

Dunn, L. C. and J. Suckling, 1956 "Studies of the genetic variability in wild populations of house mice. I. Analysis of seven alleles at locus T." Genetics 41: 344-352.

Frost, Justin, N., 1961 "Preferential segregations in triploid females." Genetics 46: 373-392.

Gershenson, S., 1928 "A new sex ratio abnormality in Drosophila obscura." Genetics 13: 488-507.

Hannah, A. M., 1955 "The effect of aging the maternal parent upon the sex ratio in Drosophila melanogaster." Z. Verebungslehre 86: 574-599.

Lefevre, G., Jr., and U. Jonnson, 1962 "Sperm relationships in twice-mated D. melanogaster females." Dros. Info. Serv. 36:85.

Lobashov, M. E., 1939 "Mixture of sperm in case of polyandry in Drosophila melanogaster." C. R. (Dokl.) de l'Acad. Sci. URSS 23: 827-830.

Muller, H. J., and F. Settles, 1927 "The non-functioning of genes in spermatozoa." Z. Verebungslehre 43: 285-312.

Novitski, E. and G. Rush, 1948 "Desemination by low temperature shocks." Dros. Info. Serv. 22:75.

Novitski, E., 1951 "Non-random disjunction in Drosophila." Genetics 36: 267-280.

Novitski, E. and L. Sandler, 1956 "Further notes on the nature of non-random disjunction in Drosophila melanogaster." Genetics 41: 194-206.

_____, 1957 "Are all products of spermatogenesis regularly functional?" Proc. Nat'l. Acad. Sci., Wash. 43: 318-324.

Oakberg, E. F., 1956 "A description of spermiogenesis in the mouse and its use in analysis of the cycle of seminiferous epithelium and germ cell renewal." Am. J Anat. 99: 391-419.

Offerman, C. A., and I. K. Schmidt, 1936 "Culture media for Drosophila." Dros. Info. Serv. 6:64.

Poulson, D. F. and B. Sakaguchi, 1961 "Nature of 'Sex-ratio' agent in Drosophila." Science 133: 1489-1490.

Sandler, L. M., and E. Novitski, 1956 "Evidence for genetic homology between chromosomes I and IV in Drosophila melanogaster with a proposed explanation for the crowding effect in triploids." Genetics 41: 189-193.

_____, 1957 "Meiotic Drive as an evolutionary force." Amer. Naturalist 91: 105-110.

Sandler, L., Yuichiro Hiraizumi and Iris Sandler, 1959 "Meiotic Drive in natural populations of Drosophila melanogaster. I. The cytogenetical basis of segregation-distortion." Genetics 44: 233-250.

Siegal, Sidney, 1956 Non-parametric Statistics for the Behavioral Sciences. McGraw-Hill, New York.

Simpson, G. G., A. Roe, and R. C. Lewontin, 1960 Quantitative Zoology. Harcourt, Brace and Co., New York.

Sturtevant, A. H. and Th. Dobzhansky, 1936 "Geographical distribution and cytology of 'sex-ratio' in Drosophila pseudoobscura and related species." Genetics 21: 473-490.

Sturtevant, A. H., 1945 "A gene in Drosophila melanogaster that transforms females into males." Genetics 30:

297-299.

Tihen, J. A., 1946 "An estimate of the number of cell generations preceding sperm formation in Drosophila melanogaster." Amer, Naturalist 80: 389-392.

Trosko, James E., 1962 The Effects of Aging Irradiated Drosophila Sperm on Sex-linked Lethal Mutation Rate and Sex Ratio. Master's Thesis (unpublished), Michigan State University.

Ward, Lenore, 1923 "The genetics of Curly wing in Drosophila another case of balanced lethal factors." Genetics 8: 276-300.

Yanders, Armon F., 1959 "The effect of X-rays on sperm activity in Drosophila." Genetics 44: 545-546.

Zimmering, S., 1959 "Modification of abnormal gametic ratio." Science 130: 1426.

_____,1960 "Modification of abnormal gametic ratios in Drosophila. I. Evidence for an influence of Y chromosome and major autosomes on gametic ratios from Bar-Stone translocation males." Genetics 45: 1253-1268.

_____, and E. Barbour, 1961 "Modification of abnormal gametic ratios in Drosophila. II. Evidence for a marked shift in gametic ratios in early vs. late sperm batches from A - type Bar-Stone translocation males." Genetics 46: 1253-1260.