STUDIES OF INSEMINATION AND SPERM STORAGE IN INTRA-AND INTER-STRAIN CROSSES OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

STUDIES OF INSEMINATION AND SPERM STORAGE IN INTRA- AND INTER-STRAIN CROSSES OF *DROSOPHILA MELANOGASTER*

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Deviations from expected progeny ratios may be the result of phenomena occurring in the meiotic, gametic, or zygotic stages. Some abnormal ratios are known to be the result of aberrations in meiosis or zygotic lethality, and it has been suggested that other abnormal ratios are the result of gametic lethality or selection. However, in studies where gametic lethality or selection is implicated as the cause of anomalous ratios, this has been as a result of eliminating other possibilities, and no mechanism has been described to account for the phenomenon. Studies of the "insemination reaction," the changes in DNA content of bovine spermatozoa after storage, and the behavior of irradiated and unirradiated *Drosophila* sperm in the female storage organs have suggested that gametic lethality or selection occurs and that some interaction between the sperm and/or seminal fluids and the female reproductive tract is involved. In this investigation, differences in the insemination indices found in crosses between four strains of *Drosophila melanogaster*, and variations in the rate of loss of sperm from the ventral receptacle when one strain of females is inseminated by different strains of males, suggest that there may be a differential viability of different types of sperm. Gametic selection, if it occurs, must be a potent evolutionary force.

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TABLE OF CONTENTS

		Page
I.	INTRODUCTION	1
II.	EXPERIMENTAL	8
	A. Experimental I--Studies of Insemination	8
	1. Materials and Methods	8
	2. Results	11
	a. Part 1	11
	b. Part 2	13
	3. Discussion	19
	B. Experimental II--Studies of Sperm Storage	23
	1. Materials and Methods	23
	2. Results	23
	a. Part 1	23
	b. Part 2	24
	3. Discussion	27
III.	GENERAL DISCUSSION	30
IV.	SUMMARY	38
V.	BIBLIOGRAPHY	39

LIST OF TABLES

Table		Page
1.	Insemination indices from reciprocal crosses of four strains of *Drosophila melanogaster*	14
2.	Insemination indices from reciprocal crosses between Oregon-R, Swedish B and F_1 hybrids	18

LIST OF FIGURES

Figure		Page
1.	Reproductive system of female Drosophila	10
2.	Insemination score patterns from crosses between Oregon-R and Swedish-B -- one hour dissections . . .	15
3.	Insemination score patterns from crosses between Oregon-R and Swedish-B -- 24 hour dissections . . .	16
4.	Insemination score patterns from crosses between Swedish-B females and four strains of males	20
5.	Insemination indices of Swedish-B females stored at 25° C.	25
6.	Insemination indices of Swedish-B females stored at 12° C.	26

INTRODUCTION

Since the time that Mendel proposed the principle of random segregation, many exceptions to this principle have been noted. A number of deviations from expected theoretical ratios have been studied, and various explanations for these discrepancies have been put forth.

In organisms such as Drosophila where sex determination is the result of a balance between the sex chromosome factors and factors on the autosomes, the female normally produces only eggs which contain one set of autosomes and one X-chromosome, while the normal male produces sperm all containing one set of autosomes and either an X-chromosome or a Y-chromosome. If the male produces equal numbers of X- and Y-bearing sperm, and if these two kinds of sperm have equal chances of fertilizing an egg, then a 1 : 1 sex ratio will be found in the progeny. In most stocks of Drosophila this normal ratio is very closely approximated. However, many deviations from it have been reported. Aberrant sex-ratios may be caused by various factors: factors affecting the production of X- and Y-bearing sperm, factors affecting the balance between autosomes and sex-chromosomes, factors affecting the viability of the different types of sperm, sex-linked lethal factors, and sex-limited factors which may result in differential viability of the sexes (Brehme, 1943).

Morgan (1911) described a modification of the sex-ratio obtained from crosses between rudimentary winged flies. He attributed these aberrant ratios to either "prematuration," an effect of the normal

allele present in the egg of the heterozygous females until extrusion of the polar bodies, or "repugnance," a reduction in fertilization when both sperm and egg carry the same factor. It has since been shown that rudimentary affects larval differentiation (Counce, 1956). Sturtevant and Dobzhansky (1936) studied a sex-ratio deviation in D. pseudobscura caused by a gene, sex-ratio, located on the X-chromosome. Cytological studies showed that in sex-ratio males the X-chromosome undergoes an equational division at each meiotic division with the Y-chromosome degenerating, thus resulting in all female progeny. Bell (1959) described a gene, daughterless, in D. melanogaster which produces all male progeny. This abnormal sex-ratio appears to be due to a lethal action against the females in the egg stage.

Sex-ratio aberrations have also been attributed to interactions between nuclear and cytoplasmic factors (Poulson and Sakaguchi, 1961; Cavalcanti, 1958). It has also been shown that continued selection within an animal stock can produce high and low sex-ratio strains; it would seem, therefore, that there are genes which when concentrated in a genotype influence either the relative production of the two kinds of gametes produced by the heterogametic sex or their relative functional ability (Crewe, 1937).

Many abnormal sex-ratios can be explained by deviations in the production of one type of gamete or in differences in viability of the zygotes of different sexes. However, there are other deviations from a 1 : 1 ratio which seem to result from differential viability of X- and Y-bearing sperm or differences in their ability to fertilize eggs. C. W. Metz (1929), studying unisexual progenies in Sciara, eliminated

the possibility of zygote mortality by egg counts and concluded that the abnormal progenies were due to selective elimination or inactivation of sperms. A sex-ratio abnormality in D. obscura described by Gershenson (1928) is attributed to the removal of spermatozoa with the Y-chromosome from the fertilization process. Novitski (1947) described an anomalous condition in D. affinis which he called male sex ratio (MSR) and which results in the production by certain affinis males of only male offspring. He noted that when females mated to MSR males are dissected and the ventral receptacles examined, large quantities of sperm are found in them although such females have produced few or no offspring. This suggests that the sperm may not be capable of fertilization.

Besides anomalous sex-ratios, deviations from other theoretical ratios have been reported. Novitski and Sandler (1957) reported unusual progeny ratios from male D. melanogaster carrying the Bar-Stone translocation. Zimmering (1960) and Zimmering and Barbour (1961), also studying Bar-Stone translocation males, found that certain Y autosome combinations seemed to be responsible for the abnormal ratios. Another cause of unusual ratios is a second chromosome locus called segregation-distorter (SD), which was discovered in a natural population of D. melanogaster (Sandler, Hiraizumi, and Sandler, 1959). This phenomenon, which has been called "meiotic drive," is explained on the basis of an aberration in meiosis which results in the SD^+-bearing gametes being rendered non-functional.

The potential causes of abnormal ratios can be classified in three categories: 1. aberrant meiotic segregations, 2. post-fertilization selection, and 3. gametic lethality or selection. If phenomena which

occur during meiosis result in some of the products of meiosis being rendered non-functional, it should be possible to demonstrate this by cytological methods. Sandler (1959) in preliminary cytological studies of segregation-distorter observed dicentrics and chromatid bridges in second meiotic divisions in testis smears. Phenomena which come into operation after fertilization and result in zygote lethality are easily demonstrated. Comparisons of egg counts to adults recovered will show whether zygote mortality has occurred in the egg stage. If gametic lethality or selection occurs, it will not be so easy to demonstrate. That it does exist is suggested by the fact that aberrant ratios occur which cannot be explained satisfactorily by the other mechanisms.

There are also other phenomena, revealed by studies of insemination and studies of the characteristics of sperm, which lend support to the possibility of gametic lethality or selection. Studies of polyandry suggest that repeated copulations may result in a mixture of sperm in the storage organs of the female Drosophila and that competition between sperm of different types may occur, resulting in a prevalence of progeny from one of the males used in the crosses (Lobashov, 1939). Kaufman and Demerec (1942) also found differences in the frequencies of different types of offspring from a multiply-inseminated female but suggested that they might be explained by differences in the quantity of sperm transferred by the various males.

It is generally held that the spermatozoon serves primarily as a transporting mechanism for the genetic information encoded in its DNA. It is further assumed that during this transport the DNA is wholly inert. Since sperm with various chromosome deficiencies and duplica-

tions are able to fertilize eggs, it is widely held that the functional ability of the spermatozoon is independent of its gene content (Muller and Settles, 1927). Recent studies of sperm DNA suggest, however, that there may be changes in the DNA of the spermatozoon during its life span and that variation in sperm DNA and variation in fertility may be related. The DNA content of cells has been found to be fairly constant within a species, and the DNA content is also correlated with the number of chromosome sets in the cell, haploid cells having one-half the amount of DNA as diploid cells. In the absence of chromosome change the DNA content of the sperm nucleus would be expected to be stable since the sperm nucleus is not thought to be so metabolically involved in cell activity as is true in other cells. Salisburg and his associates (1961) have found a loss of 30% of the original DNA content of bull spermatozoa after five days of _in vitro_ storage. This phenomenon may explain various effects of sperm aging which have been reported: early embryonic mortality in cattle (Salisburg, Birge, De La Torre, and Lodge, 1961); effects on fertility which have been found in the rat (Soderwall and Blandau, 1941), guinea pig (Soderwall and Young, 1940), and chicken (Nalbandov and Card, 1943; Dharmajan, 1950); and increased mutations in _Drosophila_. Leuchtenberger and co-workers (1955) found that the amount of DNA per spermatozoon in fertile men is constant and uniform within each individual, while, in contrast, the DNA content of sperm from men with suspected infertility showed great variability.

In _Drosophila_, sperm from a single insemination may be retained within the storage organs of the female for a period of time. In order to discover whether spermatozoa of different types behave differently,

it is pertinent to study insemination and sperm storage.

Yanders (1959) found a reduction in the number of sperm stored in the ventral receptacles of female Drosophila if the male had been irradiated before mating.

Studies of sexual isolating mechanisms have also produced some interesting findings. Sympatric species may exhibit various types and degrees of sexual isolating mechanisms which prevent the exchange of genes between them: failure of the different species to mate, matings resulting in non-viable offspring, matings resulting in sterile F_1 hybrids, and matings in which the female is inseminated but in which the sperm are inactivated or killed in the reproductive tract of the female (Patterson, 1957). Patterson (1946) has described an "insemination reaction" in some species of Drosophila which takes place in the female reproductive tract immediately following copulation. The insemination reaction occurs in both intra- and inter-specific matings (Patterson, 1947); in females from homogamic matings the vagina returns to a normal condition within eight or nine hours, but in females from heterogamic matings it may remain swollen for several days. The insemination reaction is characterized by a swelling of the vagina and the presence of a "reaction mass," which is composed of spermatozoa and substances of acidophilic staining properties (Lee, 1950). The reaction mass is expelled after a period of time together with any sperm which have failed to reach the storage organs of the female (Patterson, 1947). Patterson (1947) has found that live spermatozoa are not necessary for the induction of the insemination reaction since sterile males which produce no sperm will induce a reaction . Lee (1950) has

found by artificially inseminating D. gibberosa females with various materials that testis and testis plus accessory glands will produce a typical insemination reaction while male accessory glands alone will not. Therefore, it would seem that some factor from the testis is responsible for the induction of the reaction. The insemination reaction is perhaps related to various "immunological" types of phenomena which have been noted in fertilization processes (C. B. Metz, 1955; Hultin, 1959; Baum, et al., 1961; Rumke and Hellinga, 1959).

The previously described investigations suggest that the study of gametic lethality or selection in Drosophila should begin with studies of insemination and sperm storage. Since the behavior of sperm in the female reproductive tract may be related to the genetic constitution of the male and female, studies of crosses between races of a species of Drosophila may provide evidence bearing on this phenomenon.

EXPERIMENTAL

Experimental I--Studies of Insemination

Materials and Methods

Four geographical strains of Drosophila melanogaster were used in these experiments: Oregon-R, Canton-S, Crimea, and Swedish-B. Since each of these strains was established from a different geographical race, it is likely that they have different genetic constitutions resulting from genetic drift and varying selective pressures.

These cultures were maintained by mass transfer in pint milk bottles on a semi-synthetic nutrient medium (modified after Carpenter, 1950) in a constant-temperature room at 25° C. Flies were transferred to fresh food bottles every three or four days.

All males and females to be used in a particular cross were collected within a 24 hour period in most cases. (In a few cases not enough flies had emerged during the first 24 hours, so that the collections were extended over a 48 hour period.) Only virgin females were used in these experiments. The females were placed in vials containing minimal medium (Offerman and Schmidt, 1936) to prevent egg laying and were aged for six days at 25° C. The males were aged on nutrient medium. On the seventh day after collection of the flies, 50 females and 100 males, for each cross, were introduced, without etherization, into a pint milk bottle containing minimal medium. The mating bottles were placed in the constant temperature room (25° C.) for a

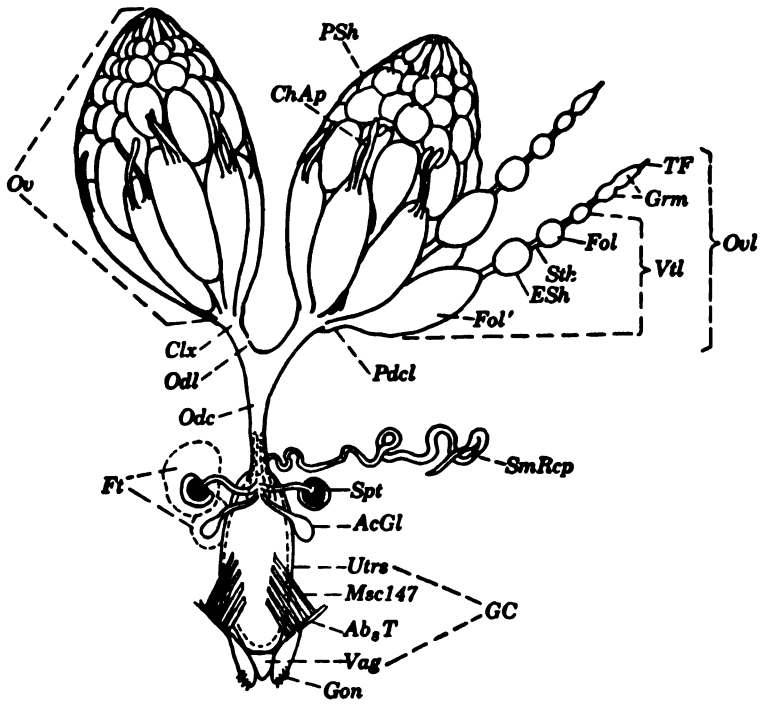
24 hour period. At the end of the mating period the flies were etherized, the females from each mating were divided into two groups of 25 flies and were placed in separate vials containing minimal medium, and the males were discarded. All of the vials were then coded by covering the labels and randomly assigning code letters to the vials. The groups were not decoded until all dissections made on a particular day were completed.

One hour after separating the males and females, one-half of the females from each mating were dissected and scored. Twenty-four hours after separation, the remaining half of the females from each mating were dissected and scored.

In Drosophila females, sperm are stored in three separate storage organs: the ventral receptacle and the two spermathecae (Figure 1). The ventral receptacle is the best organ for estimating quantity of sperm stored: the majority of sperm are stored there, the sperm are oriented longitudinally, and the walls of the ventral receptacle are transparent in contrast to the chitinous walls of the spermathecae which make observation of sperm difficult.

The ventral receptacle is easily removed intact from the female by placing an etherized female in a drop of saline on a slide, holding the female in place with a dissecting needle or fine forceps, placing the tip of another dissecting needle on the ovipositor, and gently pulling apart. This operation is carried out under low magnification using a stereomicroscope. Following dissection, a cover slip is placed on the saline preparation and the ventral receptacles are scored with a compound microscope at a magnification of 250X.

Figure 1. Reproductive system of female Drosophila.



Dorsal aspect of reproductive organs with two ovarioles separated from the ovary and the seminal receptacle uncoiled; egg in uterus and fat surrounding left spermatheca and accessory gland indicated by broken outlines. Ab_7S, seventh abdominal sternite; Ab_1T, Ab_8T, first and eighth abdominal tergites; AcGl, accessory gland; ChAp, chorionic appendage of egg; Clx, calyx; ESh, epithelial sheath; Fol, follicle; Fol', basal follicle; Ft, fat tissue; GC, genital chamber; Gon, gonopod; Grm, germinarium; Msc 14Z, ventral muscles of seventh abdominal segment; Msc 144, sternal muscles to uterus; Msc 147, tergal muscles to uterus; Odc, common oviduct; Odl, lateral oviduct; Ov, ovary; Ovl, ovariole; Pdcl, pedicel; PSh, peritoneal sheath; Rect, rectum; SmRcp, seminal receptacle; Spt, spermatheca; Stk, interfollicular stalk; TF, terminal filament; Utrs, uterus; Vag, (posterior) vagina; Vtl, vitellarium; Vul, vulva.

(from Miller, "The internal anatomy and histology of the imago of Drosophila melanogaster." in Biology of Drosophila, p. 520.)

Five categories were used in scoring the quantity of sperm in the ventral receptacle: category 0 corresponds to a complete absence of sperm, category 1 to from one spermatozoon to one-fourth fullness of the ventral receptacle, category 2 from one-fourth to one-half fullness, category 3 from one-half to three-fourths fullness, and category 4 from three-fourths to complete fullness.

Although the raw scores from the dissections of females from different matings can be compared, it is desirable to have some single figure representative of the pattern of scores which might be used in the comparisons. Such a representation, called the "insemination index" (I. I.), has been devised. (Yanders, unpublished). It is the sum of the multiple of the number of flies (n) in each category times the category number, all divided by the theoretical perfect score (four times the total number of flies dissected):

$$I.\ I. = \frac{n_0 \times 0 + n_1 \times 1 + n_2 \times 2 + n_3 \times 3 + n_4 \times 4}{4\,(n_0 + n_1 + n_2 + n_3 + n_4)}$$

Experimental I--Results

Part 1

In the first experiment, males from one of the four strains and females from each of the four strains were collected each week. Fifty females from each strain were mated to 100 males of the particular strain being tested that week. Matings, dissections, and scoring were carried out as described in the "Materials and Methods." This pro-

cedure was repeated until each of the strains of males had been mated with each strain of females. The whole procedure was then repeated so that for each of the 16 different crosses a total of 100 females was dissected. Since each mating was repeated at another time, it was possible to compare the scores and insemination indices to see whether they were reproducible. Dissections from each mating were made at one hour and 24 hours after termination of mating to determine whether any changes in the insemination scores or indices could be observed over that period of time.

A considerable variation in the score patterns and insemination indices was found in the different matings. A particular strain of males mated to the different strains of females resulted in different insemination indices. One strain of females when inseminated by different strains of males also resulted in different indices. Thus, it would seem that the quantity of sperm in the storage organs is not characteristic of the male alone or the female alone, but is dependent upon the male-female combination used in the mating.

Variation was also found in the changes in the insemination indices over a 24 hour period. In some crosses no change was found, while in other crosses a considerable change was noted. For example, matings between Swedish-B males and Canton-S females gave average insemination indices of 0.48 at one hour and 0.49 at 24 hours while matings between Oregon-R males and Canton-S females gave average insemination indices of 0.56 at one hour and 0.40 at 24 hours.

Comparisons of the indices in the two replicates of the experiment showed a degree of consistency in the indices for a particular

mating. Certain matings tended to result in comparatively high insemination indices while others gave consistently low indices. For example, matings between Canton-S males and Oregon-R females gave one hour insemination indices of 0.70 and 0.64 while matings between Swedish-B males and Oregon-R females gave one hour indices of 0.49 and 0.51. Table 1 gives the insemination indices for the reciprocal crosses of the four strains.

Comparisons of insemination score patterns also showed a fair degree of reproducibility considering the crudeness and subjectivity of the test. Figures 2 and 3 illustrate by means of bar graphs the patterns of insemination scores found in some of the crosses. By comparing the patterns of scores in dissections made at different times, it can be seen that the score patterns of a particular cross are similar.

Part 2

Since the first experiment showed that an insemination index was somewhat characteristic of the male-female combination, an experiment was devised in order to determine whether insemination index differences could be correlated with differences in the chromosome content of the males and females. Reciprocal crosses were made between Oregon-R and Swedish-B flies, and the F_1 hybrids from these crosses were used in further experiments.

The F_1 female hybrids from the reciprocal crosses will be the same in chromosomal constitution; each female will have one X-chromosome and one set of autosomes of Oregon-R origin and one X-chromosome and a set

Cross		1 Hour Dissection			24 Hour Dissection		
Male	Female	Replicate 1	Replicate 2	1 and 2	Replicate 1	Replicate 2	1 and 2
Oregon-R	Oregon-R	.76	.58	.67	.68	.62	.65
Oregon-R	Canton-S	.60	.52	.56	.38	.43	.40
Oregon-R	Crimea	.65	.52	.59	.54	.40	.47
Oregon-R	Swedish-B	.74	.57	.66	.76	.51	.63
Canton-S	Canton-S	.63	.41	.52	.57	.29	.43
Canton-S	Oregon-R	.70	.64	.67	.67	.62	.64
Canton-S	Crimea	.60	.58	.59	.46	.45	.46
Canton-S	Swedish-B	.66	.56	.61	.55	.71	.63
Crimea	Crimea	.53	.66	.60	.54	.60	.57
Crimea	Oregon-R	.64	.54	.59	.60	.58	.59
Crimea	Canton-S	.72	.65	.68	.58	.53	.56
Crimea	Swedish-B	.53	.67	.60	.67	.63	.65
Swedish-B	Swedish-B	.32	.48	.40	.43	.43	.43
Swedish-B	Oregon-R	.49	.51	.50	.55	.47	.51
Swedish-B	Canton-S	.44	.51	.48	.46	.52	.49
Swedish-B	Crimea	.54	.44	.49	.40	.60	.50

Table 1. Insemination indices from reciprocal crosses of four strains of Drosophila melanogaster

FIGURE 2: INSEMINATION SCORE PATTERNS FROM CROSSES BETWEEN OREGON-R AND SWEDISH-B — ONE HOUR DISSECTIONS

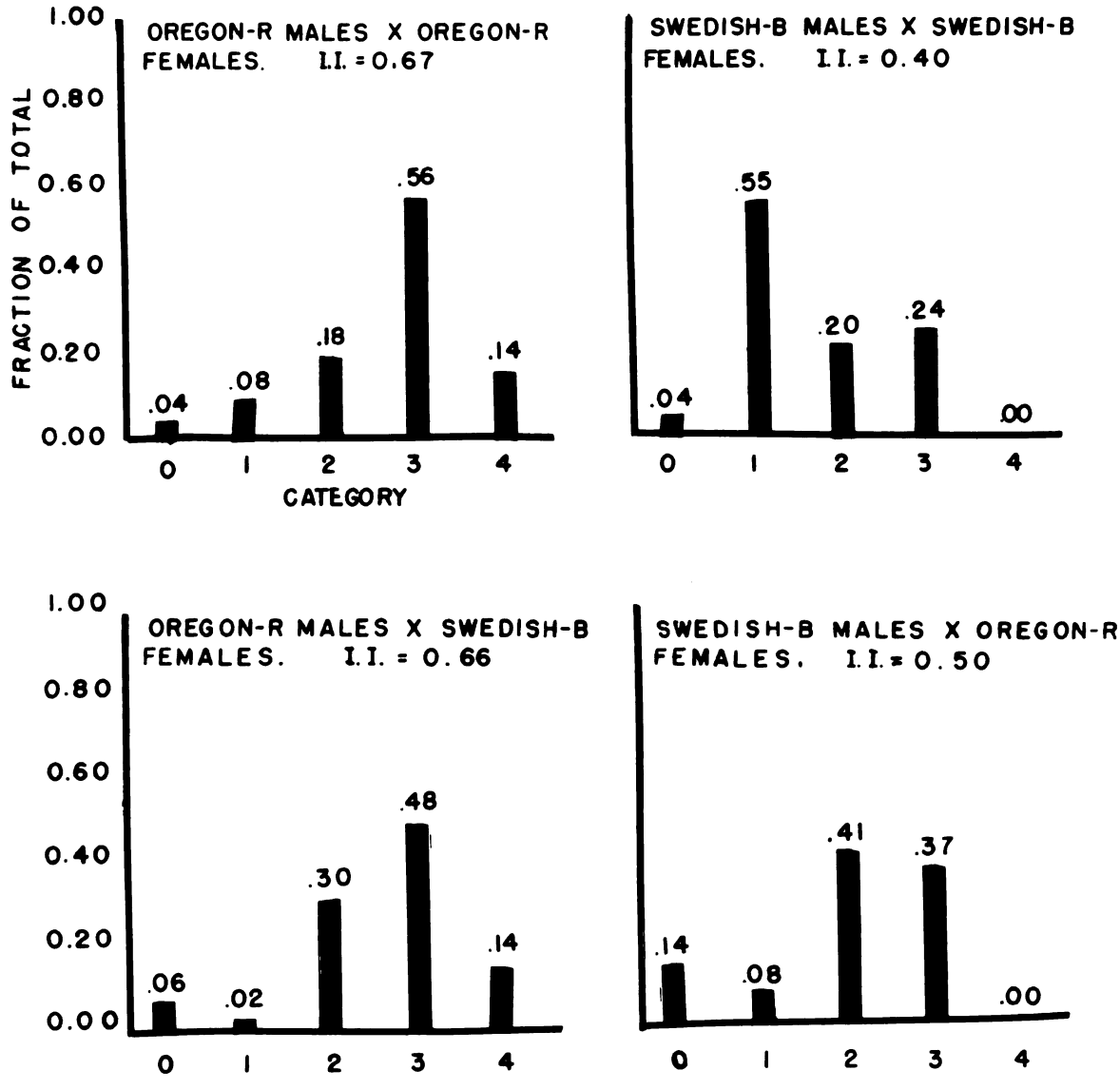
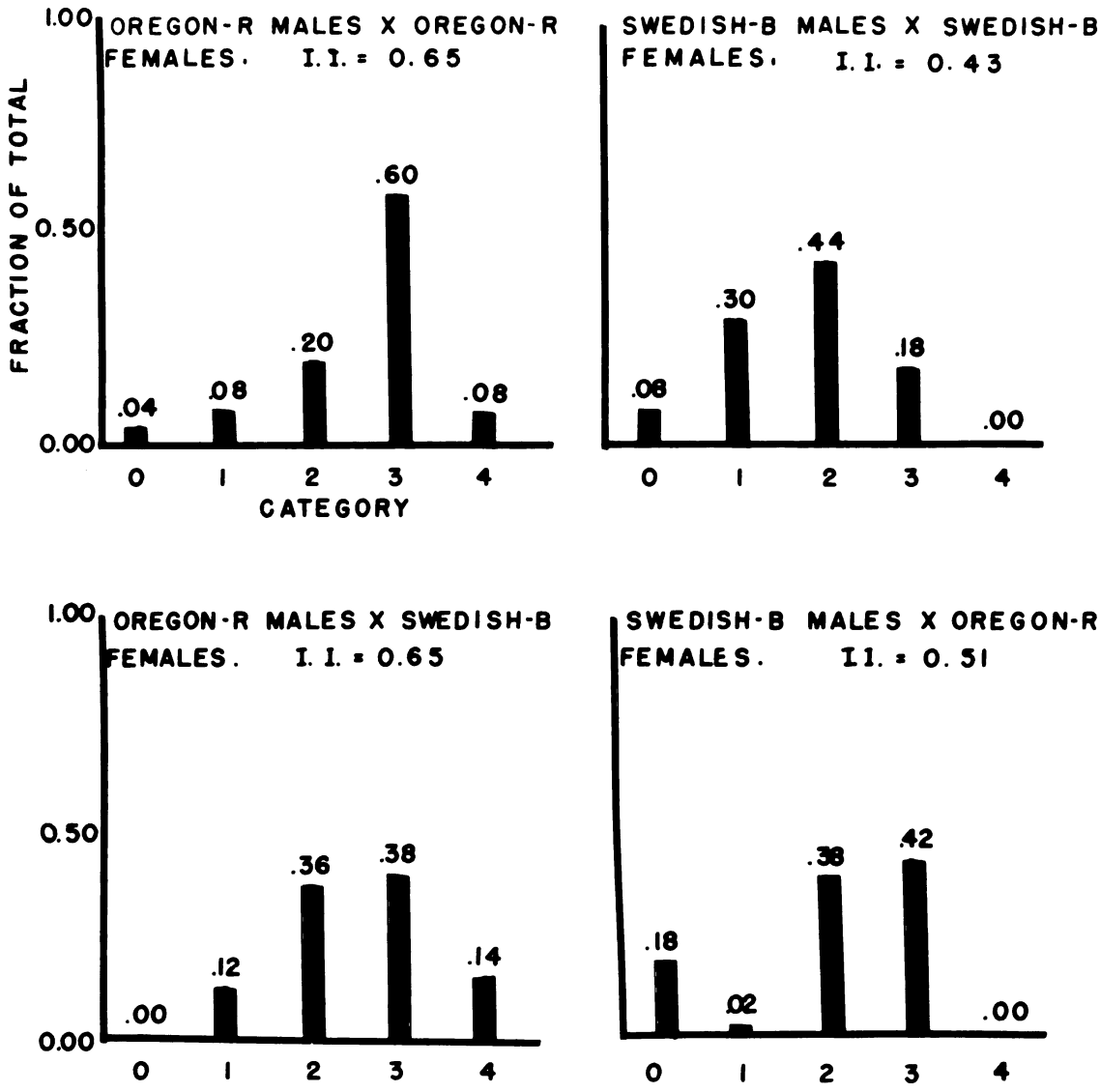


FIGURE 3: INSEMINATION SCORE PATTERNS IN CROSSES BETWEEN OREGON-R AND SWEDISH-B — 24 HOUR DISSECTIONS



of autosomes of Swedish-B origin. The F_1 male hybrids from the reciprocal crosses will differ in their chromosomal constitution. The cross Oregon-R females x Swedish-B males will result in F_1 hybrid males which have the X-chromosome and one set of autosomes from their Oregon-R mothers and the Y-chromosome and one set of autosomes from their Swedish-B fathers. This hybrid has been designated H_1. In the reciprocal cross, Swedish-B females x Oregon-R males, the F_1 hybrid males will have a Swedish-B X-chromosome and an Oregon-R Y-chromosome plus one set of autosomes from each parent. This hybrid has been designated H_2.

Reciprocal matings of Oregon-R, Swedish-B, H_1 and H_2 flies were carried out as in the previous experiments. Dissection and scoring were accomplished in the same manner as before. It was not found necessary to repeat this experiment as before, however, since the use of the F_1 hybrid females from each of the reciprocal crosses between Oregon-R and Swedish-B provided an internal check on the consistency of the test and, in addition, the insemination indices from the matings between Oregon-R and Swedish-B flies could be compared to those of the previous experiment. The insemination indices produced in these crosses can be found in Table 2. It can be seen that crosses involving Oregon-R, H_1 or H_2 males always give higher insemination indices than crosses involving Swedish-B males. Since the only characteristic common to Oregon-R, H_1 and H_2 males is the possession of Oregon-R autosomes, it would seem that greater quantities of sperm from male flies possessing Oregon-R autosomes enter or a greater number are retained in the ventral receptacles of the types of females used in these crosses.

Table 2. Insemination indices[1] from reciprocal crosses between Oregon-R, Swedish-B and F_1 hybrids[2]

		Females			
		Oregon-R	H_1	H_2	Swedish-B
Males	Oregon-R	.63	.77	.67	.62
	H_1	.69	.75	.72	.65
	H_2	.65	.73	.76	.71
	Swedish-B	.49	.63	.64	.40

One Hour Dissections

		Females			
		Oregon-R	H_1	H_2	Swedish-B
Males	Oregon-R	.64	.74	.78	.69
	H_1	.62	.70	.70	.69
	H_2	.72	.73	.74	.72
	Swedish-B	.53	.64	.68	.45

24 Hour Dissections

[1] Each insemination index is based on the dissection of 25 females.

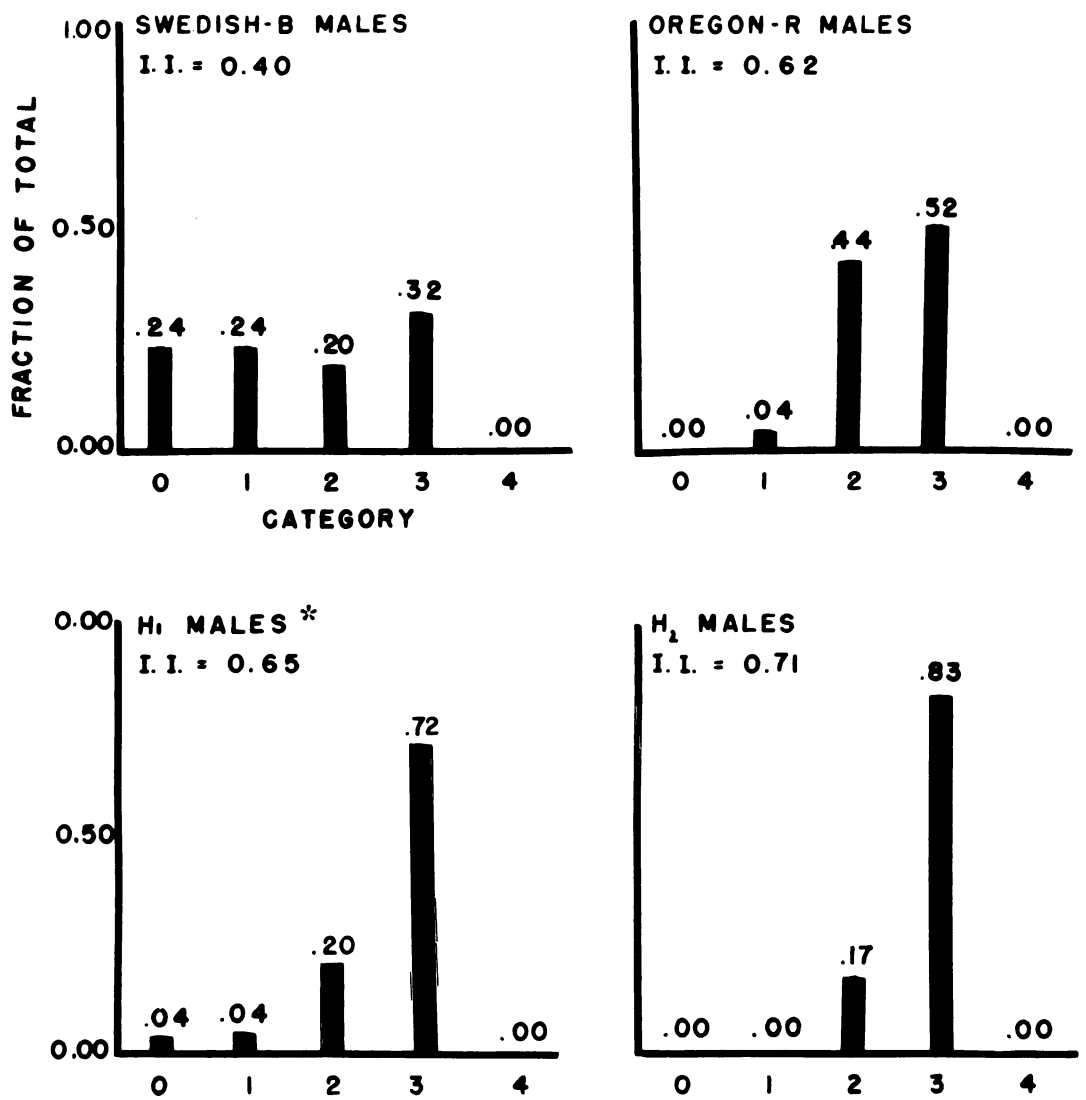
[2] Oregon-R females x Swedish-B males $\longrightarrow$ H_1
Swedish-B females x Oregon-R males $\longrightarrow$ H_2

An inspection of the insemination score patterns (Figure 4) of the four types of males when mated to Swedish-B females shows a greater similarity between the score patterns of Oregon-R, H_1 and H_2 males in comparison to the pattern found with Swedish-B males, particularly in regard to the two lowest scoring categories.

Discussion of Experimental I

It has been shown that the mating of one strain of males to the four strains of females results in different insemination indices. The mating of one strain of females with four different strains of males also results in different insemination indices. Several explanations may be proposed to explain these phenomena. The first possibility is that the number of sperm transferred in a particular mating depends upon the length of copulation and that this may vary from strain to strain. However, studies of the mating behavior in reciprocal crosses of these four strains (Yanders, unpublished) show little variation in duration of copulation and no correlation between the duration of copulation and the insemination index. Another possibility is that the number of sperm in the ejaculate of the male differs from strain to strain. That this is not the explanation is shown by the fact that a particular strain of males will give a high insemination index when mated to one strain of females and a low insemination index when mated to another strain. A third possibility is that some "incompatability" exists between the sperm and/or seminal fluids and the reproductive tract of the female. Certain types of sperm may be inactivated or adversely affected by some factor in the female repro-

FIGURE 4: INSEMINATION SCORE PATTERNS FROM CROSSES BETWEEN SWEDISH-B FEMALES AND FOUR STRAINS OF MALES



* Oregon-R females x Swedish-B males ⟶ H_1
Swedish-B females x Oregon-R males ⟶ H_2

ductive tract so that they do not reach the storage organs.

Effects of the type of female inseminated on the frequency of recovery of certain mutations and chromosome aberrations have been reported by several investigators. Hildreth and Carson (1957) found that spontaneous lethal frequencies in the X-chromosome from wild-type Samarkand males varied with the type of female mated to the Samarkand male. Bonnier (1954) and Lüning (1954) found differences in the frequencies of X-ray induced aberrations in the male gamete to be influenced by the strain of females to which the males were mated.

The interaction between the sperm and/or seminal fluids and the reproductive tract which these studies suggest may be related to the immunological types of reactions which are well documented in other organisms. Antigen-antibody reactions between gametes have been described in molluscs, annelids, echinoderms and chordates (C. B. Metz, 1955), and antibodies against sperm have been demonstrated in mammals (Hultin, 1959; Rümke and Hellinga, 1959; Baum, Boughten, Mongar, and Schild, 1961). The insemination reaction described by Patterson suggests that an immunological type of reaction might occur in the reproductive tract of female Drosophila.

An interaction between sperm and the female may also explain changes in the insemination index over a 24 hour period. If the environment of the storage organs is in some way detrimental to the sperm, over a period of time the sperm may become inactivated or killed and subsequently lost from the storage organs. This suggests that an investigation of sperm storage over longer periods of time might provide information on the behavior of sperm stored in the

females. Therefore, studies of sperm storage in one strain of females when inseminated by various strains of males were carried out and are described in the following section.

Experimental II--Studies of Sperm Storage

Materials and Methods

Oregon-R, Swedish-B, H_1 and H_2 flies were used in the following experiments. Flies were cultured, mated, dissected and scored in the same way as described for previous experiments. In these experiments, however, the females from each mating were divided into equal groups, placed in vials containing minimal medium, and stored for various lengths of time before dissection. The females were transferred to fresh vials every three or four days during their storage. In the first experiment the females were stored in a constant temperature room at 25° C. It was found that the flies could be maintained for only about three weeks on minimal medium at this temperature, so in the subsequent experiment the flies were stored in a cold room at about 12° C.

Experimental II--Results

Part 1

In the first storage experiment 100 virgin Swedish-B females were mated to 200 males of each strain. The females were separated from the males, and the females from each mating were divided into four groups of 25 flies. These females were then stored at 25° C. One group of 25 females from each mating was dissected at 24 hours after

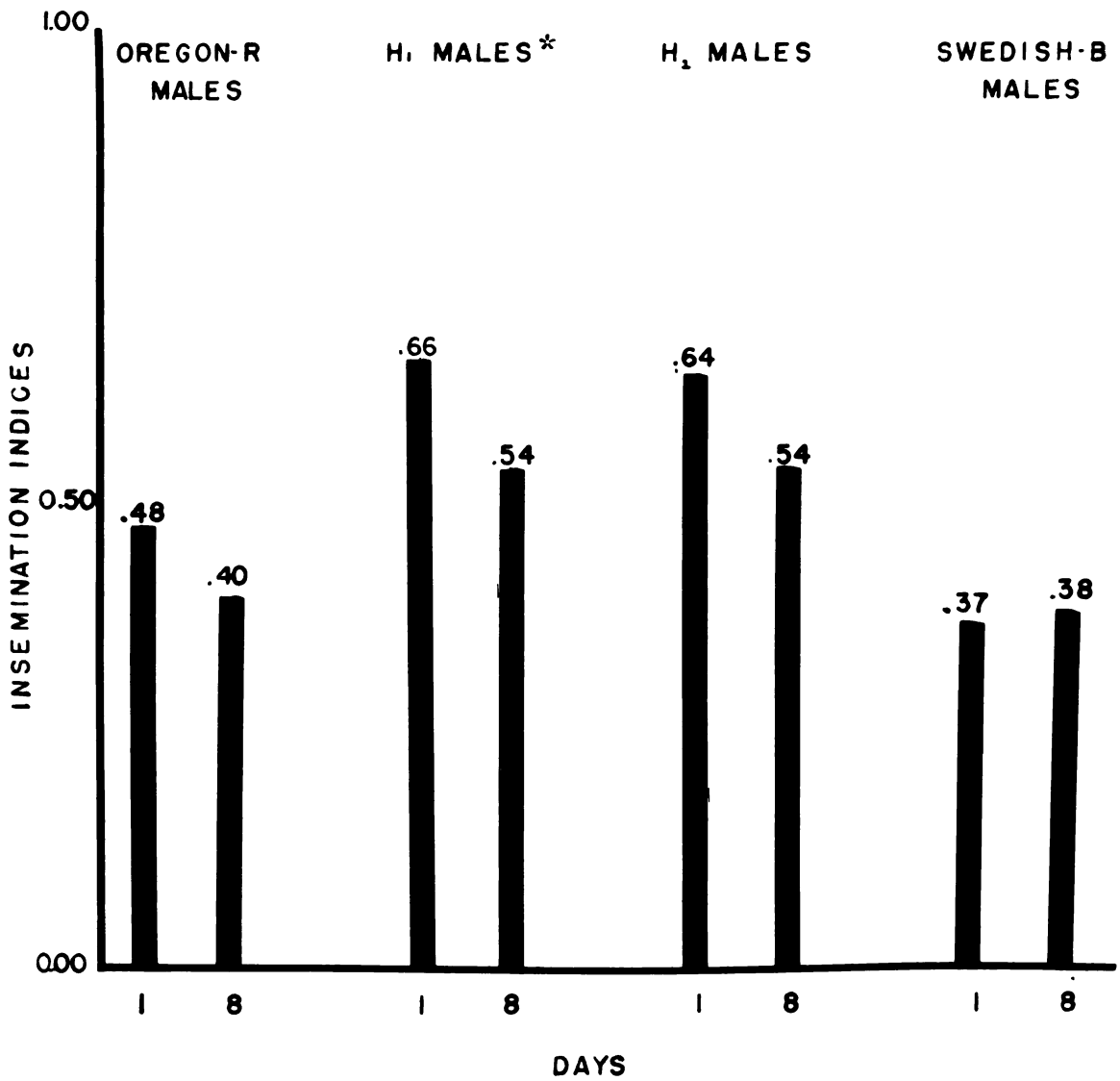
termination of mating. Another group from each mating was dissected at eight days after the mating. After this time the percentage of mortality of the females was so high that the experiment was terminated. The results of these dissections are graphically represented in Figure 5.

In the mating using Oregon-R males, the insemination index drops from 0.48 to 0.40 during the seven day period. Using H_1 males the change is from 0.66 to 0.54, and with H_2 males from 0.64 to 0.54. In the Swedish-B x Swedish-B mating there is no change during the seven days of storage, the insemination indices being 0.37 and 0.38. In this experiment, as before, it is noted that the hybrid males are more similar to the Oregon-R than to the Swedish-B males.

Part 2

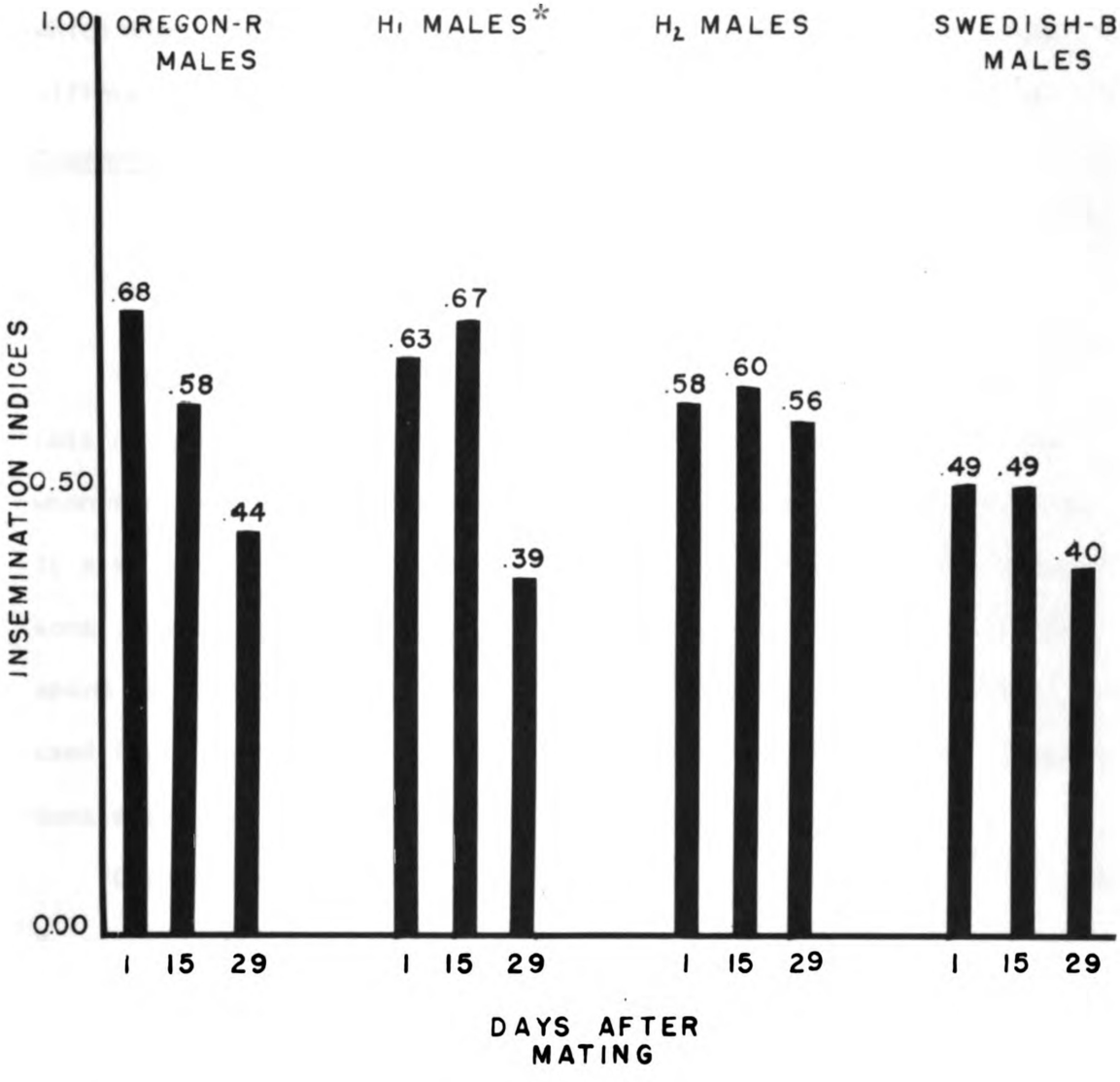
The second storage experiment was set up exactly like the first, except that the females were stored at 12° C. Since the metabolic rate of the flies is depressed at lower temperatures, the first group of females was dissected at 24 hours after mating as before, but the subsequent groups were dissected at 15 days and 29 days instead of at weekly intervals. The insemination indices (Figure 6) resulting from the mating with Oregon-R males show a gradual decline over the 29 day period similar to the decline shown in the 25° C. storage experiment. The index from the mating with H_1 males also shows a decline, but the drop in the insemination index does not begin until sometime after the fifteenth day of storage. A slight decline in the insemination index was noted when the females had been inseminated by Swedish-B males,

FIGURE 5: INSEMINATION INDICES OF SWEDISH-B FEMALES STORED AT 25° C



* Oregon-R females x Swedish-B males ⟶ H_1
Swedish-B females x Oregon-R males ⟶ H_2

FIGURE 6: INSEMINATION INDICES OF SWEDISH-B FEMALES STORED AT 12° C



* Oregon-R females x Swedish-B males ⟶ H_1
Swedish-B females x Oregon-R males ⟶ H_2

but little or no change when H_2 males were used. It may be noted that in this experiment the H_1 hybrid males, which have an Oregon-R X-chromosome in addition to a set of Oregon-R autosomes, were similar to the Oregon-R males, as in the first experiment; however, the H_2 males, which have only one set of Oregon-R autosomes, were not. It is difficult to compare the results of the two storage experiments since it is not known what the effect of temperature might be on any interaction which might take place between the sperm and storage organs. That different temperatures affect the expression of various traits in Drosophila has been widely reported.

Discussion of Experimental II

The results of the storage experiments show that the rates of loss of sperm from the storage organs of the females are different when the females have been inseminated by different strains of males. It also seems that the differences may be correlated with the chromosomal constitution of the male. The fact that the number of stored sperm is reduced over a period of time when certain types of males are used for insemination indicates some kind of incompatability between some sperm and the reproductive tract of the female.

Oliver and Anderson (1942) have reported a case of infertility in D. melanogaster due to the inactivation of sperm in the ventral receptacle. They found that when glossy females were mated to normal males and dissected at 24 hour intervals, the percentage of females with motile sperm dropped from 100% on the first day to 16% on the sixth day and to 0 on the seventh day. Over an eleven day period the

percentage of normal (wild-type) females with motile sperm in their ventral receptacles ranged from 93% to 100% throughout the test period. C. W. Metz (1956) found that when females heterozygous for autosomal lethals were mated with their heterozygous brothers, they laid only unfertilized eggs unless remated to suitable males of another composition. That the females contained stored sperm from the first mating was shown by the types of progeny recovered after the second mating. This evidence seems to indicate that inactivation of sperm may occur in the environment of the storage organs of some females.

It is not known by what mechanism loss of sperm from the storage organs occurs. Some sperm, of course, may be lost during egg laying. However, females raised on minimal medium lay few eggs, and low temperature storage also inhibits egg laying. In addition, since the females are all of the same strain, this should be fairly constant and would not explain the differences in rates of loss. It has also been shown (Yanders, unpublished) that females which have been irradiated seven days before mating with X-ray doses which cause degeneration of the ovaries, thus eliminating egg laying, still exhibit a loss of sperm from the ventral receptacles. It has been suggested (Guyenot, 1913) that sperm may be resorbed in the ventral receptacle, but in dissections of many females cellular debris (which might be expected if resorption is occurring) has not been noted. Lefevre and Jonsson (1962) have suggested that after insemination sperm may continually enter and leave the ventral receptacles; and that, finally, only the best oriented sperm are accommodated within the storage organs, and excess sperm may be found in the oviducts and vagina. It is possible

that sperm which have been rendered inactive are ejected or "leak out" from the ventral receptacle.

GENERAL DISCUSSION

In order to discuss the possible interaction which might occur between sperm and the reproductive tract of the female, as well as the implications of such interaction, it is pertinent to begin with a description of the female reproductive system of Drosophila (Figure 1). The first full account of the morphology and functioning of the reproductive organs in the Drosophila female was published by Nonidez in 1920. Descriptions have also been made by Sturtevant (1925-26), Miller (1950), and Patterson (1954). The following brief description is largely based on that of Patterson.

The female reproductive system of Drosophila occupies the posterior two-thirds of the abdomen and consists of the following organs. The paired ovaries are located at the anterior end of the tract. A lateral oviduct from each ovary joins to form the common median oviduct. The common oviduct is joined, at its posterior end, to the genital chamber, an elongated muscular chamber with a larger anterior portion, usually designated as the uterus, and a narrower posterior portion, the vagina. The ventral receptacle is a long coiled tube which arises from the ventral wall of the anterior end of the uterus. The end of the ventral receptacle proximal to the uterus has a very thick muscular wall and narrow lumen. The spermathecae are a pair of mushroom-shaped organs consisting of a brown, chitinous capsule and slender ducts which connect to the dorsal wall near the anterior end of the uterus. Finally a pair of accessory glands, the parovaria, lie

just behind the spermathecae and connect to the uterus by fine ducts.

During copulation the male deposits a large bundle of sperm, the spermatophore, in the genital chamber of the female. Kaufman and Demerec (1942) reported that the male might deposit as many as 4000 sperm in a single insemination. However, not all of these sperm will reach the storage organs of the female. Judging from the number of fertile eggs laid by a female after a single insemination (the greatest number of larvae emerging from eggs laid by a single female in Kaufman's [1942] study was 204) and even allowing for polyspermy, the number of stored sperm must be very much lower than the number originally received. Wheeler (1947) found that in D. melanogaster excess ejaculate material, including sperm, was expelled by the female at some time after copulation. Nonidez (1920) has described the ejaculate as being composed of spermatozoa suspended in a dense, sticky material secreted from the paragonia.

If the sperm reach the storage organs of the female by means of "swimming" alone, selection against less vigorous sperm may occur at this stage of the insemination process since only the sperm which reach the storage organs before the expulsion of the excess ejaculate will be available for fertilization. However, although Drosophila sperm in vitro are seen to undulate, they do not exhibit any forward movement (Yanders, unpublished). In mammals there is evidence that sperm play a very passive role during the passage from the site of deposition to the site of fertilization and that transport depends largely on the muscular contractions of the female reproductive tract (Austin and Bishop, 1957). In Drosophila the length of the journey

from the genital chamber to the storage organs is very short (less than the length of the sperm tail) in contrast to the length of the sperm's journey in mammals, suggesting that muscular contractions of the female reproductive tract may not be necessary for the transport of sperm in this organism. However, in many cases, the speed with which the sperm disentangle themselves from the spermatophore and enter the storage organs (sperm have been found in the storage organs in dissections made at less than one minute after the termination of copulation) may indicate that both sperm activity and muscular contractions of the reproductive tract play a role in sperm transport.

There is evidence in some species that physiological changes in the sperm take place after its introduction into the female reproductive tract. In some mammals it has been shown that sperm must reside in the female reproductive tract for a period of time before they attain their fertilizing capacity (Chang, 1951; Austin, 1952). Some cases of infertility, then, may be due to the failure of the environment of the female tract to induce the proper physiological changes in the sperm necessary for it to be capable of fertilization. In other cases aging of the sperm in the reproductive tract of the female may have a deleterious effect upon it. Decreased fertility resulted when sperm were aged in the reproductive tract in chickens, guinea pigs, and rats (Dharmajan, 1950; Nalbandov and Card, 1943; Soderwall and Young, 1940; Soderwall and Blandou, 1941). If the capacity of sperm to fertilize is dependent upon physiological interactions between the sperm and/or semen and the female reproductive tract, then physiological changes in either parent might cause some

modification of this interaction. If this is the case, then modifications in some progeny ratios due to aging of the parents, such as the change in the human sex ratio where a lower percentage of males is born to older parents and the change in sex ratio in Drosophila noted by Hannah (1955) due to aging of the maternal parent, may be the result of physiological changes in the parents caused by aging.

That a reaction between the ejaculate and the female reproductive tract does occur in Drosophila is evidenced by the insemination reaction. Patterson suggested that one possible function of this reaction might be as a sexual isolating mechanism preventing cross insemination between species; however, Wheeler (1947) in a study of seventy-eight species of Drosophila found that the reaction occurred in intra-specific matings as well as in inter-specific matings. He also found a range of differences in the severity of the reaction. Some species, including melanogaster, showed no apparent reaction while others showed reactions ranging from slight to severe. Wheeler points out that although no reaction is apparent in some species, changes in the vaginal mucous membrane may occur. The presence of the insemination reactions in intra-specific matings leads one to the conclusion that it is a normal part of the insemination process and may play some role in preparing the reproductive tract and/or the sperm for fertilization. The severe insemination reaction seen in matings between species may then be an exaggerated form of a normal condition. According to the fundamental immunological concept of "self-not-self," it is reasonable that heterologous sperm may be more "foreign" and induce a more violent reaction in the female.

Physiological changes in the female after insemination may be of a more generalized nature than the rather localized insemination reaction since mating is shown to stimulate egg production (Chiang and Hodson, 1950). Virgin Drosophila females may lay eggs, but egg laying increases greatly after copulation.

It would seem quite probable that interactions between the ejaculate and the female reproductive tract may play some role in the sperm's capacity to fertilize eggs, either by causing physiological changes in the sperm or by selecting against less vigorous sperm or sperm of certain antigenic constitutions.

This leads to the question of whether sperm differ in regard to their ability to fertilize. It is possible that differences in the genic constitutions of males may result in the production of sperm with physiological or antigenic differences. Shrigley (1940) observed that various types of abnormalities of sperm, occurring infrequently in both Pearlneck and Ring Doves, were increased markedly in the species hybrids. Further, the backcross hybrids which possessed Pearlneck specific antigens, and therefore at least some of the genes of Pearlneck, possessed more abnormal spermatozoa than backcross hybrids not having these genes. The motility of Drosophila sperm is also dependent upon the chromosomal make up of the male since males lacking a Y chromosome (XO) may produce two types of sperm, one containing an X-chromosome, the other without a sex chromosome (O-type). Both kinds of sperm are non-motile. Occasionally a normal XY male may produce an O-type sperm; these sperm are motile.

Differences in sperm can then be correlated with differences in

the genetic constitution of the male. Can there be differences in sperm due to their own genic content? Since sperm with deletions are able to fertilize eggs (Muller and Settles, 1927), it has been assumed that the genes in the spermatozoon are non-functional and that the ability of the sperm to fertilize is entirely independent of the gene content. However, the fact that sperm containing deletions are fertile is not compelling evidence that the genes are not functioning since genes may exert their specific action at different times during the developmental process. D. melanogaster zygotes deficient for chromosome blocks including the Notch locus and up to 45 further bands of X-chromosome are able to cleave, form blastoderm, and initiate organogenesis and histogenesis before the death of the embryo. Zygotes with the scute deficiency (up to 11 bands) develop into fully differentiated larvae before death. Therefore the loci missing in these particular deletions have no essential functions with respect to the developmental stages preceding the lethal effect (Hadorn, 1958). There is no way at present to demonstrate whether the genes in the sperm are active, and the condensed state of the chromosomes in the sperm would seem to preclude it. However, some genes in the Drosophila sperm are known to function immediately upon their introduction into the egg. The deep orange paternal gene becomes active in the egg system even prior to the formation of the zygotic nucleus (Counce, 1956), and the gene suppressor-of-erupt functions immediately after its introduction by fertilization (Glass and Plaine, 1950).

Studies of changes in DNA content upon aging of bull spermatozoa indicate that the genetic material in the sperm may not be wholly

inert (Salisburg, et al., 1961). Studies of the DNA content of mammalian sperm which are morphologically normal but unable to fertilize eggs also show that there may be a correlation between aberrant DNA content and the ability of the sperm to fertilize. However, this is not necessarily a correlation between genes and ability to fertilize (Leuchtenberger, Weir, Schrader, and Murmanis, 1955; Leuchtenberger, I. Murmanis, L. Murmanis, Ito, and Weir, 1956).

It is also possible that differences in sperm may be due to the accumulation of gene products in the cytoplasm during spermiogenesis, but prior to the final meiotic division.

In reference to the data from my experiments, it would seem that the differences found in the insemination indices in various crosses and the variation in rates of loss of sperm from the ventral receptacles may reflect a differential viability of different types of sperm within the reproductive tract environment of different types of females. However, whether this is due to a physiological inferiority of some sperm or an immunological reaction between sperm and female tract, and whether this is a reflection of the genetic constitution of the males and females or of the sperm cannot be determined from these experiments.

If gametic selection exists, it must represent a significant evolutionary force. If complete selection occurred against a completely recessive gene in the gametic state, the gene would be eliminated in one generation; but if zygotic selection occurs, only the homozygotes will be selected against, and genes in the heterozygous condition will be retained in the population. Also, gametic selection would not alter

the reproductive fitness of the population; in zygotic selection the number of progeny is reduced, but in gametic selection it is not, thus changing the genotypic makeup of the population more rapidly. Li (1955) has pointed out that the length of time required to effect a specific change in the frequency of a gene by a fixed low intensity selection is less for gametic than zygotic selection. It would seem that any phenomenon of such evolutionary importance should be more thoroughly investigated.

SUMMARY

One of the possible causes of aberrant progeny ratios may be gametic lethality or selection. In order to discover whether there is any evidence of a selective mechanism operating against male gametes stored in the female Drosophila melanogaster, studies of insemination and sperm storage in intra- and inter-strain crosses were carried out.

In reciprocal crosses between the four strains used in these experiments, it was noted that the quantity of sperm stored in the ventral receptacle of the female varied in the different crosses but seemed to be characteristic of the particular male-female combination. Studies of the rate of loss of sperm from the ventral receptacles over a period of time showed differences in the rates of loss when a strain of females was inseminated by different strains of males. The differences in quantity of stored sperm and differences in the rate of loss of sperm from the storage organs of the female suggest that there may be a differential viability of sperm within the environment of the female reproductive tract.

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