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# EFFECT OF AGE, REARING AND MATING EXPERIENCES ON FREQUENCY DEPENDENT SEXUAL SELECTION IN *DROSOPHILA PSEUDOOBSCURA*

BY

ANITA M. PRUZAN

A dissertation submitted to the Graduate
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Figure 2. Ratio of homogamic to heterogamic males observed mating with _Drosophila pseudoobscura_ Chiricahua females. Females were aged, exposed to males, exposed to copulating couples or allowed to mate prior to tests. Homozygous Chiricahua (CH) and orange-eyed (_or_, autosomal recessive) Standard _Drosophila pseudoobscura_ males were used.

# CHAPTER I

# INTRODUCTION

"I think it will be admitted by naturalists, without my entering on details, that secondary sexual characteristics are highly variable ... The cause of the original variability of these characters is not manifest; but we can see why they should not have been rendered as constant and uniform as others, for they are accumulated by sexual selection, which is less rigid in its action than ordinary selection, as it does not entail death, but only gives fewer offspring to the less favoured males." (Darwin, 1859).

Behavioral analyses of sexual selection have most often utilized species of the genus Drosophila for several reasons. Since the early nineteen hundreds, the "fruit fly" has been the organism of choice for laboratory genetics investigations, and much information has accumulated to date. Most species of Drosophila develop from egg to adult in less than two weeks, are both easy and inexpensive to rear, and under normal conditions a single gravid female can produce hundreds of offspring. The low chromosome number (most diploid numbers range from six to twelve), the ease of isolating mutants found in nature or produced in the laboratory, and the well-understood mechanics of heredity make it possible to obtain a large number of flies of "identical" genotype and thus to investigate the relationship between behavioral measurements and genetic background employing concrete, reliable and highly specialized experimental strategy. If a particular gene mutation is found to be related to a change in behavior, for example, use of genetic mosaics can localize the site of gene's action to a specific region of the fly (Hotta and Benzer, 1972). Further studies can specify the biochemical, morphological and/or developmental changes which intervene between the gene and the behavior.

Numerous studies have focused on courtship and mating behavior of Drosophila. Bastock and Manning (1955) and Manning (1959) described in detail the sexual behavior of *Drosophila melanogaster* and *Drosophila similans*. Spieth (1968, 1969 and references therein) studied the courtship and mating behavior in 200 species of Drosophila, including the entire nasuta subgroup. Brown (1964, 1965) described *Drosophila pseudoobscura* and its relatives; Ehrman and Strickberger (1960) presented a pictorial record of *Drosophila paulistorum* matings and Grossfield (1972) studied *Drosophila palustris*.

General patterns of courtship and mating behavior of Drosophila males emerge from the selected studies itemized above. A courting male circles a female, taps her with his forelegs, vibrating one or both wings, licks the genitalia of the female and finally mounts the receptive female. Permutations in this pattern between males of different species, between males of the same species who differ either in genotype or karyotype and between individual males provide the basis for sexual selection described by Darwin (1859) as "... the struggle between the individuals of one sex, generally the males, for the possession of the other sex."

Petit (1958) and Ehrman (1965) independently showed in *Drosophila melanogaster* and *Drosophila pseudoobscura* respectively, that sexual selection is, in some instances, dependent upon the proportion of males in a breeding situation or "frequency dependent." If two kinds of males, A and B, are present in different proportions in the observed population, then the number of matings per A individual is greater when A is rare, whereas B males are relatively more successful in acquiring mates when their kind is in the minority. Both Petit (1958) and Spiess (1974) have shown that the frequency of females is of relatively little importance in determining sexual selection; hence, this is sometimes also known as the"rare male mating advantage." The generality of the phenomenon has been documented in recent years for additional species of Drosophila. Spiess, for example, employed *Drosophila persimilis* homozygous for two autosomal inversions, Klamath (KL) and Whitney (WH), to show that the relative mating success of each type of male may be altered by varying their ratio; a clear advantage was awarded to the minority homokaryotype. Similar results were reported for other Drosophila species, namely *D. melanogaster*, *D. pseudoobscura*, *D. immigrans*, *D. persimilis*, *D. willistoni*, *D. tropicalis*, *D. equinoxialis*, and *D. funebris* (Spiess, 1968; Spiess and Spiess, 1969; Petit and Ehrman, 1969; Ehrman, 1970a, 1972; Borisov, 1970). The phenomenon of rare male advantage has also been reported in the flour beetle, Tribolium (Sinnock, 1970), and in the wasp Mormoniella, (Grant, Snyder and Glessner, 1974).

While relatively little is known about mating advantages of rare genotypes in natural environments, the few existing reports (Ehrman, 1970b, Borisov, 1970; Anderson, personal communication and Anderson, 1974) are of great evolutionary importance. If rarity confers a higher Darwinian fitness upon carriers of a given genotype (Anderson and Watanabe, 1974), the frequency of the initially rare type would be expected to increase until an equilibrium is reached. Frequency-dependent selection would in such cases, offer a supplementary explanation for maintenance of genetic and chromosomal polymorphisms in nature (Dobzhansky, 1970; Murray, 1972). Genetic diversity within a species will be preserved even if heterozygous individuals are not super or in fitness to homozygotes.

Rare male advantages in Drosophila depend in large part on the ability of males to copulate several times in succession, and are apparently lost when males remain _in copula_ for lengthy intervals of time (Ehrman, 1972a). Males will court females or any other object of appropriate size, until they find one which is receptive, and immediately after copulation will repeat courtship and mounting. Once a virgin Drosophila female has mated, however, she rejects for a time all further mating attempts. Reports of occasional repeated female matings, however, were confined mainly to _Drosophila melanogaster_ (Lefevre and Jonson, 1962). Recent work with _D. melanogaster_ (Fuerst, Pendlebury and Kidwell, 1973), _D. pseudoobscura_ (Dobzhansky and Pavlovsky, 1967), and _D. paulistorum_ (Richmond and Ehrman, 1974) indicate that multiple matings may occur more frequently than previously expected in the laboratory as well as in the field (Pyati, 1972; Crumpacker, Pyati and Ehrman, 1974). Studies with _D. melanogaster_ (Manning, 1962, 1967) have shown the inhibition of receptivity to be due to two components: (a) the mechanical effect of copulation itself, lasting up to 48 hours; (b) the effect ascribed to the presence of live sperm in up to three sperm receptacles. This latter effect wears off once a female exhausts her supply of sperm in egg laying and she is then ready to mate again.

The demonstration of the basis of female sexual responsiveness (Spieth, 1966; Grossfield, 1972; Ewing, 1967), the elucidation of the reproductive physiology of Drosophila (Patterson & Stone, 1952; King, 1970) and the well-documented existence of frequency-dependent sexual selection in a number of species of this genus make it possible to investigate experimentally the significance of such factors as age, rearing experiences and previous matings of females upon their subsequent choice of male mates.

# CHAPTER 2

# MATERIALS AND METHODS

The three strains of Drosophila pseudoobscura utilized here have been described in detail by Ehrman, Spassky, Pavlovsky and Dobzhansky (1965); Dobzhansky and Spassky (1962); Wright and Dobzhansky (1946); and Ehrman (1968a). Arrowhead (AR) and Chiricahua (CH) strains are wild types and homokaryotypic for their respective third chromosome inversions while the orange-eyed mutant, or, is homokaryotypic for the Standard gene arrangement and homozygous for an autosomal recessive gene altering eye color. Individual experiments employed either AR females and males confined with or males, or CH females and males with or males. The or males are easily distinguishable from either AR or CH males with the aid of a four-power hand lens.

Groups of females were either aged or given different experiences prior to direct observation in mating chambers originally described by Elens and Wattiaux (1964) and Ehrman (1965; see Ehrman, 1968b, for a description of their use). Virgin females were housed separately for three days, and were then subjected to one of the following conditions prior to direct-observation tests.

1. Females were allowed to mate with either homogamic males (AR females X AR males or CH females X CH males) or heterogamic males (AR females X or males or CH females X or males) for 24 hours in half pint bottles containing food, after which time the males were discarded and the females placed in individual vials containing food and left to oviposit for seven days. Presence of larvae in a vial indicated that mating had taken place, and only females producing larvae were used further. Prior to tests, the females were

removed without etherization from these vials and placed in half pint bottles containing food, approximately fifty females per bottle, for six hours. Results of tests with females so handled (previously called "widows," Pruzan and Ehrman, 1974) may be found under the heading "Mating Experience" followed by the karyotype or genotype of their first mates in Tables 1-7.

2. Females were placed on one side of a two-compartment cage containing food and separated from either homogamic or orange-eyed males. Pilot studies indicated that a fine micromesh divider between compartments prevented matings from taking place; however the openings were sufficiently large to allow light and airborne stimuli such as odor and vibration to pass through. Females and males often clustered on either side of the divider and were observed extending their probosces toward each other. It is assumed, therefore, that tactile stimulation was also possible. After 24 hours the males were discarded and approximately fifty females were placed in each half pint bottle containing food for seven days. Results of mating tests using these females are summarized under headings "Exposure to" followed by karyotype or genotype of males present in the cage in Tables 1-7.
3. Females were placed on one side of a two compartment cage (described above) and exposed for 24 hours to either homogamic or heterogamic females and males. The karyotype of females on both sides of the mesh was always the same.

Thus, if one side of the compartment contained virgin AR females, the other side contained either AR females and AR males or AR females and or males. After 24 hours these virgin females were placed in half pint bottles containing food, approximately fifty females per bottle, for seven days; the other flies were discarded. Results of mating tests employing these females are summarized under heading "Exposure to" followed by the karyotype and/or genotype of the couples present in Tables 1-4.

4. Because all the females under the above conditions were eleven days old at the time of testing, females in this group were aged in half pint bottles containing food for an equivalent length of time and served as a control group. Results of tests employing such females are summarized under the "11 day virgins" headings in Tables 1-7.
5. This group of females were four day old virgins. Because published reports documenting frequency-dependent mating selection have in the past used young virgins exclusively, females in this group served as an additional control.

"Female choice" experiments were conducted to evaluate the effects of age, previous mating and rearing experiences on frequency-dependent sexual selection. Twenty males and twenty females were placed into each observation chamber without etherization. The females exposed to conditions 1-4 above were 11 days old when tested, whereas all the males were four days old, and each chamber contained two types of males in varying proportions. Thus, AR females subjected to one of the pre-test conditions one through five above were observed with AR males and _or_ males in 1:1, 1:4 and 4:1 ratios respectively. Similarly, twenty CH females from each of the pre-test groups were observed with twenty males of CH and _or_ types in 1:1, 1:4, and 4:1 ratios per chamber. In all, AR females and CH females were each studied after eight types of experience, and the number of matings as well as the karyotype or genotype of male with which each female mated were noted for the three ratios of males present. All chambers were observed for three-hour periods.

# CHAPTER 3

# RESULTS

Tables 1 and 2 summarize the results of experiments in which 20 *Drosophila pseudoobscura* females and males were introduced into each observation chamber. The columns in each table represent the experimental manipulations administered to females prior to direct observation for mating preferences, as well as the ratio of the homogamic to heterogamic males present in each chamber. Rows labeled "♀ matings" give both the observed number of matings with males of each karyotype and numbers of expected matings if the males mated randomly, acquiring mates according to the frequencies of their genotypes or karyotypes present in the observation chambers. The percentage of females mating declined from a high of 90% for 4-day-old virgins, to approximately 60% for eleven-day virgins, and a low of 16% for females with previous mating experience. In order to obtain approximately fifty matings for each condition, numbers of replications were varied. Chi square values were calculated to indicate the significance of deviations in mating preferences which were observed from those that were expected.

Insert Tables 1 and 2 about here

Both Arrowhead (AR, Table 1) and Chiricahua (CH, Table 2) 4-day-old virgin females conferred a mating advantage upon those males which were in the minority, whereas males homogamic with the females mated more frequently than expected when the two types of males were present in equal proportions. Eleven-day-old virgin AR females awarded a mating advantage to AR males when they were either in the minority or present in numbers equal with *or* males, whereas mating was random when *or* males were in the minority. However, CH females of the same age(11-days), when tested with CH and *or* males, mated significantly more often with CH males whether rare, common or present in equal numbers when compared to *or* males.

Both AR and CH females who were exposed to airborne and tactile stimuli generated across a screen by either males alone or by females and males of their same type, yielded equivalent results when subjected to direct-observation tests for mate selection. For example, when AR females were exposed to AR males and then tested with AR and *or* males in a 1:1 proportion, AR males had a significant mating advantage ($p < 0.05$). Similarly, when AR ♀♀ were exposed to AR ♀♀ and ♂♂ AR males mated significantly more often than *or* males when both were equally numerous ($X^2 = 4.09$; $p<0.05$; Table 1, columns 4 and 5). The same relationship between $X^2$ values obtained from data concerning mating with females exposed either to males alone or to copulating couples was observed for AR and CH females regardless of the proportion of males present in these observation chambers. (Tables 1 and 2, columns 4, 5 and 7, 8).

When the effect of exposure was compared with the effect of no exposure, some differences in sexual selection became apparent. Eleven-day-old AR virgins conferred a highly significant mating advantage upon AR males ($X^2 = 20.64$; $p < 0.01$). After exposure to either *or* males or AR females plus *or* males however, similarly aged virgin AR females mated randomly ($X^2 = 0.32$ and 1.03 respectively). In both instances the observation chambers contained an equal number of AR and *or* males (Table 1; columns 2, 7 and 8; row 7).

Eleven-day-old virgin CH females mated more frequently with CH than with _or_ males whether the former were equal in number, common or rare ($X^2$ = 19.27, 5.96 and 5.90 respectively). CH females which were exposed either to CH males or to copulating CH couples mated randomly only when _or_ males were in the minority, whereas CH females exposed to _or_ males or to CH females plus _or_ males mated randomly in chambers containing all proportions of CH and _or_ males tested (Table 2, columns 2, 4, 5, 7 and 8).

Of particular interest are the results obtained when females had a previous mating experience when younger (see columns 3 and 6, Tables 1 and 2, for AR females and for CH females, respectively). Females awarded a mating advantage to males of the same karyptype or genotype as their first mate in chambers where these males were either equal in number or in the minority, and matings were random when the males encountered for the first time were rare. The effect is most pronounced in tests with females whose first mating experience was with _or_ males, because it is _only_ under these conditions that 11-day-old females confer a mating advantage upon _or_ males (Tables 1 and 2 column 6).

While $X^2$ values reported in Tables 1 and 2 measure departures from random mating here, they do not assess the degree of isolation or the relative mating advantage of the two types of males observed under each condition. At the suggestion of Professor Howard Levene, Departments of Biological Sciences and of Mathematical Statistics, Columbia University, log odds were used to evaluate the relative mating success between heterogamic and homogamic males. This measure, sometimes known as "lodds," is the natural logarithm of the ratio of homogamic to heterogamic males observed mating. In cases where the two types of males are present in different proportions, a correction factor for the unequal ratios is included:

where N = Total number of matings,
X = Number of homogamic males mating, and
Y = N-X = Number of heterogamic males mating,

then if the two types of males are present in equal numbers, the logarithmic index = In $\left(\frac{X}{Y}\right)$.

For unequal ratios of males present in the experimental population, the index becomes:

$$= \text{In}\left(\frac{X}{aY}\right)$$

$$= \text{In}\left(\frac{X}{Y}\right) - \text{In } a$$

where $a = \frac{\text{number of homogamic males present}}{\text{number of heterogamic males present}}$

and represents a correction factor.

$$\text{S.D.} = \sqrt{\text{variance}} = \pm\sqrt{\frac{1}{X} + \frac{1}{Y}}$$

Since log odds is an approximately normally distributed index for the determination of statistical significance, its value is converted to score:

$$Z = \frac{\text{log odds}}{\text{S.D.}}$$

Ratios of homogamic to heterogamic males which were observed mating expressed as a natural logarithm and the statistical significance of this Relative Mating Success index (RMS) between two types of males with the females present are itemized in Tables 3 and 4 for AR males with *or* males and for CH males with *or* males. Log odds values ± 2 S.D. are presented graphically in Figures 1 and 2, permitting a direct comparison of RMS values between the two types of males under different conditions. Positive log odds values indicate mating advantages for homogamic males, whereas the relatively greater success of *or* males is shown by negative values. When lines, each representing the log odds values and their standard error for different proportions of males per chamber, are clearly separated, the differences between isolation indices are significant. Lines which wholly overlap indicate no significant differences, but lines which overlap only partially need to be evaluated further statistically:

$$\text{Difference log odds} = \text{log odds condition}_1 - \text{log odds condition}_2$$

$$\text{S. D. of difference} = \sqrt{\text{var}_1 + \text{var}_2}$$

I. Data obtained using AR females with AR males and *or* males:

Insert Table 3 and Figure 1 about here

A. Effect of Age of AR females on Relative Mating Success index between AR and *or* males:

Table 3 shows that the Relative Mating Success index was highly significant ($p < 0.01$) when AR and *or* males were observed with 4-day-old virgin AR females for all three proportions of males tested (columns 2, 4 and 6, row 1). AR males were favored when they were in the minority or when they were common, whereas *or* males were more successful at mating only when they were rare (Figure 1). The difference between the Relative Mating Success indices for chambers containing 1:1 versus 1:4 proportion of AR to *or* males were nonsignificant ($z = 0.90$; $p > 0.05$), but each of these indices taken individually differed significantly from the Relative Mating Success index obtained when *or* males were rare (4:1).

A significant difference in relative mating success was found for matings with 11-day-old virgin females in observation chambers where AR males were either equal in number to *or* males or in the minority, and nonsignificant differences were recorded for chambers where *or* males were rare (Table 3, columns 2, 4, 6; row 2). The difference between Relative Mating Success indices for AR and *or* males were significant in comparison of chambers containing rare *or* males and chambers containing either equal numbers of these males or minority AR males. No significant differences were found between mating successes of AR males in 1:1 versus 1:4 proportions (Figure 1, 11-day-old virgins).

No significant differences were found when comparisons were made between relative success of AR and *or* males mating with 4-day-old virgin females versus 11-day-old virgin females, even in the case where *or* males were in the minority (this, despite the difference in $X^2$ values in Table 1, column 1 and 2, row 14 or Z values, Table 3, column 4, rows, 1 and 2).

B. Effect of exposure of AR females to either copulating couples or only to males on the Relative Mating Success index comparing AR and *or* males.

Comparisons between Relative Mating Success indices were made between AR and *or* males paired with 11-day-old virgin females versus females exposed to males only, and finally, between matings with AR females exposed to females and males versus females exposed to males only. Results summarized in Table 5a show no significant differences between values obtained after exposure to copulating couples versus exposure to males only. Since 11-day-old virgin females were housed together and were therefore exposed to other AR females, the effect of exposure can be attributed to the presence of only males. Further analyses were therefore made between the Relative Mating Success indices obtained from matings with 11-day-old virgin females and females exposed to either homokaryotypic or heterokaryotypic males.

Insert Table 5a about here

1) Exposure to AR males.

Both $X^2$ values (Table 1, columns 2 and 4) and log odds values (Table 3, rows 2 and 4) show similar trends in comparisons of mating behavior with 11-day-old virgins and AR females exposed to AR males. Matings were random when _or_ males were in the minority, and AR males were favored when they were either equally as numerous as _or_ males or were rare. Comparison of differences in log odds values between these conditions for 1:1, 4:1 and 1:4 proportions also showed no significant differences. In tests with 11-day-old virgins a significant difference in mating success was found between AR and _or_ males in 1:1 versus 4:1 proportions. This difference was obliterated after exposure to AR males (Table 3, columns 1 and 2, rows 2 and 4; see also Table 1, same conditions). Apparently, AR males lose some advantage when they are either equally numerous as _or_ males or when AR males are rare after females have exposure to AR males (Table 5, row 14).

Insert Table 5b about here

2) Exposure to or males.

Both the Relative Mating Success indices and $X^2$ values indicated random mating when or males were either equal in number or rare, (Table 3, columns 1, 3, row 7; Table 1, column 7) but minority AR males were favored. When comparisons were made between log odds values for matings with 11-day-old virgins and log odds values for matings with females exposed to or males for each of the 1:1, 4:1 and 1:4 proportions of males in the population, the advantage of or males increased in chambers containing the 1:1 proportion of males ($p < 0.01$), but showed a decreased advantage when or males were rare (Table 5b, rows 4-6).

A trend is apparent in experiments in which AR females were exposed to either AR males or to or males. Exposure to males prior to tests lowers the mating advantage of males of the same karyotype or genotype when they are in the minority.

C. Effect of prior mating experience of females on Relative Mating Success index of AR males and or males.

1) Mating experience with AR males.

Females which were permitted to mate with AR males and then tested with AR and or males, awarded a highly significant mating advantage to AR males when such males were either equal in numbers or rare, but not when they were in the majority (Table 1, column 3). Relative Mating Success index between AR males and or males was highly significant for the same ratios of males in the population as above, and nonsignificant when or males were rare (Table 3, row 3).

Results of statistical tests between log odds using 11-day-old virgin females and 11-day-old females with previous mating experiences showed no significant differences in index values (Table 5, rows 7-9). Significant difference in isolation existed between AR males and *or* males in 1:4 and 4:1 proportions, but this difference was smaller than that found between males in the same proportions as described above.

2) Mating experience with *or* males.

Orange-eyed males had a highly significant advantage as measured both by $X^2$ values (Table 1, column 6) and log odds values (Table 3, row 6) when they were equal in number to AR males and when they were rare, but matings were random when they were common. Comparison of log odds values calculated for matings with 11-day-old virgins and matings with *or* experienced females showed a highly significant difference within the 1:1 proportion of males, and no significant difference in chambers with 1:4 or 4:1 proportions of AR to *or* males (Table 5b, rows 12-14). The trend for all proportions was towards an increased advantage for *or* males tested with *or* experienced females (Figure 1). Therefore, the effect of a previous mating experience is to increase mating advantages for males of the same karyotype or genotype as the initial mate, both in the cases of *or* and AR males (note one exception however, when AR males are in 1:1 proportion).

For all conditions of AR females tested, the advantage of rare AR males can be summarized:

♀♀ experienced with *or* ♂♂ < ♀♀ exposed to AR ♂♂ < ♀♀ exposed to *or* ♂♂ < 11-day-old virgins < 4-day-old virgins < ♀♀experienced with AR

♂♂;

and the advantage of rare *or* males tested with AR females with various experiences can be summarized:

> ♀♀ experienced with AR ♂♂ < ♀♀ exposed to *or* ♂♂ < ♀♀ exposed to AR ♂♂ < 11-day-old virgins < 4-day-old virgins < ♀♀ experienced with *or* ♂♂.

It appears therefore, that rare males enjoy greatest advantage with females who had previously mated with males of the same karyotype or genotype as the rare one.

II. Data obtained using CH females with CH males and with *or* males.

Table 6 summarizes the differences in Relative Mating Success indices between CH and *or* males. Significant differences were found for comparisons between matings with 11-day-old virgins and females exposed to *or* ♂♂ (1:1) and 11-day-old virgins and females with initial copulatory experiences with *or* ♂♂ (1:1 and 4:1; rows 4, 10 and 12).

Rather than following the format for data analyses presented in part I, data obtained using CH females were compared directly with data obtained using AR females for each experimental condition as well as for each proportion of males present in the test situations (Table 7). Table 4 and Figure 2 show the Relative Mating Success indices for CH data only.

Insert Table 4, Table 6, Table 7 and Figure 2 about here

The only difference which was statistically significant was between matings with 11-day-old virgins where the heterogamic males were in the minority (Table 7, column 2, row 6). The Relative Mating

Success index between AR males and _or_ males was nonsignificant (Table 3, column 2, row 2, and figure 1), whereas CH males were favored over minority _or_ males in matings with CH females (Table 4, column 2, row 2, and Figure 2). Differences in Relative Mating Success indices between AR and _or_ males and CH and _or_ males for matings with females exposed to males, exposed to females and males, 4-day-old virgins, 11-day-old virgins and females with previous mating experience were nonsignificant for the three proportions of males tested.

# CHAPTER 4

# DISCUSSION

The generality of frequency-dependent sexual selection in Drosophila has been confirmed in a series of published reports (Spiess, 1974; Petit and Ehrman, 1969; Kojima and Yarbrough, 1967). Results obtained here using 4-day-old virgin Arrowhead and Chiricahua females of the often-described and employed *Drosophila pseudoobscura* (Ehrman, 1968a; Ehrman, Spassky, Pavlovsky and Dobzhansky, 1965; Dobzhansky and Spassky, 1962; Dobzhansky and Epling, 1944) concur. Homozygous Arrowhead and Chiricahua females conferred mating advantages on AR, CH and *or* males, but homogamic males had a mating advantage when they were equal in numbers to *or* males.

The trend in recent experiments has been to study the possible mechanism(s) by which rare male mating advantages are mediated. Ehrman (1969) used a "double chamber" to explore the role of olfactory stimuli as the basis for this discrimination. She found that the minority advantage disappears in the front part of a cage if a current of air is introduced into it which had first passed through a rear section containing courting and copulating couples of "rare" type. Further evidence suggestive of the fact that olfactory stimuli mediate male courtship and mating behavior has come from studies by Shorey and Bartell (1970). They found that a volatile sex pheromone produced by *Drosophila melanogaster* females stimulates males to courtship (see also Averhoff and Richardson, 1974). In addition, experiments conducted in our laboratory show that substances mediating rare male behavior between *Drosophila pseudoobscura* of the Arrowhead and Chiricahua strains are susceptible to chemical purification and analysis. Reliable changes in frequency-dependent sexual selection were obtained when the floor of the mating chamber was spotted with active substances extracted from whole flies (see Leonard, Ehrman and Pruzan, 1974 for a review and Leonard, Ehrman and Schorsch, 1974 for a description of the bioassay). Spotting with extract from Arrowhead males either abolished the mating advantage normally accorded rare Arrowhead males in chambers containing both Arrowhead and Chiricahua males or induced the rare male advantage for Chiricahua males in chambers containing equal numbers of these two strains. While extract obtained from Chiricahua males acted in a parallel way, there is evidence suggesting that the two substances differ chemically (Ehrman, 1972b); acetone solvent must be used to extract the active substance(s) from Arrowhead flies and petroleum ether is the solvent of choice for Chiricahua males.

The effect of different physical environments upon sexual selection have been widely investigated. For example, as early as 1945 Mayr and Dobzhansky described selective matings between strains of different geographical origins in _Drosophila pseudoobscura_ and _Drosophila persimilis_. Grossfield (1966, 1968) studied the relative importance of vibration and light, and Ehrman (1966) investigated the effects of temperature on sexual selection. She was able to induce a mating advantage for Arrowhead _Drosophila pseudoobscura_ originally collected at Mather, California by raising this strain at different temperatures: 16° and 25°C. Males raised at 16°C had a significant mating advantage over flies of the same strain raised at the higher temperature when they were in a 1:1 proportion. This advantage was erased however, when 25°C males were rare; matings here were random. Within the limits tested, flies raised at lower temperature are consistently larger and Drosophila females prefer larger males. It is evident therefore, that environmental factors have a great influence on sexual selection (for additional references see Petit, 1972).

Until now relatively little was known about the effects of prior sexual experiences participated in by Drosophila females on their subsequent choice of mates. A notable exception is the study by Dobzhansky and Pavlovsky (1967). They examined multiple matings in mass cultures using different strains of *Drosophila pseudoobscura*. Each type of female tested was confined with three types of males for five days, left to oviposit for five days, and again confined with the same three types of males for the second cycle of matings. Results based on examination of larval chromosomes of resulting progeny indicated that many of these females mated with the same type of male in their second mating cycle as they did during their initial mating cycle. Since the females were confined with males for five days during each of the two cycles, the possibility of more than one mating during each of the confinements could not be excluded.

Results obtained in this current study show that copulatory experiences prior to direct observation tests have a profound effect on frequency-dependent sexual selection. The phenomenon was most apparent when homozygous Arrowhead and Chiricahua females first mated with *or* males. Control Chiricahua females which were equivalently aged (11 days) but which had no prior experiences with males conferred mating advantages upon Chiricahua males over *or* males whether the latter were common, rare or equal in numbers. Arrowhead females which were 11-day-old virgins also mated preferentially with Arrowhead males when they were in the minority or in proportions equal to *or* males, but chose mates randomly when *or* males were rare. In contrast, females of the above strains whose first mating experience was with *or* males mated significantly above chance expectations with this type of male when they were in the minority or equal in numbers to homogamic males, and matings were random when homogamic males were rare.

In view of the important implications that frequency-dependent mating selection contains for evolutionary theories, equilibria between the gene frequencies of rare and common types would be reached much more rapidly if sexual selection is altered by prior mating experiences (Hedrick, 1972).

Tests with females who were exposed to but were not allowed to mate with males were conducted in order to evaluate the relative importance of these two experiences. Figures 1 and 2 show that males of the type to which the females were exposed lose advantages when rare in observation chambers, relative to matings with 11-day-old virgins, although the differences are not statistically significant. It appears therefore, that minority Arrowhead males enjoy their greatest advantage in observed matings with Arrowhead females in the following sequence:

♀♀ experienced with AR ♂♂ > 4-day-old virgins > 11-day-old virgins > ♀♀ exposed to *or* ♂♂ > ♀♀ exposed to AR ♂♂ > ♀♀ experienced with *or* ♂♂; and the advantage of rare *or* males tested with Arrowhead females with various experiences can be summarized:

♀♀ experienced with *or* ♂♂ > 4-day-old virgins > 11-day-old virgins > ♀♀ exposed to AR ♂♂ > ♀♀ exposed to *or* ♂♂ > ♀♀ experienced with AR ♂♂. When Chiricahua females with various experiences were observed with Chiricahua and *or* males the results showed the same trend.

Results obtained in this study therefore indicate that Drosophila females modify their behavior as a result of experience, and for some this is a sufficient although minimal definition of learning ( Lefrancois, 1972; Kendler, 1974). The question of learning in Drosophila, long debated (Yeatman and Hirsch, 1971) has recently been affirmed by Quinn, Harris and Benzer (1974) in a paper dealing with conditioned behavior in *Drosophila melanogaster*.

The reorganization of behavior as a result of copulatory experiences reported in this study, particularly evident when a female's initial mating is with a heterogamic male raise many questions which require further investigation. Experiments are now underway to test whether the effect can properly be called learning and concurrently to determine whether the muscular changes resulting from copulation with *or* males and/or the endocrine changes resulting from transfer of sperm are the necessary conditions for this phenomenon to take place.

# CHAPTER 5

# SUMMARY

Frequency-dependent sexual selection, documented in several *Drosophila* species, has previously been demonstrated using only young virgin females. This present study investigated the effects of age, copulatory experiences and previous exposure to males or females and males on minority advantages.

*Drosophila pseudoobscura* females homokaryotypic for Arrowhead (AR) or for Chiricahua (CH) third chromosome inversions were observed directly for sexual preferences in observation chambers containing both AR and orange-eyed (*or*, autosomal recessive) Standard males, or CH and *or* males respectively in 1:1, 1:4 or 4:1 proportions. Drosophila females comprising the two control groups were both virgins at time of test but one group was 4 days old while the second group consisted of females 11 days old. Females comprising the six experimental groups were also eleven days old when direct observation tests were conducted but were either exposed to, but prevented from mating with:

1. males of the same karyotype as the females (AR or CH males);
2. orange-eyed (*or*) males;
3. females and males of the same karyotype as the females (AR ♀♀ and AR ♂♂ or CH ♀♀ and CH ♂♂); or
4. mixed couples (AR ♀♀ and *or* ♂♂ or CH ♀♀ and *or* ♂♂).

Females in this group were allowed to mate and their copulatory experiences were of two sorts:

5. females were confined and allowed to mate with males of the same karyotype as the females; or
6. females were confined and allowed to mate with *or* males.

Unlike four-day-old virgin females which conferred mating advantages upon all minority males tested (AR, CH, and _or_) as well as upon males of the same karyotype as the females when the former were in 1:1 proportion, eleven-day-old virgin females mated more often with CH males whether the latter were in the minority, majority or equivalent in number to _or_ males ($p < .05$). AR females of the same age awarded a mating advantage to AR males when they were either in the minority or present in numbers equal with _or_ males.

No significant differences were found between the effects of exposure to females and males and exposure to males only. However, males of the karyotype or genotype to which females were exposed lost mating advantages when rare. Of particular interest were the results of direct observation tests where the females had copulatory experiences with _or_ males, for it is only in such cases that _or_ males had mating advantages with 11-day-old females. A Relative Mating Success index was described and utilized in these analyses. Results obtained in this study indicated therefore that sexual selection in _Drosophila pseudoobscura_ is both age and experience dependent.

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TABLE 1. *Number of* Drosophila pseudoobscura *female matings: Females varied according to age and previous experiences; all were homozygous Arrowhead. Males varied according to genotype, karyotype and proportions present. AR = Arrowhead;* or *= orange-eyed, homozygous orange (autosomal recessive) genotype, ST karyotype $\chi^2 = 3.84, p<0.05$; $\chi^2 = 6.65, p<0.01$. a = significant in favor of AR makes; b = significant in favor of* or *males. E = expected number of matings given panmixia. O = observed number of matings. Total number of observed matings = 1324.*

| Ratio of AR♂♂:or♂♂ | Measures | | | CONDITION OF FEMALES | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 4 day virgins | 11 day virgins | Mating experience AR♂♂ | Exposure to AR♂♂ | Exposure to AR♀♀ +AR♂♂ | Mating experience or♂♂ | Exposure to or♂♂ | Exposure to AR♀♀ +or♂♂ |
| 1:1 | Replicates | | | 3 | 4 | 10 | 3 | 4 | 15 | 4 | 4 |
| | % ♀♀ mating | | | 90 | 70 | 28 | 91 | 77 | 25 | 63 | 77 |
| | ♀ Matings | AR♂♂ | O | 37 | 45 | 40 | 37 | 35 | 18 | 23 | 35 |
| | | | E | 27.0 | 28.0 | 28.5 | 27.5 | 27.5 | 33.0 | 25.0 | 31.0 |
| | | or♂♂ | O | 17 | 11 | 17 | 18 | 20 | 48 | 27 | 27 |
| | | | E | 27.0 | 28.0 | 28.5 | 27.5 | 27.5 | 33.0 | 25.0 | 31.0 |
| | $\chi^2$ | | | 7.41 a | 20.64 a | 9.28 a | 6.56 a | 4.09 a | 13.64 b | 0.32 | 1.03 |
| 4:1 | Replicates | | | 3 | 4 | 7 | 4 | 4 | 8 | 4 | 4 |
| | % ♀♀ mating | | | 90 | 68 | 33 | 73 | 73 | 35 | 71 | 68 |
| | ♀ Matings | AR♂♂ | O | 35 | 41 | 39 | 46 | 46 | 34 | 49 | 43 |
| | | | E | 43.2 | 44.0 | 36.0 | 46.4 | 46.4 | 45.6 | 45.6 | 43.2 |
| | | or♂♂ | O | 19 | 14 | 6 | 12 | 12 | 23 | 8 | 11 |
| | | | E | 10.8 | 11.0 | 9.0 | 11.6 | 11.6 | 11.4 | 11.4 | 10.8 |
| | $\chi^2$ | | | 7.78 b | 1.02 | 1.25 | 0.02 | 0.02 | 14.75 b | 1.27 | 0.00 |
| 1:4 | Replicates | | | 3 | 6 | 7 | 4 | 4 | 9 | 4 | 4 |
| | % ♀♀ mating | | | 79 | 57 | 28 | 64 | 68 | 36 | 68 | 65 |
| | ♀ Matings | AR♂♂ | O | 20 | 28 | 22 | 16 | 19 | 18 | 19 | 17 |
| | | | E | 9.0 | 13.6 | 8.0 | 10.2 | 11.0 | 13.0 | 11.0 | 10.4 |
| | | or♂♂ | O | 25 | 40 | 18 | 35 | 36 | 47 | 36 | 35 |
| | | | E | 36.0 | 54.4 | 32 | 40.8 | 44.0 | 52.0 | 44.0 | 41.6 |
| | $\chi^2$ | | | 16.81 a | 19.06 a | 30.63 a | 4.12 a | 7.27 a | 2.40 | 7.27 a | 6.28 a |

TABLE 2. *Number of* Drosophila pseudoobscura *female matings: Females varied according to age and previous experiences; all were homozygous Chiricahua. Males varied according to genotype, karyotype and proportions present. CH = Chiricahua;* or *= orange-eyed, homozygous orange ( autosomal recessive) genotype, ST karyotype.* $\chi^2 = 3.84$, $p < 0.05$; $\chi^2 = 6.65$, $p < 0.01$. *a = significant in favor of CH males; b = significant in favor of* or *males. E = expected number of matings given panmixia. O = observed number of matings. Total number of observed matings = 1416.*

| Ratio of CH♂♂:or♂♂ | Measures | | | CONDITION OF FEMALES | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 4 day virgins | 11 day virgins | Mating experience CH♂♂ | Exposure to CH♂♂ | Exposure to CH♀♀ + CH♂♂ | Mating Experience or♂♂ | Exposure to or♂♂ | Exposure to CH♀♀ + or♂♂ |
| 1:1 | Replicates | | | 3 | 5 | 9 | 4 | 4 | 15 | 4 | 4 |
| | % ♀♀ Mating | | | 85 | 60 | 28 | 63 | 72 | 24 | 68 | 65 |
| | ♀ Matings | CH♂♂ | O | 36 | 47 | 38 | 35 | 39 | 27 | 28 | 25 |
| | | | E | 25.5 | 30.0 | 25.5 | 25.0 | 29.0 | 36.5 | 27 | 26 |
| | | or♂♂ | O | 15 | 13 | 13 | 15 | 19 | 46 | 26 | 27 |
| | | | E | 25.5 | 30.0 | 25.5 | 25.0 | 29.0 | 36.5 | 27 | 26 |
| | $\chi^2$ | | | 8.65[a] | 19.27[a] | 12.25[a] | 8.00[a] | 6.90[a] | 4.95[b] | 0.07 | 0.08 |
| 4:1 | Replicates | | | 4 | 8 | 11 | 4 | 4 | 15 | 4 | 4 |
| | % ♀♀ Mating | | | 86 | 53 | 25 | 72 | 75 | 23 | 63 | 63 |
| | ♀ Matings | CH♂♂ | O | 48 | 77 | 48 | 45 | 44 | 45 | 42 | 40 |
| | | | E | 55.2 | 68.0 | 43.2 | 46.4 | 48.0 | 55.2 | 40 | 40.8 |
| | | or♂♂ | O | 21 | 8 | 6 | 13 | 16 | 24 | 8 | 11 |
| | | | E | 13.8 | 17.0 | 10.8 | 11.6 | 12.0 | 13.8 | 10 | 10.2 |
| | $\chi^2$ | | | 4.70[b] | 5.96[a] | 2.67 | 0.21 | 1.66 | 9.42[b] | 0.50 | 0.08 |
| 1:4 | Replicates | | | 3 | 6 | 15 | 4 | 5 | 17 | 4 | 4 |
| | % ♀♀ Mating | | | 90 | 49 | 17 | 74 | 61 | 16 | 84 | 80 |
| | ♀ Matings | CH♂♂ | O | 19 | 19 | 18 | 13 | 18 | 14 | 12 | 15 |
| | | | E | 10.8 | 11.6 | 10.4 | 11.8 | 12.2 | 11.2 | 13.4 | 12.8 |
| | | or♂♂ | O | 35 | 39 | 34 | 46 | 43 | 42 | 55 | 49 |
| | | | E | 43.2 | 46.4 | 41.6 | 47.2 | 48.8 | 44.8 | 53.6 | 51.2 |
| | $\chi^2$ | | | 7.78[a] | 5.90[a] | 6.94[a] | 6.94[a] | 3.45 | 0.88 | 0.18 | 0.47 |

Table 3. Relative Mating Success index (RMS, described more fully in text) between Arrowhead (AR) and orange-eyed (*or*, autosomal recessive) Standard *Drosophila pseudoobscura* males observed mating with AR females. Females were aged, exposed to males, exposed to copulating couples or allowed to mate prior to direct observation tests. Negative RMS values = *or* males favored; positive RMS values = AR males favored. Calculated RMS values contain adjustment factors for the different proportions of males present in experimental populations. Z=1.96, $p < 0.05$; Z = 2.57, $p < 0.01$.

| | Proportion of AR to *or* males present in population | | | | | |
|---|---|---|---|---|---|---|
| Condition of ♀♀ | 1:1 | | 4:1 | | 1:4 | |
| | RMS ± S.D. | Z | RMS ± S.D. | Z | RMS ± S.D. | Z |
| 4-day virgins | 0.78± 0.29 | 2.69 | -0.78± 0.28 | 2.79 | 1.16± 0.30 | 3.87 |
| 11-day virgins | 1.41± 0.34 | 4.15 | -0.31± 0.31 | 1.00 | 1.03± 0.25 | 4.12 |
| Mating experience AR ♂♂ | 0.86± 0.29 | 2.97 | 0.49± 0.44 | 1.11 | 1.59± 0.32 | 4.97 |
| Exposure to AR ♂♂ | 0.72± 0.29 | 2.48 | -0.04± 0.32 | 0.13 | 0.60± 0.30 | 2.00 |
| Exposure to AR ♀♀ + AR ♂♂ | 0.56± 0.28 | 2.00 | -0.04± 0.32 | 0.13 | 0.75± 0.28 | 2.68 |
| Mating experience *or* ♂♂ | -0.98± 0.28 | 3.50 | -1.00± 0.27 | 3.70 | 0.43± 0.28 | 1.54 |
| Exposure to *or* ♂♂ | -0.16± 0.28 | 0.57 | 0.43± 0.38 | 1.13 | 0.75± 0.28 | 2.68 |
| Exposure to AR ♀♀ + *or* ♂♂ | 0.26± 0.26 | 1.00 | -0.02± 0.38 | 0.05 | 0.66± 0.30 | 2.20 |

Table 4. Relative Mating Success index (RMS, described more fully in text) between Chiricahua (CH) and orange-eyed (*or*, autosomal recessive) Standard *Drosophila pseudoobscura* males observed mating with CH females. Females were aged, exposed to males, exposed to copulating couples or allowed to mate prior to direct observation tests. Negative RMS values = *or* males favored; positive RMS values = CH males favored. Calculated RMS values contain adjustment factors for the different proportions of males present in experimental populations. Z = 1.96, $p < 0.05$; Z = 2.57, $p < 0.01$.

| | Proportion of CH to *or* males present in population | | | | | |
|---|---|---|---|---|---|---|
| Condition of ♀♀ | 1:1 | | 4:1 | | 1:4 | |
| | RMS ± S.D. | Z | RMS ± S.D. | Z | RMS ± S.D. | Z |
| 4-day virgins | 0.88 ± 0.31 | 2.84 | -0.56 ± 0.26 | 2.15 | 0.78 ± 0.28 | 2.79 |
| 11-day virgins | 1.29 ± 0.31 | 4.16 | 0.88 ± 0.37 | 2.38 | 0.67 ± 0.28 | 2.39 |
| Mating experience CH ♂♂ | 1.07 ± 0.32 | 3.34 | 0.69 ± 0.43 | 1.60 | 0.75 ± 0.29 | 2.59 |
| Exposure to CH ♂♂ | 0.85 ± 0.31 | 2.74 | -0.15 ± 0.31 | 0.48 | 0.12 ± 0.31 | 0.39 |
| Exposure to CH ♀♀ + CH ♂♂ | 0.72 ± 0.28 | 2.56 | -0.37 ± 0.29 | 1.28 | 0.52 ± 0.28 | 1.86 |
| Mating experience *or* ♂♂ | -0.53 ± 0.24 | 2.21 | -0.76 ± 0.25 | 3.04 | 0.29 ± 0.31 | 0.94 |
| Exposure to *or* ♂♂ | 0.07 ± 0.27 | 0.26 | 0.27 ± 0.39 | 0.69 | -0.14 ± 0.32 | 0.44 |
| Exposure to CH ♀♀ + *or* ♂♂ | -0.08 ± 0.28 | 0.29 | -0.10 ± 0.34 | 0.29 | 0.20 ± 0.30 | 0.67 |

Table 5a. Differences in Relative Mating Success (RMS) indices between Arrowhead (AR) and orange-eyed (*or*, autosomal recessive) Standard *Drosophila pseudoobscura* males observed mating with Arrowhead females. $Z = 1.96$, $p < 0.05$; $Z = 2.57$, $p < 0.01$. Ratio of AR to *or* males present in observation chamber: 1:1, 4:1, 1:4.

(a) Comparison between effect of exposure to females and males and exposure to males only.

| Female condition at mating | | | | AR: *or* ♂♂ | Differences in RMS ± S.D. | Z |
|---|---|---|---|---|---|---|
| 11-day virgins | vs. | Exposure to | AR ♂♂ | 1 : 1 | 1.41 - 0.72 = 0.69 ± 0.45 | 1.53 |
| " | vs. | " | AR ♀♀ + AR ♂♂ | 1 : 1 | 1.41 - 0.56 = 0.85 ± 0.44 | 1.93 |
| " | vs. | " | AR ♂♂ | 1 : 4 | 1.03 - 0.60 = 0.43 ± 0.39 | 1.10 |
| " | vs. | " | AR ♀♀ + AR ♂♂ | 1 : 4 | 1.03 - 0.75 = 0.28 ± 0.65 | 0.43 |
| " | vs. | " | AR ♂♂ | 4 : 1 | -0.31 + 0.04 =-0.27 ± 0.44 | 0.61 |
| " | vs. | " | AR ♀♀ + AR ♂♂ | 4 : 1 | -0.31 + 0.04 =-0.27 ± 0.44 | 0.61 |
| 11-day virgins | vs. | Exposure to | *or* ♂♂ | 1 : 1 | 1:41 + 0.16 = 1.57 ± 0.44 | 3.57 |
| " | vs. | " | AR ♀♀ + *or* ♂♂ | 1 : 1 | 1:41 - 0.26 = 1.15 ± 0.44 | 2.61 |
| " | vs. | " | *or* ♂♂ | 1 : 4 | 1.03 - 0.75 = 0.28 ± 0.37 | 0.76 |
| " | vs. | " | AR ♀♀ + *or* ♂♂ | 1 : 4 | 1.03 - 0.66 = 0.37 ± 0.39 | 0.95 |
| " | vs. | " | *or* ♂♂ | 4 : 1 | -0.31 - 0.43 =-0.74 ± 0.49 | 1.51 |
| " | vs. | " | AR ♀♀ + *or* ♂♂ | 4 : 1 | -0.31 + 0.02 =-0.29 ± 0.49 | 0.59 |

(Table 5b) Comparison between Relative Mating Success indices between AR and *or* males mating with 11-day-old virgin females and females with exposure or copulartory experiences.

| Female condition at mating | AR: *or* ♂♂ | Difference in RMS ± S.D. | Z |
|---|---|---|---|
| 11-day virgins vs. Exposure to AR ♂♂ | 1 : 1 | 1.41 - 0.72 = 0.69 ± 0.45 | 1.53 |
| " vs. " | 1 : 4 | 1.03 - 0.60 = 0.43 ± 0.39 | 1.10 |
| " vs. " | 4 : 1 | -0.31 + 0.04 =-0.27 ± 0.44 | 0.61 |
| 11-day virgins vs. Exposure to *or* ♂♂ | 1 : 1 | 1.41 + 0.16 = 1.57 ± 0.44 | 3.57 |
| " vs. " | 1 : 4 | 1.03 - 0.75 = 0.28 ± 0.37 | 0.76 |
| " vs. " | 4 : 1 | -0.31 - 0.43 =-0.74 ± 0.49 | 1.51 |
| 11-day virgins vs. Mating with AR ♂♂ | 1 : 1 | 1.41 - 0.86 = 0.55 ± 0.45 | 1.22 |
| " vs. " | 1 : 4 | 1.03 - 1.59 =-0.56 ± 0.40 | 1.40 |
| " vs. " | 4 : 1 | -0.31 - 0.49 =-0.80 ± 0.54 | 1.48 |
| 11-day virgins vs. Mating with *or* ♂♂ | 1 : 1 | 1.41 + 0.98 = 2.39 ± 0.44 | 5.43 |
| " vs. " | 1 : 4 | 1.03 - 0.43 = 0.60 ± 0.37 | 1.62 |
| " vs. " | 4 : 1 | -0.31 + 1.00 = 0.69 ± 0.41 | 1.68 |

Table 6. Differences in Relative Mating Success (RMS) indices between Chiricahua (CH) and orange-eyed (*or*, autosomal recessive) Standard *Drosophila pseudoobscura* males observed mating with Chiricahua females. $Z = 1.96$, $p < 0.05$; $Z = 2.57$, $p < 0.01$. Ratio of CH to *or* males present in observation chamber: 1:1, 4:1, and 1:4.

| Female condition at mating | CH: *or* ♂♂ | Difference in RMS ± S.D. | Z |
|---|---|---|---|
| 11-day virgins vs. Exposure to CH ♂♂ | 1 : 1 | 1.29 - 0.85 = 0.44 ± 0.44 | 1.0 |
| " vs. " | 1 : 4 | 0.67 - 1.22 =-0.55 ± 0.42 | 1.31 |
| " vs. " | 4 : 1 | 0.88 - 0.14 = 0.74 ± 0.49 | 1.51 |
| 11-day virgins vs. Exposure to *or* ♂♂ | 1 : 1 | 1.29 - 0.07 = 1.22 ± 0.41 | 2.98 |
| " vs. " | 1 : 4 | 0.67 + 0.14 = 0.81 ± 0.42 | 1.93 |
| " vs. " | 4 : 1 | 0.88 - 0.27 = 0.61 ± 0.53 | 1.15 |
| 11-day virgins vs. Mating with CH ♂♂ | 1 : 1 | 1.29 - 1.07 = 0.22 ± 0.45 | 0.49 |
| " vs. " | 1 : 4 | 0.67 - 0.75 = 0.08 ± 0.40 | 0.20 |
| " vs. " | 4 : 1 | 0.88 - 0.69 = 0.19 ± 0.57 | 0.33 |
| 11-day virgins vs. Mating with *or* ♂♂ | 1 : 1 | 1.29 + 0.53 = 1.82 ± 0.40 | 4.55 |
| " vs. " | 1 : 4 | 0.67 - 0.29 = 0.38 ± 0.42 | 0.90 |
| " vs. " | 4 : 1 | 0.88 + 0.76 = 1.64 ± 0.45 | 3.64 |

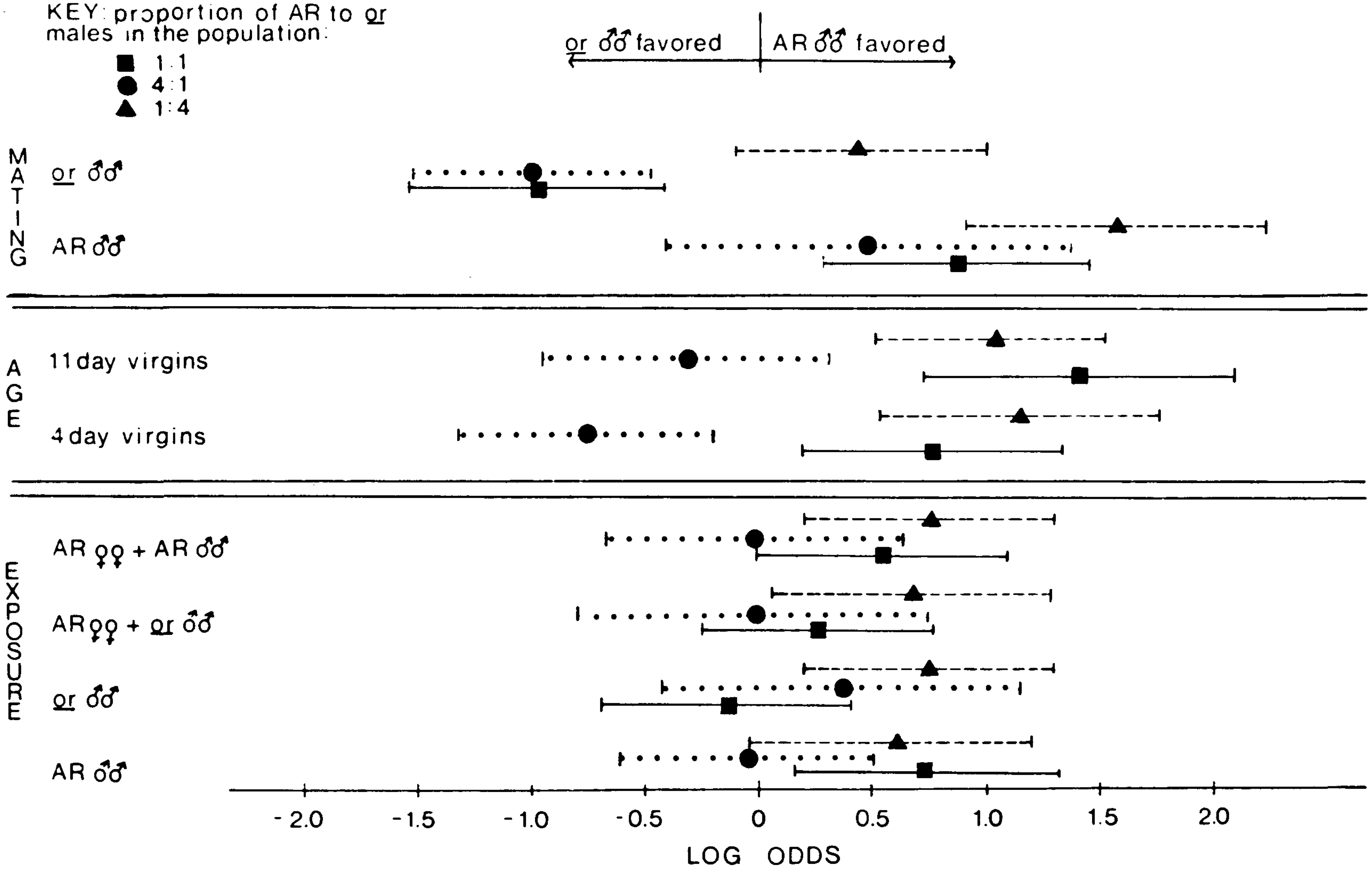
Table 7. Differences in Relative Mating Success index values between AR with *or* males and CH with *or* males, mating with *Drosophila pseudoobscura* AR and CH females respectively. Females were aged, exposed to heterogamic (He) and homogamic (Ho) males, exposed to copulating couples or allowed to mate prior to tests. Z = 1.96, $p < 0.05$; Z = 2.57, $p < 0.01$.

| | Proportion of Homogamic to Heterogamic males present in population | | | | | |
|---|---|---|---|---|---|---|
| 0 condition at mating | 1:1 | | 4:1 | | 1:4 | |
| | Difference in RMS ± S.D. | Z | Difference in RMS ± S.D. | Z | Difference in RMS ± S.D. | Z |
| Exposure to Ho ♂♂ | 0.72-0.85 =<br>-0.13 ± 0.42 | 0.38 | -0.04+0.15 =<br>0.11+0.44 | 0.25 | 0.60-0.12 =<br>0.48 ± 0.43 | 1.12 |
| Exposure to Ho ♀♀ + ♂♂ | 0.56-0.72 =<br>-0.16 ± 0.39 | 0.41 | -0.04+0.37 =<br>0.33 ± 0.43 | 0.77 | 0.75-0.52 =<br>0.23 ± 0.39 | 0.59 |
| Exposure to He ♂♂ | -0.16-0.07 =<br>-0.23 ± 0.39 | 0.59 | 0.43-0.27 =<br>0.16 ± 0.54 | 0.30 | 0.75-0.14 =<br>0.61 ± 0.42 | 1.45 |
| Exposure to Ho ♀♀ + He ♂♂ | 0.26+0.08 =<br>0.34 ± 0.38 | 0.89 | -0.02+0.10 =<br>0.08 ± 0.51 | 0.16 | 0.66-0.20 =<br>0.46 ± 0.42 | 1.10 |
| 4-day virgins | 0.78-0.88 =<br>-0.10 ± 0.42 | 0.24 | -0.78+0.56 =<br>-0.22 ± 0.38 | 0.58 | 1.16-0.78 =<br>0.38 ± 0.41 | 0.93 |
| 11-day virgins | 1.41-1.29 =<br>0.12 ± 0.46 | 0.26 | -0.31-0.88 =<br>-1.19 ± 0.48 | 2.48 * | 1.03-0.67 =<br>0.36 ± 0.37 | 0.97 |
| Mating experience Ho ♂♂ | 0.86-1.07 =<br>-0.21 ± 0.43 | 0.49 | 0.49-0.69 =<br>-0.20 ± 0.38 | 0.53 | 1.59-0.75 =<br>0.84 ± 0.44 | 1.91 |
| Mating experience He ♂♂ | -0.98+0.53 =<br>-0.45 ± 0.37 | 1.22 | -1.00+0.76 =<br>-0.24 ± 0.37 | 0.65 | 0.43-0.29 =<br>0.14 ± 0.42 | 0.33 |

# FIGURE CAPTIONS

Figure 1. Ratio of homogamic to heterogamic males observed mating with *Drosophila pseudoobscura* Arrowhead females. Females were aged, exposed to males, exposed to copulating couples, or allowed to mate prior to tests. Homozygous Arrowhead (AR) and orange (*or*, autosomal recessive) Standard *Drosophila pseudoobscura* males were used. Log odds = natural logarithm of ratio of males observed mating (AR matings / *or* matings) minus natural logarithm of ratio of males present (AR/*or*). Negative log odds values indicate *or* males are favored; positive values indicate that AR males are favored. Standard error bars indicate the 95% confidence level.

Figure 2. Ratio of homogamic to heterogamic males observed mating with *Drosophila pseudoobscura* Chiricahua females. Females were aged, exposed to males, exposed to copulating couples or allowed to mate prior to tests. Homozygous Chiricahua (CH) and orange-eyed (*or*, autosomal recessive) Standard *Drosophila pseudoobscura* males were used. Log odds = natural logarithm of ratio of males observed mating (CH matings/*or* matings) minus natural logarithm of ratio of males present (CH/*or*). Negative log odds values indicate that *or* males are favored; positive log odds values indicate CH males are favored. Standard error bars indicate the 95% confidence level.


KEY: proportion of AR to or males in the population:
■ 1:1
● 4:1
▲ 1:4
or ♂♂ favored
AR ♂♂ favored
MATING
or ♂♂
AR ♂♂
AGE
11day virgins
4day virgins
EXPOSURE
AR ♀♀ + AR ♂♂
AR ♀♀ + or ♂♂
or ♂♂
AR ♂♂
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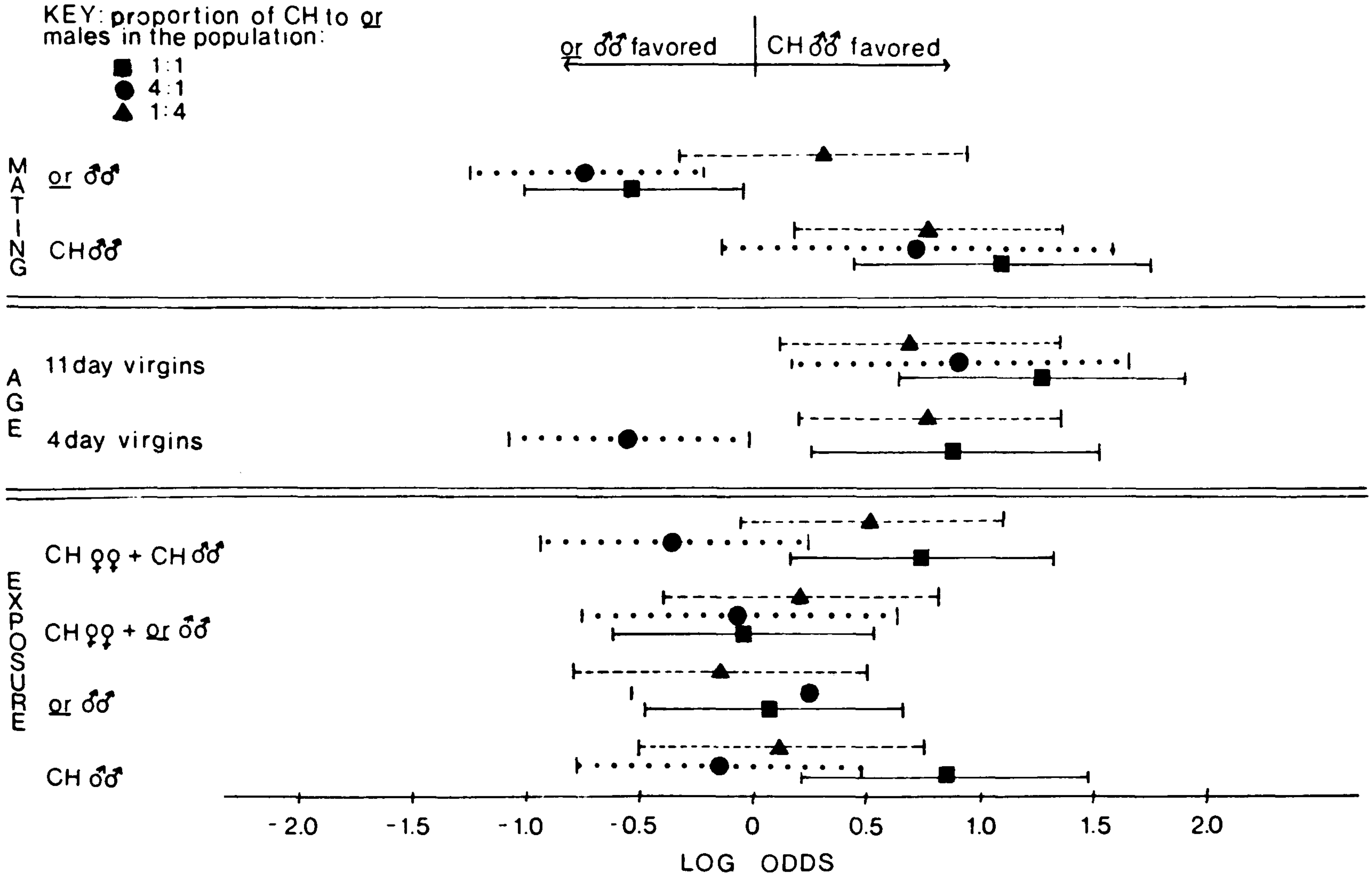
-1.5
-1.0
-0.5
0
0.5
1.0
1.5
2.0
LOG ODDS


KEY: proportion of CH to or males in the population:
■ 1:1
● 4:1
▲ 1:4
or ♂♂ favored
CH ♂♂ favored
MATING
or ♂♂
CH ♂♂
AGE
11day virgins
4day virgins
EXPOSURE
CH ♀♀ + CH ♂♂
CH ♀♀ + or ♂♂
or ♂♂
CH ♂♂
-2.0
-1.5
-1.0
-0.5
0
0.5
1.0
1.5
2.0
LOG ODDS