

A RARE-MALE ADVANTAGE IN THE HOUSEFLY INDUCED BY WING CLIPPING AND SOME GENERAL CONSIDERATIONS FOR DROSOPHILA

EDWIN H. BRYANT, AYKUT KENCE¹ AND KAY T. KIMBALL²

Department of Biology, University of Houston, Houston, TX 77004

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ABSTRACT

Multiple-choice crosses among five geographic strains of the housefly, *Musca domestica* L., were carried out in equal (10:10) and low-frequency (4:16) ratios. Initially, a low-frequency-male mating advantage was apparent, but further analyses related this minority advantage to a reduction of male mating success during marking by wing clipping. When there are fluctuating differences in the level of sexual vigor between competing male types over replicate trials of a cross, a mating advantage will accrue to the minority type. Even if males from the two competing strains are equally vigorous, such fluctuating differences will occur during sampling of flies. Harming the flies during marking will serve to enhance this effect and make significant departures toward greater mating success of rare males highly likely. This statistical bias in favor of minority males was substantiated in simulations of the KENCE-BRYANT model of mating success and compared with our results of a minority advantage in the housefly and with published results of a minority advantage in *Drosophila*. Our evidence, though circumstantial, that an advantage to minority males could have been induced by such an experimental bias suggests that a re-examination of existing data, as well as new experimentation, is necessary to discern whether or not a real rare-male advantage exists.

ONE of the more intriguing discoveries in experimental population genetics of *Drosophila* has been that a given male type enjoys greater mating success when rare than when common. This minority, or rare-male, advantage has been reported for *D. melanogaster* (PETIT 1958; TARDIF and MURNIK 1975; PETIT and NOUAUD 1975; MOLIN 1979; SPIESS and SCHWER 1978; SPIESS and KRUCKEBERG 1980), *D. pseudoobscura* (EHRMAN *et al.* 1965; EHRMAN 1966, 1967, 1968, 1970a; LEONARD and EHRMAN 1976; SPIESS 1968; FONTDEVILA and MÉNDEZ 1979; EHRMAN, ANDERSON and BLATTE 1977), *D. persimilis* (SPIESS and SPIESS 1969), *D. tropicalis*, *D. willistoni* and *D. equinoxialis* (EHRMAN and PETIT 1968; PETIT and EHRMAN 1968), *D. funebris* (BORISOV 1970), although possibly not for *D. immigrans* or *D. paulistorum* (EHRMAN 1971, 1972a). It has been less well documented for other insects, but an apparent low-frequency mating advantage has been reported for *Tribolium castaneum* (SINNOCK 1969, 1970) and

¹ Present address: Department of Life Science, Middle East Technical University, Ankara, Turkey.

² Present address: Southwest Research Institute, 3600 Yoakum Blvd., Houston, Texas 77006.

for the wasp *Mormoniella vitripennis* (GRANT, SNYDER and GLESSNER 1974; GRANT *et al.* 1980). If such frequency-dependent mating success is widespread among organisms, it would have special significance in population genetics as a mechanism for maintaining polymorphisms in natural populations without genetic load at equilibrium (*e.g.*, KOJIMA 1971) and for promoting outbreeding and exchange of genes among populations (AVERHOFF and RICHARDSON 1974; LEWONTIN 1974).

In carrying out mating tests among laboratory strains of the housefly, however, CHILDRRESS and McDONALD (1973) were unable to detect frequency-dependent mating success and our attempts with mutant strains of this species proved equally unproductive (BRYANT, unpublished). CHILDRRESS and McDONALD suggested that rare-male advantage may be elicited only in organisms with elaborate courtship patterns and not in organisms having simple mating behavior, such as the housefly (MURVOSH, FYE and LABRECQUE 1964; TOBIN and STOFFOLANO 1973) and *D. immigrans* (see also EHRMAN 1972a). Nevertheless, in studying mating among geographic strains of the housefly, we subsequently detected increased mating advantage of rare males (KIMBALL 1976). The apparent validation of a rare-male advantage in these studies when previous attempts had failed to do so for this species prompted us to consider possible differences among the various experiments that could have led to such disparate results.

The primary difference between the various tests on the housefly was the use of wild-type strains in our latter tests, while mutant marker strains had been used previously. This necessitated marking competing strains by the customary technique of clipping the distal margin of one wing on both sexes of one strain (*e.g.*, EHRMAN 1966). Preliminary tests indicated that this marking had no significant effect upon the performance of the flies. After examining the ensemble of all crosses in our experiment, however, it became apparent that the marked males (but not marked females) were invariably less successful than unmarked males of the same strain. This was rather surprising, but we assumed that this adverse affect of marking would be balanced over the strains when we rotated clipping from strain to strain over the replicate mating trials of a cross.

After further deliberation, we decided that alternating clipping between strains over replicates might not balance these adverse marking effects when the two competing male types were in unequal frequencies. A rare-male type can lose only a few potential matings when harmed during marking, while the potential loss to common males by such marking is much greater. This can be seen clearly if males were incapacitated by marking: at a low ratio of competing male types, say 2:18 pairs per strain, marked rare males would incur a loss of two potential matings (from random expectation), while marked majority males would lose up to 18 potential matings. If all females mated, the resultant mating success of rare:common males would be closer to 50:50 than the expected ratio of 2:18. When the effects of marking are less severe, a statistical advantage to minority males should remain, even though marking is rotated between strains. The advantage accrued to minority males would depend upon the magnitude of harm inflicted upon competing males while being marked for identification.

Considering the widespread acceptance of the minority mating advantage in genetics and population biology, we believe that exploring the possible inducement of such an effect through a bias in experimental design is warranted. The purpose of this paper is manifold: (1) To present an analysis of low-frequency-male advantage in multiple-choice crosses among geographic strains of the housefly, *Musca domestica* L., in relation to decreased performance of marked males. (2) To provide general computer simulation of the effects of alternately harming rare and common males upon resultant mating success of rare males by using the KENCE-BRYANT model of mating success in flies (KENCE and BRYANT 1978). (3) To compare results of these simulations with data on rare-male advantage in *Drosophila* from the literature.

MATERIALS AND METHODS

Multiple-choice crosses in the housefly: Flies were collected by sweep net from 5 localities throughout the western United States and returned to the laboratory to initiate stock cultures: Redding, California; Kingman, Arizona; Craigmont, Idaho; Osborne, Kansas; and Sheldon (Houston), Texas. After 4 to 5 generations in the laboratory, eggs for mating tests were collected by placing cups of CSMA larval medium into the cages for 6 hr (see BRYANT 1969 for medium preparation), randomized to break up individual egg batches and counted into sets of 80 eggs per 18 g CSMA larval medium for rearing at 27°. Emerging adults were collected at least every 12 hr and held separately by sex and strain for 4 to 5 days. All flies were then lightly anesthetized with CO₂, the end of 1 wing of both sexes of a strain was slightly clipped for identification and the males and females of a cross were placed on opposite sides of a 15 × 15 × 4 cm Plexiglass chamber. Flies were allowed to recover for at least 1 hr, and the partition separating the sexes was removed to observe subsequent matings for 3 hr by direct observation (*e.g.*, ELENS and WATTIAUX 1964).

Three multiple-choice observation chambers were set up simultaneously employing 20 pairs of flies per chamber in frequencies of 4:16, 10:10 and 16:4 pairs per strain, respectively. The 3 chambers constituted a replicate trial of a cross between 2 geographic strains and each such trial was repeated 5 times, totaling 100 possible matings for each frequency of a cross (assuming females mate only once). All possible pair-wise crosses among the 5 geographic strains were carried out in the morning with constant light at 27°.

The model: A detailed account of the KENCE-BRYANT model of mating success can be found elsewhere (KENCE and BRYANT 1978); only a brief summary is provided here.

Courtship involves the mutual exchange of excitatory and inhibitory signals between partners; the difference between partners determines the duration and eventual success of a courtship bout (BASTOCK and MANNING 1955; BASTOCK 1956; MANNING 1959; SPIETH 1952, 1974; TOBIN and STOFFOLANO 1973). A simple hyperbolic relationship was proposed between the linear differential of these signals and mating speed (time to mating, t), as $t = 1/(a_{\sigma} - a_{\phi})$, where a_{σ} and a_{ϕ} represent the intensity of courtship signals of the courting male and female respectively, and the linear differential of the signals the "effective excitation" of BASTOCK and MANNING (1955).

Since all males and females of a given genotype strain do not exhibit identical levels of sexual intensity, we assumed that variation among individual flies was normally distributed and that the mean intensity of signals for males and females was the same within a strain (although increased activity of males results in increased mating success, while in females it results in increased reluctance to mate). In the simulations, matings were accomplished by randomly drawing males and females from a specified normal distribution and the times to mating determined by the reciprocals of their effective excitations. In the model, mating was possible only when the male intensity exceeded that of the female, creating a balance between the sexes of a

genotypic strain whereby not all pairs of flies should mate. This concept of a balance between the sexes seems to be amply supported in the literature (*e.g.*, BASTOCK 1956; SPIETH 1968; BÖSIGER 1962, 1963, 1965; KENCE and BRYANT 1978).

Multiple-choice crosses were simulated by considering 2 competing strains with their respective means and standard deviations of sexual intensity. Males and females were randomly drawn from the appropriate distributions and the courtship times determined from their effective excitations; if these were negative or sufficiently small to prolong courtship beyond a typical observation period (3 hr), the courtships were terminated. Upon completion of a copulation, males were free to remate with remaining females; females, however, were limited to a single mating. A male could also be randomly paired with a female being courted by another male, and we allowed that a more vigorous male (higher intensity of signal) could usurp the accumulated courtship of a less vigorous male. The input to the program thus consisted of various numbers of males and females of each strain, their respective means and standard deviations of sexual vigor and the average copulation time.

Simulations of an induced minority advantage were carried out by alternately lowering the average signal intensity (vigor) of rare and common males over the replicates of a given cross. By varying the degree of rarity, mating speed (standard deviation of vigor; KENCE and BRYANT 1978; BRYANT 1980), copulation time and the degree of harm inflicted during marking, a response surface was obtained of the effect of these changes on the performance of minority and majority males.

The concept of intensity of signal (vigor) utilized here represents a general trait affecting mating success and would encompass a variety of actual behavioral signals, including visual (BASTOCK and MANNING 1955), auditory (BENNET-CLARK and EWING 1967, 1970) and pheromonal communication (CARLSON *et al.* 1971; ROGOFF *et al.* 1964; SHOREY and BARTELL 1970). Thus, we are simulating a general loss in sexual vigor during marking that affects the subsequent performance (mating success) of males in multiple-choice crosses.

RESULTS

Multiple-choice crosses of the housefly: The performance of the males of each geographic strain over the four crosses with the other strains is given in Table 1 as the percent mating by a given male type over the crosses, PETIT's coefficient of mating success for these males (see PETIT and EHRMAN 1969) and chi-square tests for departures from random mating. With HALDANE's correction for bias (HALDANE 1956), PETIT's coefficient of mating success is computed as

$$K = \frac{X/x}{(Y+1)/y}$$

where X , Y and x , y are the observed and expected (under random mating) numbers of matings by each male type, respectively. Under random mating, the expected value of K is 1.0; values greater or lesser than 1.0 indicate, respectively, greater or lesser mating success by male type X than expected under random mating.

The chi-square values in Table 1 indicate that Idaho, Kansas and California males showed significantly greater mating success than expected at low frequency. Texas males were significantly less competitive at equal and high frequencies, but were able to mate randomly over all crosses when they were rare. The relative performance of the males at the three frequencies is clearly indicated by the coefficients of mating success in column 3; there was always an increase in the mating success of low-frequency males in comparison with their

TABLE 1

Mating performance of males of each geographic strain pooled over the four multiple-choice crosses with the other strains

Geographic strain	Mating ratio	Total matings	Percent by males of this strain	Coefficient of male mating success	χ^2 (1 df)
Idaho	4:16	372	25.3	1.350	6.45**
	10:10	383	56.6	1.299	6.51**
	16:4	377	80.4	1.010	0.03
Kansas	4:16	359	27.9	1.538	13.84***
	10:10	382	54.2	1.176	2.68
	16:4	377	77.7	0.862	1.23
California	4:16	380	24.2	1.273	4.33*
	10:10	373	49.5	0.974	0.05
	16:4	361	74.6	0.728	5.68**
Arizona	4:16	385	20.4	1.021	0.06
	10:10	383	47.6	0.906	0.47
	16:4	365	78.9	0.921	0.46
Texas	4:16	382	18.1	0.879	0.90
	10:10	393	42.2	0.728	9.47**
	16:4	397	73.3	0.639	11.14***

Data from KIMBALL (1976). PETIT's coefficient of mating success with HALDANE's correction given in text. Significance levels are: * = $P < 0.05$; ** = $P < 0.025$; *** = $P < 0.01$.

performance at equal or high frequency. Hence, an apparent rare-male advantage was more-or-less present in each of the geographic strains.

Marking of males for identification impaired their subsequent mating performance, and we hypothesized that such a marking effect could have induced a rare-male advantage in our data. According to this hypothesis, rare males should lose fewer matings when marked than they are able to gain when common males are marked, creating an imbalance over the replicates of a cross in favor of rare males. In Table 2, we give the coefficients of mating success for marked and unmarked males for each strain in the low-frequency crosses, in comparison with the coefficients of mating success for equal frequency from Table 1. For each strain, the coefficient of mating success for marked males was nearer to that of the equal-

TABLE 2

Coefficients of mating success for marked and unmarked males of each geographic strain at low frequency (4:16) in comparison with mating success at equal frequency (10:10) from Table 1

Strain	Coefficients of mating success		
	4:16 (Marked)	10:10	4:16 (Unmarked)
Idaho	1.047	1.299	1.679
Kansas	1.118	1.176	1.984
California	1.073	0.974	1.507
Arizona	0.881	0.906	1.213
Texas	0.696	0.728	1.065
Average	0.963	1.017	1.490

frequency crosses than was the corresponding coefficient for unmarked males. The ratios of these coefficients indicate that, when rare males were marked, their success was nearly the same as that at equal frequency ($0.963/1.017 = 0.95$), while the success of unmarked males was almost $1\frac{1}{2}$ times that at equal frequency ($1.490/1.017 = 1.46$). The effects of marking were not symmetrical about the performance of males at equal frequency and hence, averaging mating success of marked and unmarked rare males would create an imbalance in favor of these minority males.

We might also expect that if the degree of harm inflicted upon males during marking varied over crosses, so might the degree of minority gain. This would cause an association between the differential performance of marked and unmarked males and the amount of minority gain. This can be investigated by looking at the mating success of unmarked and marked males in equal-frequency crosses and using this to predict mating success of males in the associated low-frequency crosses. For the 20 crosses among our geographic strains, we plot in Figure 1 the ratio of the coefficients of mating success for marked *versus* unmarked males of a cross at equal frequency against the ratio of the coefficients of mating success for males of the same cross at low *versus* equal frequency. There was considerable variation both in the degree of harm inflicted and in the minority gain among the crosses, but overall there was a significant association between them ($r = 0.53$; $P < 0.05$). Thus, the minority advantage we observed in our housefly data was not only related to an imbalance in the effect of marking, but also the degree of gain was related to the level of harm inflicted upon the males. Both of these factors offer strong evidence that the minority advantage was induced by our marking procedure.

Computer simulations: Simulations with the KENCE-BRYANT model of mating success were utilized to determine a more general relationship between the effects of marking and minority advantage. The average sexual vigor of competing males was alternately lowered for rare and common males, and the subsequent advantage of rare males was determined over a series of replicate trials with 100 simulated mating pairs per trial. Since the initial sexual vigor of competing males was the same, deviations from random mating in favor of rare males can be attributed to the simulated effect of marking.

Figure 2 gives the simulated success of males for various degrees of rarity in the population and for various combinations of parameters in the model. Figure 2A shows mating success of minority males over a range of copulation times, for a fixed level of harm of one standard deviation of sexual vigor. Figure 2B gives the effects of different degrees of harm inflicted for a fixed copulation time of 10 min. In all cases, alternately harming rare and common males resulted in *an increase in the relative performance of rare males*. The magnitude of advantage increased with shorter copulation time and with the degree of harm inflicted upon the competing males. There was a clear differential advantage over the range of frequencies with the greatest deviations from random mating occurring at intermediate frequencies (30% to 40%). These proportional deviations from random expectation are greatest before a given male type becomes too rare in the population;

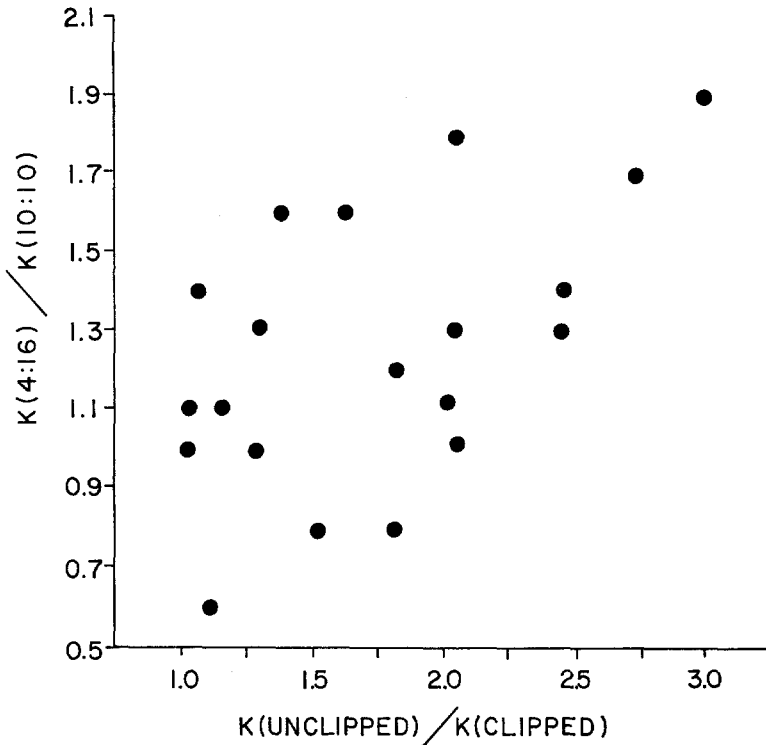


FIGURE 1.—Relative mating success of unmarked and marked males, expressed as the ratio of their coefficients of mating success, and the resultant minority advantage of these males, expressed as the ratio of their coefficients of mating success at low (4:16) and equal (10:10) frequency, for the 10 crosses among the geographic strains of the housefly. PETER's coefficient of mating success with HALDANE's correction factor is given in the text. Correlation between the marking effect and the minority gain was 0.53, $P < 0.05$.

however, from the statistical viewpoint, the chi-square values for these deviations (dividing by the expectation) would be greatest at the lowest frequencies.

Is the minority gain in the housefly consistent with the observed effect of marking? The average mating success of marked males was 77% of their unmarked performance, corresponding roughly to a loss in sexual vigor near 0.5 standard deviation units (KENCE and BRYANT 1978, Figure 5). The expected percent mating by minority males for 20% frequency, a marking effect of 0.5σ and a copulation time of 10 min would be about 30% from Figure 2B. However, this calculation does not account for the longer copulation time for the housefly.

In other simulations, we observed that the separate effects of copulation time and degree of harm were reasonably additive upon the resultant magnitude of rare-male advantage. The minority advantage for a copulation time of 70 min *versus* 10 min from Figure 2A at 20% frequency would be approximately $30\%/40\% = 0.75$. Thus, the expected proportion of matings by rare males would be $30\% \times 0.75 = 22.5\%$. This corresponds to the observed performance of rare housefly males of 23.2%, from the average percentage mating at 4:16 in Table 1,

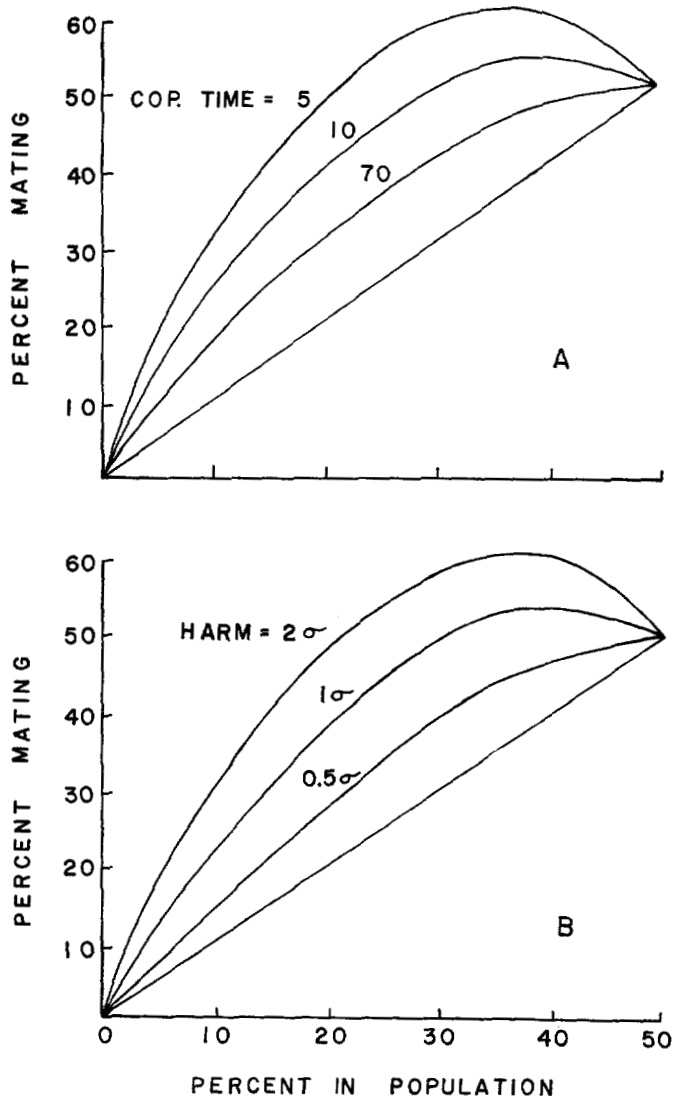


FIGURE 2.—Computer simulations of the percent mating success of competing males in multiple-choice crosses over a range of frequencies of male types. Figure 2A gives the mating success of males over a range of copulation times for a fixed level of putative harm inflicted during marking of one standard deviation unit of vigor. Figure 2B gives mating success of males for varying degrees of harm inflicted during marking (in standard deviation units of vigor) for a fixed copulation time of 10 min. In all simulations, rare and common males were alternately depressed in their sexual vigor and the resultant mating success of the rare males summed over a series of such replicated mating trials. All strains had the same initial level of sexual vigor and the standard deviation of vigor (mating speed) was set at 0.10, a typical level of this parameter among flies (BRYANT 1980). Quadrant bisector gives random expectation.

column 2. These data show an excellent correspondence between the average minority gain and that predicted by our model based upon the observed degree of harm inflicted on males during marking.

General considerations for Drosophila: The question arises as to whether the generally accepted rare-male advantage in *Drosophila* might also be induced by marking procedures. Without a close scrutiny of the individual trials alternately marking each type of male for the published reports of the minority advantage, this cannot be easily ascertained. Even so, a magnitude of harm from 0.5 to 1.0 standard deviation units of sexual vigor, sufficient to induce a rare-male advantage, would be difficult to detect in individual trials with the number of mating pairs normally used in these tests. Assuming a normal distribution of sexual vigor, for example, more than 60 marked and unmarked males, respectively, would have to be compared to detect a marking effect of 0.5 standard deviation units. As we did for the housefly in Figure 1, the overall performance of marked and unmarked males may have to be compared over an entire experiment. As an alternative, we ask here whether the *pattern* of mating success over a range of input frequencies of males for published *Drosophila* data is consistent with our simulations, for appropriate combinations of parameters.

To conform to our simulations, mating tests should have the following characteristics: (1) The males of the two competing strains should have equivalent mating success when equally frequent. (2) Mating tests should be carried out over a wide range of input frequencies of both male types. (3) Mating tests should use wild-type strains requiring identifying marking. The data by SPIESS and SPIESS (1969, Table 1) on *D. persimilis* and by EHRMAN (1967, Table 1: positive geotactic strains) on *D. pseudoobscura* meet these criteria. The performance of one of the male types over the range of input frequencies is given for each data set in Figure 3, along with the predictions based upon the simulations of Figure 2. For these comparisons, a copulation time of five to 10 min and marking effects of 0.75σ (*D. persimilis*) and 1.0σ (*D. pseudoobscura*) were assumed.

The correlations between the observed and predicted performances of the males were 0.94 for *D. persimilis* ($P < 0.001$) and 0.83 for *D. pseudoobscura* ($P < 0.01$). The estimated minority advantage for both species is therefore consistent with a gain induced by a marking effect of one standard deviation of vigor or less. The particular estimates of harm were chosen to give the best overall fit to each data set and thus the fit may be less than convincing. However, this level of harm seems reasonable, given the more complex courtship pattern of these flies in relation to that of the housefly, where we observed a marking effect of 0.5σ . Far more interesting, perhaps, is the nonlinearity of the response at intermediate frequencies in both data sets predicted by the model. This is not inherent in existing models of frequency-dependent mating success (see DISCUSSION) and is evidence that the minority advantage in these data may have been elicited by a marking effect.

If the rare-male advantage in *Drosophila* is indeed based upon alternately harming rare and common males, such an effect should not be present when mutant and wild strains not requiring marking are utilized. In general, this seems to be true. The pioneering work of PETIT (1951, 1954, 1958) on frequency-

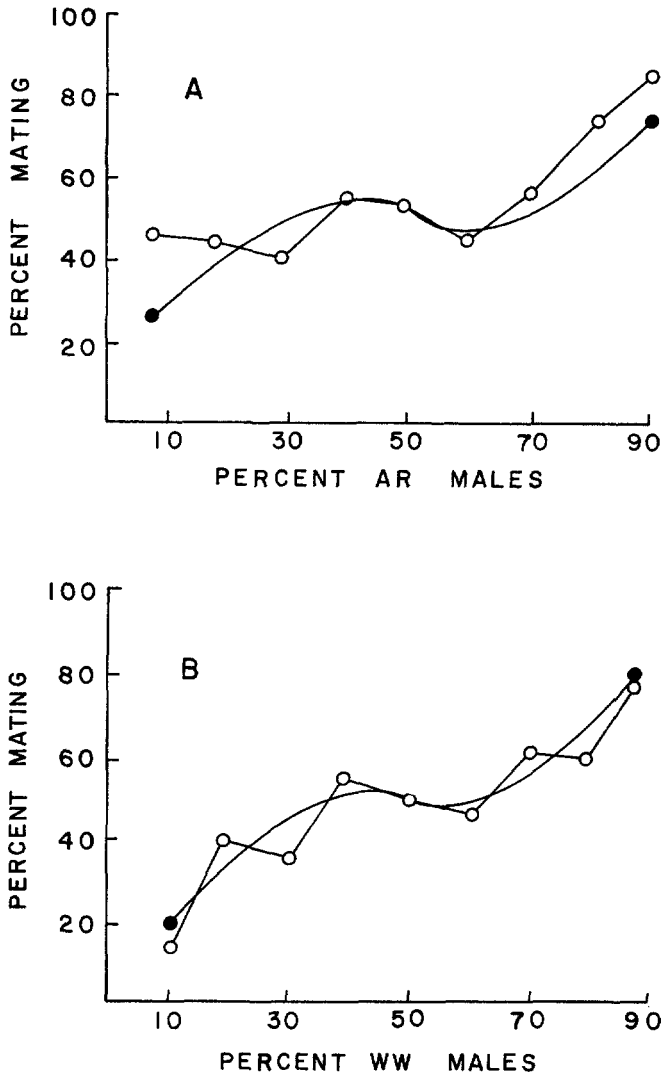


FIGURE 3.—Goodness-of-fit of the model of alternately harming rare and common males over a range of input ratios (●—●) to the empirical data of EHRMAN (1967) for *D. pseudoobscura* (Figure 3A, ○—○), and of SPIESS and SPIESS (1969) for *D. persimilis* (Figure 3B, ○—○). The clipping effect was assumed to be 0.75σ for *D. persimilis* and 1.0σ for *D. pseudoobscura*. Correlation between simulated and observed male performance was 0.83 for *D. pseudoobscura* ($P < 0.01$) and 0.94 for *D. persimilis* ($P < 0.01$).

dependent mating success in *D. melanogaster* displayed a more complex response than an advantage to rare males (e.g., white showed greater mating success at low as well as at high frequency). MARKOW (1978) and MARKOW *et al.* (1980) were unable to demonstrate minority success among mutant genotypes of *D. melanogaster*, and ANDERSON and MCGUIRE (1978) found no consistent deviations from panmixia by analyzing offspring of *D. pseudoobscura* karyotypes from large ex-

perimental populations, even though a minority advantage has been found previously when such karyotypes are marked for direct observation (*e.g.*, EHRMAN *et al.* 1965). Finally, in the same study, KLOBUTCHER (1977) did not find a significant rare-male advantage using mutants of *D. pseudoobscura* in competition with wild type, but did find minority mating success in parallel tests using karyotypes of this species that required marking for identification. On the other hand, EHRMAN (1966) reported a minority advantage using mutant strains of *D. pseudoobscura* in competition with wild type, and SPIESS and SCHWER (1978) and SPIESS and KRUCKEBERG (1980) have recently reported a rare-male advantage among eye-color mutants of *D. melanogaster*. These two cases will be addressed separately, since we believe at least two different factors may have contributed to these results.

In EHRMAN's (1966) data on *D. pseudoobscura*, the wild-type males were considerably more successful than the mutant males at equal frequency. When males differing in sexual vigor compete for mates, the advantage to the more vigorous males will increase when they are rare. This occurs because these vigorous males compete for mates against other vigorous males when common, but against numerous less vigorous males when rare. This will create a one-sided frequency-dependent mating advantage to rare vigorous males. Such one-sided frequency-dependent effects on developmental viability have been noted for some time (*e.g.*, BHALLA and SOKAL 1964; KENCE 1973; DEJONG 1976), but have been less well appreciated in the behavioral literature (but see DEBENEDICTIS 1977; ADAMS and DUNCAN 1979).

To examine EHRMAN's data for such one-sided advantages in favor of wild-type males, simulations were carried out with a 1 σ difference in sexual vigor between wild-type and mutant males, estimated from the average mating success of these wild males at equal frequency. Figure 4A gives the simulated percent mating success of the more vigorous type male over a range of input frequencies in comparison with the performance of wild-type *D. pseudoobscura* males, averaged over the tests with the mutant strains. As predicted, the advantage to the more vigorous males increased with their rarity and mimicked the results for *D. pseudoobscura*. The slope of the regression of output to input frequencies of these wild-type males was significantly less than 1.0 at $P < 0.05$, indicating a rare-male advantage; however, this slope was not significantly different from that of the simulations; thus, a two-sided minority advantage is unnecessary to account for these results.

In the data of SPIESS and SCHWER (1978) and SPIESS and KRUCKEBERG (1980), the strains were more evenly matched; hence, these results may offer greater support for a minority advantage without marking. It is important to note, however, that if sexual vigor were the same in two strains, when flies are sampled from the population, the difference in sexual vigor between the sampled lines would not be zero. Paired random normal variates, for example, would be separated by an expected distance of 1.128 standard deviation units (PEARSON and HARTLEY 1962, Table 27; see also BRYANT 1979). Since the deviations toward greater sexual vigor in one male type or the other would be equally frequent, the difference in vigor between competing males would fluctuate over replicate trials of a cross, even

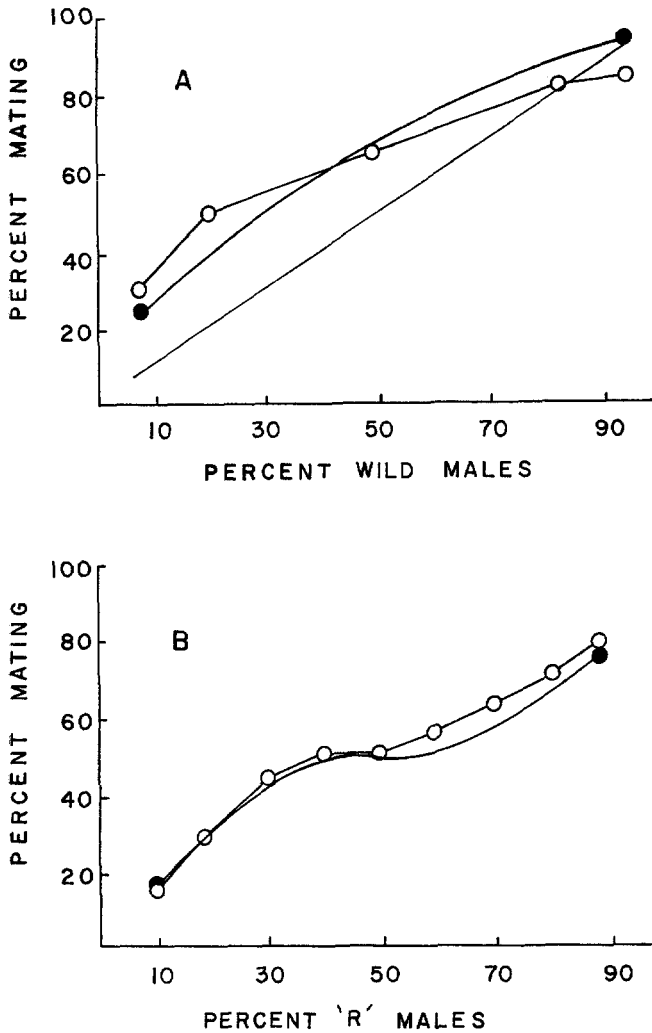


FIGURE 4.—Simulated advantage for mating tests of EHRMAN (1966) and SPIESS and KRUCKEBERG (1980), using mutant strains not requiring marking for identification. Figure 4A gives the simulated one-sided advantage in favor of the more vigorous male type (●—●) compared with average mating success of wild-type *D. pseudoobscura* males in competition with the mutant strains of Delta and orange (○—○) from EHRMAN (1966). A one standard deviation difference separated the simulated wild and mutant males. The quadrant bisector indicates random expectation. Figure 4B gives the simulated results for two unmarked strains assuming a 0.5 *c* fluctuating difference in sexual vigor between the strains due to sampling error (●—●), compared with the results for males of the *R* strain of *D. melanogaster* in competition with *O* males (○—○) from SPIESS and KRUCKEBERG (1980). There were no significant differences between the slopes of the regressions for the simulated and actual data of either set. See text for further explanations.

though average sexual vigor was the same in the two strains. This will have little or no effect when the two strains are equally frequent, but will produce the same sort of advantage to minority males as marking when the males are in unequal frequencies. This bias is inherent in the experimental design itself and will occur whether or not marking is applied or has an effect upon the performance of the males.

Is such a bias sufficient to account for the data of SPIESS and SCHWER (1978) and KRUCKEBERG (1980) on *D. melanogaster*? With 20 pairs per mating trial, the (harmonic) mean number of pairs per strain will range from 3.6 (2:18) to 10 (10:10), with an average of about six or seven pairs per strain over the range of frequencies. The mean fluctuating difference in sexual vigor between strains due to sampling would be about $1.128/\sqrt{6.5} = 0.44$. Thus, the expected minority advantage would be nearly the same as in our simulations, with a marking effect of 0.5 standard deviation units of vigor. The results of SPIESS and SCHWER (1978) do not show an overall frequency dependence by the regression of output to input frequencies (regression for *R* males was 0.96, $P > 0.05$); however, the result for these same strains reported by SPIESS and KRUCKEBERG (1980) did show a significant frequency dependence with a regression coefficient (for *R* males) of 0.72 ($P < 0.001$). These data, along with the simulation results for a marking effect of 0.5σ , are given in Figure 4B. The results closely follow the expected mating success of these males from the simulations, and there was no significant difference between the slope predicted from the model ($b=0.63$) and the actual slope. Hence, while a rare-male advantage is apparent in these data, normal sampling theory predicts a level of fluctuating difference in sexual vigor between these strains sufficient to account for the magnitude of frequency-dependent mating success observed.

DISCUSSION

If males are harmed during marking for identification such that their mating success is subsequently affected, an advantage to the rare-male type will result. In our housefly data, the overall correspondence between the observed level of harm and the performance of minority males and the correlation between the degree of harm and the associated amount of rare-male advantage strongly suggest that such a bias was operating in our experiments. We did not find a significant relationship between the level of sexual vigor in unmarked males and the loss in mating success when these males were marked. In the absence of such a confounding interaction between marking and vigor, there would be no overall effect of alternately marking males at equal frequency. Hence, in spite of an effect of marking in the housefly study, crosses among the strains at equal frequency can still be utilized to estimate geographic variation in mating success, as in KIMBALL and BRYANT (1980) and BRYANT (1980).

Our flies were marked by clipping the distal margin of one wing, but we do not know whether this in itself affected mating success or whether some other factor was involved, such as differential handling or amount of CO₂. There was

considerable variation in the degree of harm inflicted upon males over the crosses in our experiment, and it seems unlikely that small differences in the degree of wing clipping would cause such variation in mating success. We feel that handling or CO₂ effects are more likely culprits than wing clipping *per se*.

Whatever the source of the lowered mating success of marked males, it is interesting that similar marking by wing clipping has not been found to affect *Drosophila* males (e.g., EHRMAN 1966). Since *Drosophila* are smaller and harder to handle (in our experience) than houseflies, we would expect to see such a marking effect in these organisms as well. Moreover, although wing vibration is not an important signal in the housefly, it is in most *Drosophila* (BASTOCK and MANNING 1955; BENNET-CLARK and EWING 1967, 1970), and clipping by itself might alter mating ability. There is some evidence for this. When EWING (1964) removed 20%, 60% and 90% of the wings of *D. melanogaster* males he found their mating success to be 90%, 50% and 30% that of control males, respectively. Since a minority advantage has not generally been found when mutant strains not requiring marking were utilized, and available data conform to simulations based upon lowered mating success of marked males (e.g., Figure 3), it seems possible that marking may indeed affect the performance of *Drosophila* males. A large sample size is necessary to detect such marking effects; therefore, it is possible that tests reported in the literature may not have demonstrated differences in mating success between marked and unmarked males, even though they existed.

If the rare-male advantage is real, the most accepted explanation is that females recognize that there are two types of males present and change their receptivity in favor of minority males (SPIESS 1968; EHRMAN and SPIESS 1969; but see SPIESS and KRUCKEBERG 1980). Under this hypothesis, EHRMAN, PETIT and others have carried out a series of experiments to uncover the sensory basis of this recognition (EHRMAN 1966, 1967, 1969, 1970b, 1972b; LEONARD, EHRMAN and PRUZAN 1974; LEONARD, EHRMAN and SCHORSCH 1974; LEONARD and EHRMAN 1976; PETIT and NOUAUD 1975). While these results, even by the same authors, have been inconsistent in many cases, the series of experiments by EHRMAN and her colleagues seem to implicate pheromones as a basis of communication in *D. pseudoobscura*. However, a rare-male advantage was found between flies of this species reared at different temperatures (EHRMAN 1966), which suggests that flies synthesize a qualitatively different pheromone when reared at different temperatures. This would seem to jeopardize the very nature of pheromones as species-recognition agents. EHRMAN (1971) also concluded that sexual isolation among strains of *D. paulistorum* adversely affected minority advantage. Thus, two antagonistic pheromones, one for sexual isolation (preference) and one for minority recognition, might be required for the olfactory-cue basis of the rare-male advantage.

PETIT and NOUAUD (1975) found auditory signals, rather than odor, responsible for minority recognition in *D. melanogaster*, and they reasoned that the disparity of their results (auditory) and those for *D. pseudoobscura* (pheromonal) could be due to the different species involved. However, AVERHOFF and

RICHARDSON (1974) concluded that pheromones were responsible for genotypic recognition in their experiments with *D. melanogaster*, and MOLIN (1979) found odor (but not pheromones) as the basis of olfactory cues in the rare-male advantage of *D. melanogaster*. PETIT and NOUAUD also concluded that the minority advantage occurred through competition for space around the female in conjunction with female conditioning to male signals. On the other hand, SPIESS and KRUCKEBERG (1980) concluded that such conditioning (specifically, habituation) was not operating in their studies on *D. melanogaster*, but that females tended to reject males of the first courting type. These discrepancies among experiments, both within and among species, emphasize that, if the rare-male advantage exists at all, a number of causative factors may be involved.

It is possible that some of these discrepancies derive from specific mating characteristics of the strains and species involved; however, EHRLMAN (1967, 1969) also reported varying and ambiguous results in utilizing the same strains under different circumstances. Some differences among mating trials of the same strains can be expected to occur even when males of the two competing types are marked an equal number of times. In some trials, marking would enhance sampling differences in sexual vigor between two strains, resulting in a minority advantage, while in other trials marking may counteract sampling differences, leading to random mating. This would depend, of course, upon the relative magnitudes of sampling and marking effects, but it could result in ambiguous and misleading outcomes in searching for a sensory basis of the rare male phenomenon.

A rare-male advantage is based upon fitness values of genotypes changing with their frequency in the population (AYALA and CAMPBELL 1974; GROMKO 1977). Frequency-dependent mating success, however, can occur even though fitness values are constant, as we demonstrated for the one-sided preferences in Figure 4A. Such frequency-dependent mating success can also occur when a constant proportion of the females favors one male type over the other (DEBENEDICTIS 1977; O'DONALD 1977a, 1977b; ADAMS and DUNCAN 1979; KARLIN and RAPER 1979) and would be most evident when females are kept in equal proportions as male frequency is varied. This is seen clearly when sexual isolation occurs between two strains; as the males are varied, the male-to-female ratio for each type changes inversely with their frequency in the population. An example of this seems to be the frequency-dependent mating success for *Mormoniella vitripennis* reported by GRANT, SNYDER and GLESSNER 1974; GRANT *et al.* 1980. At equal frequency of wild and mutant strains, there was significant assortative mating (GRANT *et al.* 1974, Table 1), so that as the frequencies of males were varied with females in constant proportions, the mating success of both male types declined with their frequency. While such constant fitness or preference models may account for some of the reported frequency-dependent mating success in *Drosophila*, they seem to predict a linear response of output to input frequencies of males (*e.g.*, O'DONALD 1977a; see also ANDERSON 1969). In the data we examined in Figures 3 and 4B, there was a nonlinearity of response at

intermediate frequencies, which suggests that our explanation based upon sampling and marking effects may be more accurate.

Because of the number of possible factors that could produce frequency-dependent mating success, it may be difficult to demonstrate a real rare-male advantage in which one or both sexes of a genotypic strain change mating activity in relation to frequency. To do this one should ensure that (1) competing male types are equally vigorous, (2) females (or some of them) do not show a preference for one male type or the other, (3) marking for identification has no effect upon mating performance, and (4) the number of pairs per strain is sufficient to override sampling effects. Even so, an experimental bias could still persist. MARKOW (1980), for example, recently noted that males in the upper parts of a holding container are more sexually vigorous, and when few males are aspirated from a container they are often of the more vigorous type. Hence, more of the vigorous males may be chosen as minority than as majority participants. Since these various problems have not always been adequately considered in past analyses, we do not feel that the available data unambiguously demonstrate whether or not a real minority advantage exists. Rather, we feel that we have offered sufficient counter-evidence, so that more work needs to be done before any final conclusions are reached.

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