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Airborne Cues as a Factor in Frequency-Dependent Mate Selection in *Mormoniella vitripennis*

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AIRBORNE CUES AS A FACTOR IN
FREQUENCY-DEPENDENT MATE SELECTION
IN MORMONIELLA VITRIPENNIS

A Thesis

Presented to

The Faculty of the Department of Biology
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by
Harry C. White
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APPROVAL SHEET

This thesis is submitted in partial fulfillment of
the requirements for the degree of

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ABSTRACT

The sensory basis of frequency-dependent mate selection in two strains of the parasitic wasp Mormoniella vitripennis was investigated. Using a "double chamber" technique the rare male advantage was tested for both wild and mutant minority types. The frequency-dependent behavior observed for the mutant minority appears to be mediated by airborne cues, probably pheromonal.

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INTRODUCTION

Ironically, the phenomenon of frequency-dependent mate selection, so far noted only in the class Insecta, seems rather to have its greatest intuitive appeal in a consideration of human characteristics. The suggestion, capitalized on by advertising executives, that blondes (as a minority type) have more fun, would appear to illuminate a facet of human sexual experience; what Claudine Petit (1972) has referred to as the "charm of the exotic".

For evolutionists, however, the interest in frequency-dependent mate selection lies beyond a simple description of unique sexual behavior. Though little is known about the existence of a frequency-dependent response in natural populations (Borisov, 1970), this mode of sexual selection may prove to play an important role in the maintenance of certain balanced polymorphisms.

Petit (1958) and Ehrman et al. (1965) independently observed that for Drosophila melanogaster and D. pseudoobscura, respectively, mating success in the laboratory was dependent on the relative frequencies of the two types of males. The minority male type showed an increased mating efficiency. The advantage of the rare male has since been described in eight more species of the genus Drosophila; D. equinoxialis, D. funebris, D. gaucha, D. immigrans, D. pavani, D. persimilis, D. tropicalis, and D. willistoni (Ehrman and Petit, 1968; Ehrman, 1966, 1972b; Ehrman et al., 1972; Spiess,

1968; Spiess and Spiess, 1969; and Borisov, 1970); the parasitic wasp Mormoniella vitripennis (Grant et al., 1974); and suggested in the red flour beetle Tribolium castaneum (Sinnock, 1969). Females have been shown to possess the ability to distinguish strains of males carrying different chromosomal inversions, of different geographic origins, reared at different temperatures, and wild versus mutant types (Ehrman, 1967, 1970b; Petit and Ehrman, 1969).

Recent work has been concerned with the behavioral components of frequency-dependent mate selection, the mechanism(s) by which males in the minority may gain a mating advantage over those in the majority, thus making a disproportionate contribution to the gene pool.

Ehrman (1969, 1970a, 1972a) has demonstrated that frequency-dependent behavior depends most heavily on information transferred to the females by airborne olfactory cues. Frequency-dependent mate selection has been experimentally induced with equal frequencies of male types or, conversely, reduced when unequal frequencies were present. This can be accomplished through use of a double chamber technique, in which flies in the mating chamber perceive the odor of flies in an adjacent compensation chamber. Although a pheromone would appear to be the major mediating factor (Ehrman et al., 1973), it probably acts in conjunction with auditory, tactile, and visual sensory stimulation (Leonard et al., 1974). The situation is further complicated because of the age and experience dependence involved in mate selection (Pruzan and Ehrman, 1974; Pruzan, 1976).

Unlike the studies of the Ehrman group with D. pseudoobscura, Petit and Nouaud (1975) found that olfactory stimulation appeared to be of no importance as a mechanism for frequency-dependent mate selection in their investigation of D. melanogaster. The primary control mechanism was found to involve vibrational cues.

This paper represents a preliminary examination of the controlling factors of frequency-dependent mate selection, described by Grant et al. (1974), in the parasitic wasp Mormoniella vitripennis.

MATERIALS AND METHODS

Mormoniella vitripennis is a small wasp of the superfamily Chalcidoidea, which in its larval stages parasitizes certain Dipteran pupae. In this experiment the female wasp was allowed to oviposit her eggs in the pupae of the fleshfly Sarcophaga bullata. Females range from 1.0 to 3.5 mm. in length, males from 0.6 to 2.4 mm. The general biology of Mormoniella has been reviewed by Whiting (1967). The wasps show a very complex courtship behavior, predominantly characterized by the drumming of the male upon the head of the female with his antennae and prothoracic limbs. Individuals are able to copulate immediately upon emergence from the Sarcophaga pupal case and may mate more than once. For a more complete review of the mating behavior, see Barrass (1960).

The strains employed in this experiment, wild type and a double mutant for purple body and peach eye, were obtained originally from Carolina Biological Supply Company in 1972.

The wasps were cultured in small shell vials in a laboratory incubator at 21-22°C with a twelve hour (9 AM, 9PM, EST) light-dark cycle. Generation time at such temperatures is approximately 21 days. In order to obtain virgin wasps, the Sarcophaga pupal cases, within which the wasps have already matured, are opened several hours prior to emergence. Mating is effectively prohibited.

within the host pupal cases due to extremely tight crowding. Upon release from the host puparium the adult wasps were immediately anesthetized with CO_2 and sorted as to sex. Individuals were segregated according to sex and eye color (wild or mutant) in glass vials. All wasps were allowed a four hour recuperatory period prior to the mating experiments.

The mating observation chambers, made from modified glass shell vials (see Figure 1), were designed to provide ease of construction and maximum visibility. The end of a vial was cut off, resulting in a glass cylinder 90 mm. long with a diameter of 22 mm. A 4 mm. section of a two-hole rubber stopper divided the cylinder into equal halves. The holes on both sides of the disc were covered with fine metal screen. Free air flow was thus permitted between halves of the double chamber vial, but visual cues between the wasps of the adjacent chambers were severely restricted and any physical contact between the wasps was prevented.

Each end of the chamber was fitted with a one-hole rubber stopper into which a short length of metal tubing was attached; a gentle current of air could then be pumped through the length of the chamber and out the excurrent end. The air line was attached to an air compressor and filtered through charcoal and spun glass to remove impurities. The velocity of the air outflow was measured by its ability to deflect a needle gauge. A gentle air flow of constant value was maintained throughout the course of the experiment.

If the basis of frequency-dependent mate selection were ol-

factory in nature, then presumably these olfactory cues could be transmitted from one section of the double chamber to the other. The presence of the minority males in the right half of the observation chamber, designated chamber A (adjustment), could be used to counterbalance the frequency-dependent mate selection from the unequal ratio of males in the adjoining chamber to the left, designated chamber M (mating) where both sexes are present and mating is observed and scored.

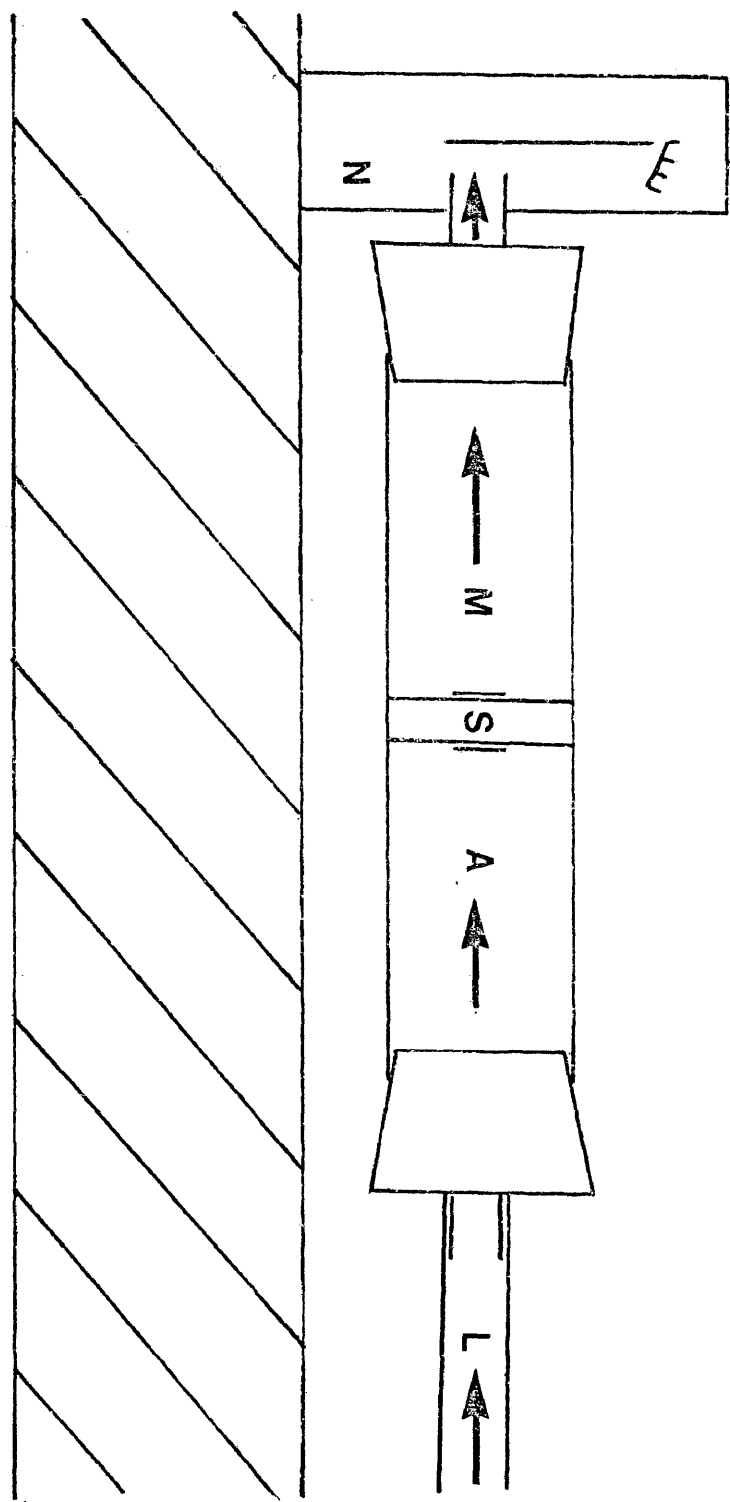
Three frequencies of mutant to wild type males were examined under conditions both adjusted for the minority male (wasps in chamber A) and unadjusted (chamber A empty). No visual or physical contact was permitted between the adjoining chambers, but the transfer of the hypothetical pheromone between the chambers was provided for by the flow of air over the "minority" in chamber A into the other half of the observation chamber (M).

In all trials female wild to mutant ratios were held constant at 5:5; air flow was maintained for all trials, with or without the adjusting minority males in chamber A. In chamber M male ratios were varied from 5:5, 8:2, and 2:8 wild to mutant type. In those trials in which the rare males of chamber M were to be adjusted by the presence of like genotype in chamber A, 6 males were used to compensate to equality. Thus if a pheromone were involved in mate recognition, an 8:8 ratio of wild to mutant pheromone could be assumed.

Matings for all trial runs were observed for 30 minutes with the aid of a dissecting scope and scored as to the type of male and female in a given mating pair. Mutant wasps were identified

by eye color, as body color differences are subtle. Sexes were identified by conspicuous differences in wing length and the positions assumed in courtship and mating. The chambers and rubber stoppers were thoroughly washed between trials to avoid any possible odorous contamination. Approximately 200 matings were observed for each test: 5:5 wild to mutant male; 8:2, unadjusted; 8:2, adjusted; 2:8, unadjusted; and 2:8, adjusted.

Fig. 1. The double observation chamber. M = chamber M (mating); A = chamber A (adjustment); S = two-hole rubber stopper and screens; N = needle gauge; L = air line.



RESULTS

The results have been analyzed with respect to two factors: mating discrimination and mating success. Mating discrimination is a measurement of the tendency to mate with like or unlike genotypes. An individual that mates only with members of its own genotype is said to show positive assortative mating. Mating success or performance describes an individual's relative mating efficiency. If wasp A mates three times for every two matings of wasp B, then wasp A can be said to be more successful at mating.

Following Schaffer (1968), Table 1 presents the results of tests for mating discrimination between wild and mutant strains for each of the five tests. Schaffer's measure represents a quantification of assortative mating and may range from +100% to -100%, the sign indicating either positive or negative assortative mating. All data indicate some discriminatory mating patterns (non-zero values) in the direction of positive assortative mating. However the results of an adjusted heterogeneity chi-square test for significance shows only the mutant minority unadjusted data to be statistically significant at a $p < 0.05$ level.

In Table 2 the data have been rearranged for the presentation of mating performance. The mating performance of the females, regardless of the male frequencies, never varied significantly

from the expected 5:5 ratios according to chi-square tests. All expected values for unequal frequencies (8:2, 2:8; adjusted and unadjusted) for the male performance data have been uniformly corrected to correspond to the non-random mating patterns observed at the 5:5 control. At equal frequencies wild males showed a significant advantage over the mutant males in mating success ($\chi^2=9.10$, $p<0.005$). By dividing the matings in which wild males were involved (123) by the total matings for the 5:5 control (203), it is calculated that wild males were involved in .606 of the total matings, rather than the expected .500 under conditions allowing for random mating. In order to compensate for the wild type advantage, a correction value of .106 N (where N is the total number of matings) was consistently added to the expected wild type mating frequencies and subtracted from the expected mutant type mating frequencies at all unequal frequencies. Thus at a frequency of .800 wild males, the expected number of wild male matings would be corrected to .906 N and the expected number of mutant male matings would be shifted down from .200 N to .094 N. Likewise at a wild to mutant ratio of .200 to .800 the number of expected matings would be .306 N for the wilds and .694 N for the mutants.

For both the wild minority unadjusted and the wild minority adjusted tests, no significant deviations from the corrected expected mating frequencies were observed. In each case the wild males approximated their inherent .106 mating advantage. With such corrections frequency-dependent mate selection was not indicated for the wild minority tests.

The phenomenon of frequency-dependent mate selection was shown for both the mutant minority adjusted and unadjusted tests. Although both deviations from the expected were significant, the χ^2 value for the adjusted mutant minority was a much lower value. To determine whether the difference between the two treatments was significant, the two samples were ranked in accord with the Mann-Whitney U test (see discussion in Siegel, 1956). The measure of probability U was determined to be 65.5, which proved to be significant at the $p \approx 0.001$ level (for 1 degree of freedom).

The lessening of the effect of frequency-dependent mate selection for the adjusted mutant minority is shown graphically in Figure 2. Mating success K is plotted against the male genotype frequency. The value K is termed the coefficient of mating success (Petit and Ehrman, 1969). A K value equal to 1.0 is indicative of random mating where no mating advantage for any type of individual exists. When $K < 1.0$, individuals are at a mating disadvantage and exhibit decreased mating success relative to the other type. $K > 1.0$ indicates an increase in mating success.

For the mutant:

$$K_m = (A/a) : (B/b)$$

$$\text{the variance of } K_m = K_{vm} = (b^2/a^2)(NA/B^3)$$

$$\text{standard deviation of } K_m = K_{vm}^{1/2}$$

where a=number of mutant males, A=number of females inseminated by mutant males, b=number of wild-type males, B=number of females inseminated by wild-type males, and $N=A+B$ = total number of inseminated females. To determine mating success for the wild-type

males, simply interchange appropriate case B and A in the preceding equations.

In Figure 2 regions 1 and 4 indicate the wild minority data, both adjusted and unadjusted, in which no evidence for frequency-dependent mate selection is found. Regions 2 and 3 indicate frequency-dependent mate selection for the mutant minority males; the K values having been shifted significantly toward the control values as a direct result of the presence of the adjusting mutant minority males in chamber A.

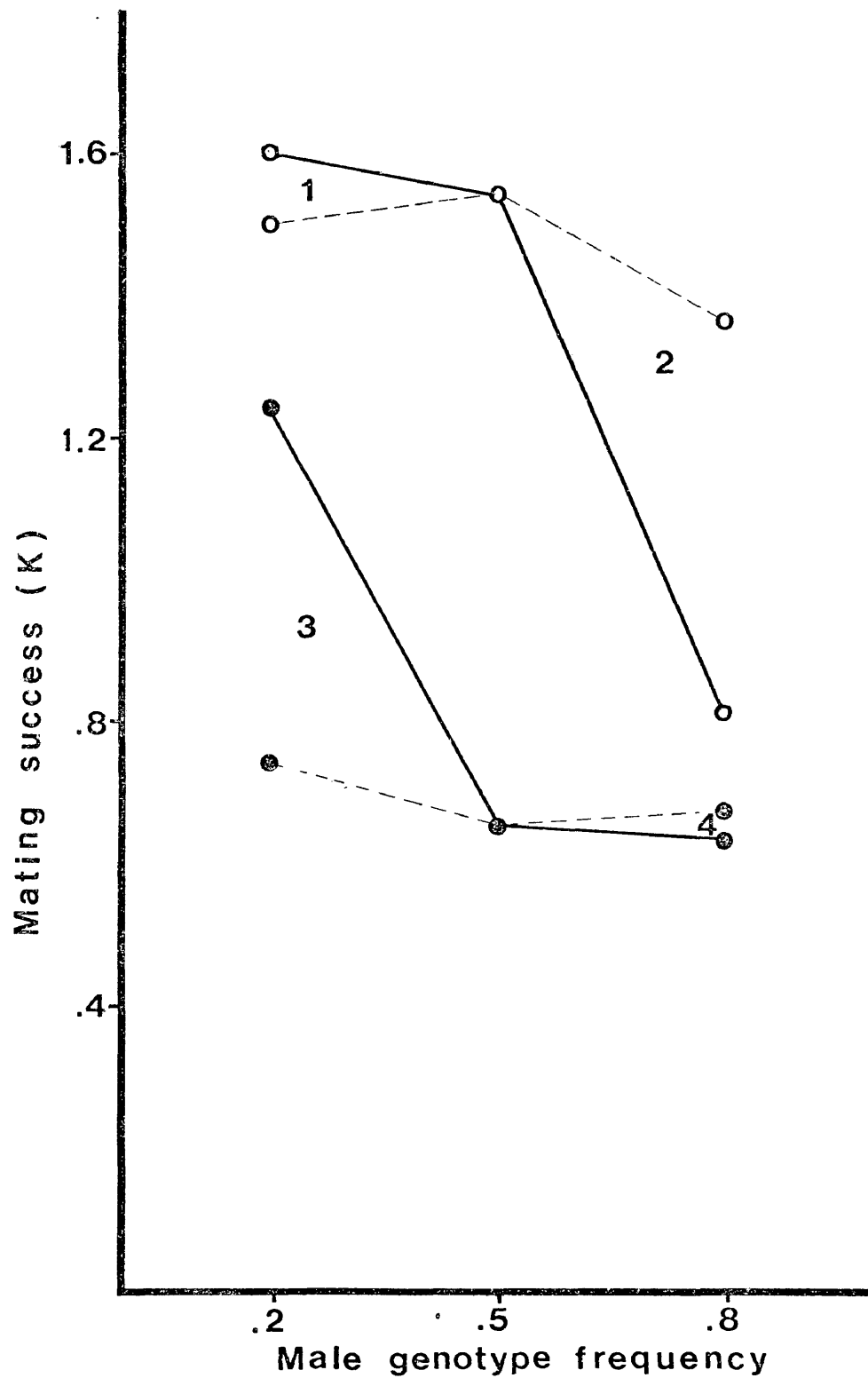
Table 1. Mating discrimination tests shown as numbers of matings observed. ($\leftarrow .6$) indicates adjusted minority.

Male frequencies	Females .5+ .5mut		Total matings	Schaffer's measure	Adj. Het. χ^2	Prob.
.2+ .8mut	36 77	24 74	211	+6.726	1.06	n.s.
.2+ ($\leftarrow .6$) .8mut	30 77	25 70	202	+1.559	0.013	n.s.
.5+ .5mut	64 38	59 42	203	+4.235	0.24	n.s.
.8+ .2mut	83 17	75 32	207	+11.41	4.08	$p < 0.05$
.8+ .2mut ($\leftarrow .6$ mut)	86 15	88 17	206	+1.102	0.005	n.s.

Table 2. Mating performance. Female wild to mutant ratios held constant at 5:5. Expected values for males have been corrected to 5:5 male frequency control. Statistical tests have been performed on corrected data. (\leftarrow .6) indicates adjusted minority. For females obs./exp. refers to random expected.

Ratio of males wild:mutant	Number of matings	Males		χ^2	Females		χ^2
		wild	mutant		wild	mutant	
2:8	211	60	151	.47	113	98	1.06
	expected r (random)	42.2	168.8	(n.s.)	105.5	105.5	(n.s.)
	expected c						
	(corrected)	64.6	146.4				
	obs./exp. c	0.93	1.03		1.07	0.93	
2:8(\leftarrow .6+)	202	55	147	1.08	107	95	0.72
	expected r	40.4	161.6	(n.s.)	101	101	(n.s.)
	expected c	61.8	140.2				
	obs./exp. c	0.89	1.05		1.06	0.94	
5:5	203	123	80	9.10	102	101	0.004
	expected r	101.5	101.5	(p<0.005)	101.5	101.5	(n.s.)
	obs./exp. r	1.21	0.79		1.01	1.00	
8:2	207	158	49	49.27	100	107	0.078
	expected r	165.6	41.4	(p<0.005)	103.5	103.5	(n.s.)
	expected c	187.5	19.5				
	obs./exp. c	0.84	2.51		0.97	1.03	
8:2(\leftarrow .6mut)	206	174	32	9.04	101	105	0.236
	expected r	164.8	41.2	(p<0.005)	103	103	(n.s.)
	expected c	187.5	19.5				
	obs./exp. c	0.93	1.65		0.98	1.02	

Fig. 2. Mating success as indicated by K computed for both wild and mutant males plotted as a function of genotype frequency. Solid line = unadjusted; broken line = adjusted for minority; open circles = wild males; closed circles = mutant males. For description of regions 1, 2, 3, and 4 see text.



DISCUSSION

The results of the double chamber experiment described in this paper demonstrate that the recognition of one wasp by another as required for frequency-dependent mate selection is, at least in part, non-visual and non-tactile. Although the possibility of a vibrational stimulus still exists, the much more likely recognitional factor is chemical stimulation, i.e., pheromonal. However, pheromones alone do not appear to mediate the frequency-dependent response. Even when the rare males have been compensated to equality, the frequency-dependent mate selection has not been completely eliminated. It is possible that in Mormoniella frequency dependent mate selection may be the result of a multi-component system.

Unlike the previous work on frequency-dependent mate selection in Mormoniella (Grant et al., 1974), the data accumulated here are asymmetric. A rare-type mating advantage was found for the mutant minority males, but not for the wild minority males. There are several possible explanations for this discrepancy. Due to the fact that the wild-type males showed a significant mating advantage at a 5:5 ratio, an advantage which was indicated by Grant et al. at a non-significant level ($\chi^2=2.81$, $p<0.10$), it might be the case that a more extreme ratio is required to eliminate their inherent advantage. The experimental method employed

here differed from earlier efforts with respect to two variables: the air blown across the wasps and a mating chamber of one-half the volume. It is not known what effect, if any, density has on the mating behavior of the wasps, but obviously more experimental work is required on possible density related effects.

The interpretation of these results provide no hard and fast explanations as to the mechanism of frequency-dependent mate selection in Mormoniella. They do provide limitations of what is possibly occurring and allow for the phrasing of questions in a more rigorous fashion. This experiment is a necessary small step in what may prove to be a long series of experimental problems required to resolve the issue. The next projected experiment will hopefully provide clarification on the question of whether these airborne cues are vibrational or olfactory. The substitution of freshly killed males for the live ones of chamber A will result in the elimination of any possible auditory stimuli. If the frequency-dependent response is still curtailed, olfaction will have been established as the controlling sensory input transferred by air.

At the present time the importance of frequency-dependent mate selection remains primarily theoretical in thrust. Until such questions as how frequency-dependent mate selection has been observed for such slight differences in Drosophila males as being cultured at different temperatures or on different media (Manning, 1972) and whether the mating advantages can be demonstrated in natural populations, the overall significance of this phenomenon, the maintenance of genetic variation, will remain conjectural.

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