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Lack of Minority Advantage in *Drosophila melanogaster* Mutants

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Abstract:The objective of this study was to test the generality of frequency-dependent fitness in the mating behavior of *Drosophila*, called the minority effect and to investigate the effect of artificial olfactory cues and marking the flies by wing clipping on the frequency-dependent mate choice. In the mating experiments using wild type (oregon) and mutant (white-eyed, sepia, ebony) strains of *Drosophila melanogaster*, the mating success of mutant males did not increase as their frequency decreased. In addition, the olfactory cue hypothesis was not supported by the results of the experiments with artificial scent. The results of this study did not support the contention that rare male advantage is a general phenomenon. Therefore rare male advantage is probably not an important factor maintaining genetic variation in natural populations of *Drosophila*.

Key Words: *Drosophila*, minority advantage, artificial scent, wing clipping.

Drosophila melanogaster Mutantlarında Azınlık Avantajının Yokluğu

Özet: Bu çalışmanın amacı *Drosophila*'nın eşeyssel davranışlarında azınlık etkisi adı verilen frekansa bağlı uyumun genelliğini sınamak, yapay koku sinyallerinin ve sinekleri kanat keserek işaretlemenin frekansa bağlı eş seçimi üzerindeki etkilerini araştırmaktır. *Drosophila*'nın normal (oregon) ve mutant (white-eyed, sepia, ebony) soyları arasında yapılan deneylerde mutant erkeklerin çiftleşme başarısı frekansları azaldığında artmadı. Ayrıca, yapay koku kullanılarak yapılan deney sonuçları koku sinyalleri hipotezini desteklemedi. Bu çalışmanın sonuçları azınlık erkek avantajının genel bir olgu olduğu savını desteklemedi. Dolayısıyla azınlık etkisi *Drosophila* toplumlarında genetik çeşitliliğin korunmasında önemli bir faktör olmayabilir.

Anahtar Sözcükler: *Drosophila*, azınlık avantajı, yapay koku, kanat kesme.

Introduction

The phenomenon called "rare male mating advantage" or "rare male effect" constitutes an interesting form of deviation from random mating in population genetics. In *Drosophila* mating experiments, it has been repeatedly observed that rare males mate more often than expected when two types of males (genotypes, strains) are present in a population at unequal frequencies (1-5). As a form of frequency dependent selection, rare male advantage (RMA) could be an

important mechanism in maintaining genetic variation in natural populations; because a genotype that is becoming rare will increase its fitness through an increase in mating success of males. As the rare type becomes more common in the population its advantage will decrease, leading to a stable equilibrium frequency. Two factors are involved in sexual selection: male competition and female choice. Differences among males are recognized by females and females choose males of high fitness (6). Because of its evolutionary significance in maintaining genetic polymorphism, RMA has received considerable attention from population biologists.

RMA was observed for almost all the species, strains, genotypes and phenotypes of *Drosophila Pseudoobscura* by Ehrman (1). He also showed the presence of RMA even when two male types differed only with respect to the temperature in which they were raised.

In an attempt to explain the better than expected performance of rare males in mating, it was proposed that "Females recognise that there are two types of males present and change their receptivity in favor of the minority males" (7). According to this proposal, a female being courted is stimulated by some cue from the male (either chemical or auditory). Adult flies have hydrocarbons which act as courtship-inducing pheromones (8). Females are usually reluctant to courting males that they first encounter. Therefore a female will receive more than one stimulus, since she will encounter more than one courting male. If the common males produce a set of stimuli leading to a "sensory adaptation", the difference in stimulus produced by a rare male may induce a female to accept a courting male, just because his cue is different. Molin (9) claimed that RMA could be observed between *Drosophila melanogaster* types that were raised in different cultures, with and without peppermint essence, even if all the flies belonged to the same genotype. Observation of RMA in organisms other than *Drosophila*, established the phenomenon as a general form of frequency dependent selection (10-12). However, it was not possible to demonstrate any RMA in houseflies (13) and in *Tribolium* (14). Although RMA had been believed to be general phenomenon in population biology, there has been a surge of negative reports on the presence of RMA in *Drosophila* populations (15-22). Various authors also raised doubts about the biological reality of the phenomenon (20-24). The technique used by a researcher can be important for the outcome of a mating experiment. According to Knopien (28) RMA may be an artifact caused by the different storage condition of the flies.

The majority of the experiments on RMA have been carried out using CH and AR strains of *Drosophila pseudoobscura*. It was necessary to mark one strain by wing clipping to identify different strains during experiments, since it is impossible to distinguish these investigation strains. Marking was alternated between the strains from one experimental run to another to prevent bias that might be due to marking only one strain. Kence (23) suggested that RMA could be produced as an experimental artifact by pooling the data from experimental runs where the rare strain is marked with data from runs where it is not, if marking has an effect on the mating success of males. Computer simulations and experiments with houseflies also supported this conclusion (25).

The objectives of the present study were to test the generality of RMA by using a number of mutant strains where marking is not needed and to repeat some experiments which were claimed to show RMA.

Materials and Methods

Strains of *Drosophila*

Oregon. A wild type strain of *D. Melanogaster*.

White (w). This *D. Melanogaster* strain carries the sex-linked eye colour mutation, white (w) in homozygous condition in females and in hemizygous condition in males.

Sepia (se). This *D. Melanogaster* strain is homozygous for autosomal (3rd chromosome) eye colour mutation sepia (se). Eye colour is darker than that of the wild type.

Ebony (e). This *D. Melanogaster* strain is homozygous for autosomal (3rd chromosome) body colour mutation ebony (e). Body colour is black in this strain.

Experimental Procedure

Flies were raised on instant *Drosophila* medium (Formula 4-24) supplied by the Carolina Biological Supply Company. Cultures were kept in a constant temperature chamber set at 25°C. Flies used in experiments were obtained by allowing 10 fertilized females to lay eggs on the medium for four days. Newly emerged virgin flies were collected, separated and sexed under light ether anaesthetization, and marked by clipping the distal margin of the wing(s) if necessary. Virgin flies were stored in tubes containing instant *Drosophila* medium in groups of 25-30 individuals. One day before the mating experiments, flies were transferred to fresh medium. Flies used in the mating experiments were 4 days old. Mating experiments were generally carried out in the morning. Ten males and ten females were introduced into an Elens-Wattiaux observation chamber (29) by aspiration. Two types of flies were combined in 1:4, 1:1 and 4:1 ratios in the observation chambers. Matings were observed for a period of three hours and the type and time of matings were recorded. Mating pairs were not removed from the chambers so that they could mate more than once. Females once mated do not accept mating attempts by other males, so that they mate only once during an experiment (30).

Experiments With Artificial Scent

In these experiments, two groups of wild type *Drosophila melanogaster* from stock oregon were used. Group N (Normal medium) larvae were raised on instant *Drosophila* medium. Group P (Peppermint medium) larvae were raised under the same conditions as N larvae but with four drops of peppermint essence added to each tube before the introduction of the adult flies. P and N flies were genetically the same but group P males presumably had an artificial scent due to the culture medium in which they developed. To detect the effect of clipping the wings, two sets of experiments were run. The number of trials for each ratio were twenty. The type of flies clipped alternated from the first set of experiments to the second. During the separation, marking was made by clipping the distal margin of the wings with a razor blade and a drop of peppermint essence was placed on the stoppers of the tubes containing P flies. The steps of the general experimental procedure explained above were followed in these mating experiments. All female flies were from N groups. The wings of the females were not marked.

Results of the experiments were evaluated by computing X^2 values and Petit's coefficient of mating success (K). If there are two competing strains, strain I and strain II, and their frequenc

ies a and b, and A and B are the number of mating by strain I and strain II (males or females) then the coefficient of mating success (K) of strain I can be computed from the formula (31).

$$K = \frac{a}{b} \cdot \frac{B}{A}$$

K ranges between 0 and ∞. If K is greater than 1 for a given strain, that strain is considered to be more successful in mating than its competitor. The standard error of K value is computed from the formula:

$$\text{Standard error} = K (A+B) / (AxB)$$

Results

The results of experiments where wild type males (oregon) competed with mutant males (white-eyed, sepia, ebony) are shown in Tables 1, 2 and 3. In experiments with oregon and white-eyed flies, the coefficient of male and female mating success (K) of oregon flies was greater than that of white-eyed flies (Table 1). While the X² values for males were highly significant (P<0.001). The results of mating experiments with oregon and sepia showed that at all ratios K values of wild type males and females were greater than one. Oregon flies were more successful in mating than sepia flies (Table 2). Deviations from random mating were significant at 1:1 ratio for males (P<0.01) and at 2 oregon: 8 sepia ratio for females (P<0.001). The rest

Table 1. The results of mating experiments between oregon and white-eyed strains.

Oregon: White eyed A:B	Number of Runs	Number of matings (malexfemale)				Have Mated				K		X ²	
		AxA	AxB	BxA	BxB	A	B	A	B	A	A	Males	Females
						Male	Male	Female	Female	Male	Female		
8:2	20	88	17	0	0	105	0	88	17	∞	±0.343	26.250***	0.952ns
5:5	20	42	34	2	7	76	9	44	41	±2.975	±0.232	52.81***	0.183ns
2:8	20	10	22	0	12	32	12	10	34	±4.068	±0.4.23	76.545***	0.204ns

ns: Not significant

***: P<0.001

Table 2. The results of mating experiments between oregon and sepia strains.

Ratio Oregon: Sepia A:B	Number of Runs	Number of matings (male:female)				Have Mated				K		X ²	
		AxA	AxB	BxA	BxB	A Male	B Male	A Female	B Female	A Male	A Female	Males	Females
		8:2	22	89	16	21	4	105	25	110	20	1.141 ±0.263	1.375 ±0.334
5:5	22	38	31	23	17	69	40	61	64	1.700 ±0.339	1.129 ±0.210	7.259***	0.426ns
2:8	22	6	20	20	54	26	74	26	40	1.425 ±0.325	2.6 ±0.654	0.391ns	15.51***

ns: Not significant

** : P<0.01

***: P<0.001

Table 3. The results of mating experiments between oregon and ebony strains.

Ratio Oregon: Ebony A:B	Number of Runs	Number of matings (male:female)				Have Mated				K		X ²	
		AxA	AxB	BxA	BxB	A Male	B Male	A Female	B Female	A Male	A Female	Males	Females
		8:2	22	85	22	8	0	107	8	93	22	3.344 ±1.226	1.057 ±0.0250
5:5	22	44	49	11	11	93	22	55	60	4.227 ±1.002	0.916 ±0.170	43.834***	0.217ns
2:8	22	11	29	12	52	40	64	23	81	2.540 ±0.513	1.135 ±0.268	22.837***	0.290ns

ns: Not significant

***: P<0.001

of the X² values in table 2 were not significant. The results of the experiments with oregon and ebony are shown in Table 3. K values for males and females of the oregon strain were always greater than 1.0, except the K value for females at 1:1 ratio. The deviations from random mating for males were highly significant (P<0.01) at all ratios, while deviations from random mating for females were not significant at any ratio. Unlike the wild type males, three type of mutant males (white-eyed, sepia, ebony) showed a lower percentage of mating than expected. White-eyed males showed the largest deviation from random mating compared with ebony and sepia males. Sepia males showed the least deviation from random mating among the three mutant strains.

The results of the mating experiments with peppermint essence using the oregon strain are presented in Tables 4 and 5. In the first set of experiments with peppermint essence P males

Table 4. The results of mating experiments between flies raised in normal medium (N) and medium with peppermint essence (P). The wings of male flies raised in medium with peppermint essence (P) were clipped by a razor blade.

The figures in parentheses are the expected mating frequencies computed according to Kence (1981) which take into account the reduction in probability of mating by marked males (s) estimated to be 0.1017.

Ratio N:P	Number of runs	Number of mating by males		K rare	χ^2
		Rare	Common		
8:2	19	21	92	0.913±0.053	
P clipped		(20.7)	(92.3)	(0.89707±0.059)	0.577ns
2:8	19	21	90	0.933±0.235	
P clipped		(24.2)	(86.8)	(1.11520±0.053)	0.081ns
Pooled totals	38	42	182	0.923±0.158	
		(44.9)	(179.1)	(1.00279±0.167)	0.218ns

Control N:P	Number of runs	Number of mating by males		K _{NW}	χ^2
		NW	CW		
5:5					
P clipped	19	59	53	1.113±0.211	0.321ns

Ns: not significant

NW: Normal wing

CW: Clipped wing

(males raised in medium with peppermint essence) were marked by clipping the distal margin of their wings (Table 4). In the second set of experiments with N males (males raised in normal medium) were clipped (Table 5). In both sets of experiments the K values for rare males were similar. Neither P males nor N males showed any rare male advantage. In mating experiments with equal numbers of P and N males, deviations from random mating were not significant, although K value for N males were greater than 1.0 in both cases (Tables 4 and 5). The percentage of matings of male flies was very close to the expected values at all ratios. In these experiments, the effect of wing clipping appears to be negligible.

Discussion and Conclusion

If the number of matings, by a given type of male is negatively frequency dependent, when more than one type of male compete for females in a population, this is called rare male advantage (RMA). The adaptive significance of genetic polymorphism related to the frequency dependent selection has been a subject of numerous population studies (32). Thus in RMA, the relative mating success of individual males of each type increases as the frequency of that type in the male population declines. The RMA in *Drosophila* was first reported by Petit (33). In

Table 5. The results of mating experiments between flies raised in normal medium (N) and medium with peppermint essence (P). The wings of male flies raised in normal medium (N) were clipped by a razor blade. The figures in parentheses are the expected mating frequencies computed according to Kence (1981) which takes into account the reduction in mating success by N males due to marking. The reduction in probability of mating by marked males (s) was estimated to be 0.12.

Ratio N:P	Number of runs	Number of mating by males		K rare	χ^2
		Rare	Common		
8:2	20	21	86	0.977±0.238	
N clipped	20	(19.3)	(87.7)	(0.88027±0.221)	0.009ns
2:8		20	83	0.964±0.240	
N clipped	20	(22.8)	(80.2)	(1.13716±0.269)	0.022ns
Pooled totals	40	41	169	0.970±0.240	
		(42.1)	(167.9)	(1.00298±0.172)	0.029ns

Control N:P	Number of runs	Number of mating by males		K _{NW}	χ^2
		NW	CW		
5:5		50	44	1.136±0.235	
N clipped	20				0.382ns

Ns: not significant
 NW: Normal wing
 CW: Clipped wing

experiments involving white-eyed mutant and wild type males, he observed that white-eyed males were more successful in mating than wild type males when their proportions were less than 40% or more than 80% in the population. Although white-eyed males were not exactly negatively frequency dependent in mating success, since they also showed advantage when they were common, this study has been widely cited as the first observed case of RMA in the literature. Nevertheless, white-eyed males appeared to be more successful in mating when they were rare than when they were in equal frequencies with wild-type males.

In this study involving white-eyed and wild type (orgeon) males, no RMA could be observed. On the contrary, the mating success of white-eyed males declined as they became rare in the population. Wild-type males were more successful in mating when they were rare compared with their performance at 1:1 ratio (Table 1). These results are in contrast with the findings of Petit (33). Peterson and Merrell (20) also reported results parallel to those observed in this study. They could not demonstrate any rare male advantage in experiments involving white-eyed and oregon strains. Rahter, as in this study they found a rare male disadvantage for white-eyed males competing with oregon males although they used a different technique from that of the present study. These results agree with the predictions of the computer simulation model of Kence (23, 24). The model predicts that when two strains compete for a given resource, the weak competitor should do even worse as it becomes rare in the population.

In this study, the test of rare male advantage was extended to two other mutant strains in addition to the white-eyed strain. When sepia and oregon males competed, there was again no RMA, although oregon males mated significantly more than sepia males at 1:1 ratio. Markow (16) also could not demonstrate any RMA in mating experiments using sepia and Canton-S strains of *Drosophila melanogaster*.

In mating experiments with the ebony and oregon strains, the results were similar to those of the previous ones in that there was no RMA. At all ratios wild-type males mated more often than the ebony males ($P < 0.001$). Previously, no study on RMA using ebony strain was reported in the literature. Therefore it is not possible to compare these results with data from other studies. Spiess et al. (2) used three eye colour types of males in *Drosophila melanogaster* and concluded that sexual behavior is controlled by loci other than the markers for eye colour.

The experiments in this study involving mutant strains differ from those of Peterson and Merrell (20) and Markow (16) in one important respect. These authors tried to homogenize the genetic background of the strains they used by carrying out a series of crosses, so that wild-type and mutant individuals differed almost only with respect to mutant genes. In this study, we did not attempt to homogenize the genetic backgrounds of the strains used in mating experiments. Therefore strains should differ from each other from a large number of genes other than the mutations white-eyed, sepia and ebony. Since the genetic differences between the strains are larger in this study compared with the strains used by Peterson and Merrell (20) and Markow (16), one should have a better chance of observing RMA in the present experiments.

According to the hypothesis of Ehrman and Spiess (7), the larger the genetic differences between the strains, the easier it should be for a female to detect the differences in cues of males belonging to different strains. Terzic reported that the rare male phenomenon is not dependent on different food composition, but is associated with variations in individual genotypes (34). The results of the present study, however, do not support their hypothesis and RMA does not seem to be a general phenomenon as claimed by various authors (2, 30, 35, 36). Ehrman (37) suggested that females obtain information about the frequencies of different kinds of males by means of airborne olfactory cues, enabling them to distinguish rare males in the population. It has been claimed (37-39) that these olfactory cues are pheromones which differ among genetically different strains. They further suggested that a female would be least sensitive to the pheromone which is in highest concentration in its environment as a result of sensory adaptation. The chemoreceptor response thresholds of females would be lower to pheromones produced by rare males, because the chemoreceptors of females would be less adapted to the pheromones of rare males.

Molin (9) attempted to test this olfactory cue hypothesis by using peppermint essence and she concluded that artificial scent due to peppermint essence can be a sufficient cue to produce a rare male advantage. She used flies of the same genetic origin (oregon strain), but raised in different culture mediums, regular mediums and medium scented with peppermint essence. When the proportion of flies raised in normal and scented medium were varied, females appeared to prefer the rare types, suggesting that the olfactory cue hypothesis is valid. It should

also be noted that in these mating experiments marking by wing clipping had to be applied to distinguish between the flies raised in normal and scented mediums.

In the present study the experiments by Molin (9) were repeated. The results of these experiments did not show any rare male advantage. Therefore the olfactory cue hypothesis is not supported. Kence (23, 24) suggested that RMA observed in these experiments may be due to alternate marking of two types of males between the successive runs of an experiment. When the results of rare type marked and common type marked runs are pooled, a bias in favour of rare types is predicted by Kence (23, 24) if marking reduces the mating success of male flies. In these experiments pooled results also did not show any RMA (Tables 4 and 5). This result is not surprising since the effect of wing clipping in these experiments was very small. Indeed, the differences between observed mating frequencies and expected mating frequencies predicted by the algebraic mating model of Kence (23) was negligible. The expected K values for rare males in the pooled results were 1.002298 and 1.00298 (Tables 4 and 5 respectively). To detect an increase in K values to the order of 0.003 would require much larger sample sizes than those used in the present study. It is not possible to assess the level of marking effect in Molin's (9) study, since she presented only the pooled results.

In conclusion, the results of this study do not support the contention that RMA is a general phenomenon. Therefore RMA is probably not an important factor maintaining genetic variation in natural populations of *Drosophila*.

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