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Multiple Mating and Sperm Displacement in a Natural Population of *Drosophila melanogaster*

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Summary. Mother-offspring data for alcohol dehydrogenase genotypes of a vineyard cellar population of *D. melanogaster* are best explained by a model that allows 21% of females in the population to mate twice with an 83% level of sperm displacement. A population model with multiple mating and sperm displacement is examined theoretically. A formula for the effective population size is derived under this model. Multiple mating increases the effective population size relative to single mating.

Key words: Population genetics – *Drosophila melanogaster* – Multiple mating – Alcohol dehydrogenase

Introduction

In *Drosophila* there is considerable evidence for the occurrence of multiple paternity in both natural and laboratory populations (Dobzhansky and Pavlovsky 1967; Fuerst et al. 1973; Richmond and Ehrman 1974; Anderson 1974; Milkman and Zeitler 1974; Stalker 1976; Pruzan 1976; Cobbs 1977; Petit et al. 1980). Laboratory studies have shown that remated females of *D. melanogaster* maintain higher levels of progeny production throughout their life (Pyle and Gromko 1978), since without remating sperm storage organs become depleted (Lefevre and Jonsson 1962). However, female *D. melanogaster* are non-receptive of a second male immediately after mating. This has been firmly established from laboratory studies (Manning 1967), as has the gradual recurrence of receptivity as sperm are depleted (Gromko and Pyle 1978). A female who accepts every amorous male would likely be at a disadvantage since there would be little remaining time or energy for feeding and oviposition. Perhaps in nature there has been stabilising sexual selection among females for some optimal level of multiple insemination.

When a female is mated by more than a single male differential utilisation of the transmitted sperm occurs. This is usually described in terms of 'sperm displacement' or 'sperm precedence' (Lefevre and Jonsson 1962; Parker 1970; Fowler 1973; Cobbs 1977) and it is thought to have arisen via sexual selection among males (Prout and Bundgaard 1977).

Although we now have some insight into the type of selective processes responsible for multiple mating and sperm displacement, and of the adaptive importance of repeated mating, there has been little discussion about the population genetic consequence of concurrent multiple paternity. Two possibilities have been suggested. The increase in genetic heterogeneity among offspring of multiply-mated females may result in reduced competition for larval resources (Richmond and Ehrman 1974), and the effect of multiple mating on effective population size (Cobbs 1977).

In field studies when mating patterns are deduced from the genotypes of a female and her offspring, estimates of concurrent multiple paternity are essential. If these estimates are not accurately ascertained one general consequence is that the mating propensity of the heterozygous male may be overestimated (Milkman and Zeitler 1974; Cobbs 1977). Apparent examples of such heterozygote advantage (Richmond and Powell, 1970; McKenzie and McKechnie, 1981) need therefore to be considered with caution. Also, many estimates of multiple insemination levels have not considered sequential insemination of a female by males of the same genotype and hence they underestimate multiple paternity (Milkman and Zeitler 1974; Anderson 1974).

An excellent analysis and estimation of concurrent multiple mating in natural populations, taking sperm displacement into account, has been presented by Cobbs (1977). He used a multiple-allelic sex-linked locus in *D. pseudoobscura* and considered maximum likelihood estimates based on a probability distribution postulated to describe the mating process. In this paper we examine the distributional relationship between maternal and offspring genotypes at an autosomal two-allele locus for single and multiple mating models, the latter incorporating sperm displacement. A formula is derived for the effective population size under multiple mating; this shows an increase from a single-mating scheme. The models are then related to data collected for the alcohol dehydrogenase (*Adh*) genotypes of

Drosophila melanogaster of a vineyard cellar population and the double-mating and sperm displacement parameters in the model estimated by maximum likelihood.

Materials and Methods

Flies were collected from the cellar population of *Drosophila melanogaster* at the "Chateau Tahbilk" vineyard (McKenzie and Parsons 1972) at approximately monthly intervals for a two-year period. Individual flies were collected directly off the surface of wooden vats, where they aggregate around the seepage, into glass vials. This collection technique avoids the possibility of females mating subsequent to capture, which is a difficulty in the majority of studies where flies are attracted to baits (Richmond 1976). In this study, therefore, the progeny of a female are certainly the products of field insemination prior to capture.

Individual females were allowed to oviposit on standard medium for 4 days at 20 °C and then electrophoretically scored for *Adh* Genotype on Cellogel. The *Adh* genotypes of 22 progeny randomly chosen from the total progeny from each female were similarly determined when they emerged as adults. The data were pooled over collections as similar distributions were observed throughout the study.

Results

The observed distribution of families among genotype progeny classes for the female parents is given in Tables 1 and 2. Homozygous females can, of course, have only two possible genotypes among their offspring, while heterozygotes may have all three. In Table 2 the number of heterozygote offspring in a family is given by the difference from 22 of the homozygous offspring. The gene frequency of F in the population, estimated from a larger data set, is $\hat{p} = 0.73$. Indeed, the gene frequencies of F gametes contributed by male parents in the offspring of FF and SS female parents are 0.738 and 0.752 respectively. The estimated gene frequency of F gametes contributed by male parents in the offspring of FS female parents is 0.720.

A combined estimate of the gene frequency in the population (of males), weighted according to the number of offspring in the three groups, is thus

$$\frac{339 \times 0.738 + 59 \times 0.752 + 266 \times 0.720}{339 + 59 + 266} = 0.732 .$$

For modelling purposes we assume that the gene frequency of F in the population is 0.73.

Table 1. Offspring data from homozygous female parents

j	FF female parents. No. of families with j FF offspring	SS female parents. No. of families with j FF offspring	Homozygous fem. parents. Total no. of families with j FF (FS) offspring	Single mating	Double mating $\hat{\gamma} = 0.21$, $\hat{\pi} = 0.83$
	Observed			Expected	
0	22	4	26	29.0	23.8
1	2	0	2	10.5	9.0
2	1	0	1		
3	0	0	0		
4	1	0	1		
5	1	2	3		
6	5	0	5		
7	1	1	2		6.1
8	8	2	10	12.0	11.2
9	18	2	20	18.6	17.4
10	16	5	21	24.2	22.9
11	14	1	15	26.4	25.5
12	18	4	22	24.2	24.1
13	17	0	17	18.6	19.4
14	13	0	13	12.0	13.3
15	9	1	10	10.5	7.8
16	2	0	2		23.8
17	3	1	4		
18	6	1	7		
19	5	1	6		
20	4	0	4		
21	11	1	12		
22	162	33	195	212.1	193.8
Total no. of families	339	59	398	398.1	398.1

Table 2. Offspring from FS Females. Cells not shown have no entries in them

FF Offspring	SS Offspring															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
0	1					1		1	1	1	7	1	2		2	17
1			1		1	1		1	1							5
2								2			1					3
3						1	2	2	3	1						9
4	1	1	1	3			2	3		2	1					14
5	1		1	1	4	4	4	7	1	1	1					25
6	4	3	4	4	4	7	3	3	2	1						35
7	6	2	2	2	4	5	3	2								26
8	9	3		1	2	1	1	2								19
9	7	1		3	2	1			1							15
10	14	2		2		3										21
11	15				1											16
12	17	2			1											20
13	18	1	1													20
14	6															6
15	6															6
16	4															4
17	2															2
18																
19	2			1												3
20																
21																
22																
	113	15	10	17	19	24	15	23	9	6	10	1	2		2	266

Single Mating Model

The simplest model that one could assume is one in which:

(i) the population is in Hardy-Weinberg equilibrium;

(ii) there is no mutation or selection operating;

(iii) there is single, random mating;

(iv) fertilisation occurs randomly between the sperm of the mating male and the female gametes.

The probability that the family of an FF female has the (FF, FS) distribution of (j, 22 − j) is

$$2pq\binom{22}{j}\left(\frac{1}{2}\right)^{22}, \quad j = 1, \ldots, 21$$

since the male parent in this case must be a heterozygote. The probabilities of obtaining all FS offspring or all FF offspring are respectively

$$q^2 + (2pq)\left(\frac{1}{2}\right)^{22} \quad \text{and} \quad p^2 + (2pq)\left(\frac{1}{2}\right)^{22}.$$

These families with the same genotype for all offspring will come largely from matings with homozygous males.

Offspring types (FF, FS) from FF females, and (FS, SS) from SS females have the same probability distribution under random mating, since the same type of male gamete contributed determines the respective genotypes of the offspring.

An observed and expected distribution of (FF, FS) offspring from FF females and (FS, SS) offspring from SS females combined is given in Table 1. A goodness-of-fit test gives $X^2 = 122.4$ compared to χ^2 with 10 d.f. Single mating clearly gives a very poor fit. Under the model the distribution should be symmetric about 1 to 11 and 11 to 21: this is not so in the observed data, the lower tail being too heavy. There is also a decrease in families of only one genotype in the observed data. It is possible that a model of single mating with heterozygote advantage would explain this decrease, but this would not explain the very heavy lower tail.

Data for heterozygote female offspring are rather sparse, so as a condensation we consider the three marginal distributions of the number of FF, SS und FS offspring. The probability of having j FF offspring in a family is

$$p^2\binom{22}{j}\left(\frac{1}{2}\right)^{22} + 2pq\binom{22}{j}\left(\frac{1}{4}\right)^{j}\left(\frac{3}{4}\right)^{22-j}, \quad j = 1, \ldots, 22$$

and the probability of having no FF offspring in a family

$$p^2\left(\frac{1}{2}\right)^{22} + 2pq\left(\frac{3}{4}\right)^{22} + q^2.$$

Table 3. Distribution of offspring from FS female parents

j	Number of families with j FF offspring			Number of families with j SS offspring		
	Observed	Single mating Expected	Double mating $\hat{\gamma}=0.21, \hat{\pi}=0.83$ Expected	Observed	Single mating Expected	Double mating $\hat{\gamma}=0.21, \hat{\pi}=0.8$ Expected
0	17	19.6	16.7	113	141.9	132.9
1	5	6.2	8.0	15	6.2	6.7
2	3			10		
3	9	10.7	10.7	17	10.7	12.4
4	14	17.1	16.8	19	16.9	17.7
5	25	21.2	21.0	24	20.4	20.5
6	35	21.7	22.0	15	19.5	19.2
7	26	20.3	21.2	23	15.4	15.0
8	19	19.9	21.1	9	10.6	10.4
9	15	21.5	22.5	6	7.0	6.9
10	21	23.9	24.4	10	17.4	16.3
11	16	24.6	24.5	1		
12	20	22.1	21.6	2		
13	20	16.9	16.3	0		
14	6	10.8	10.3	2		
15	6	9.5	8.9	0		
16	4			0		
17	2			0		
18	0			0		
19	3			0		
20	0			0		
21	0			0		
22	0			0		
Total no. of families	266	266	266	266	265.9	266

The SS distribution is similar, but with p and q interchanged. The probability of having j FS offspring in a family is

$$\binom{22}{j}\left(\frac{1}{2}\right)^{22}, \quad j=0,1,\ldots,22$$

not depending on p.

Observed and expected frequencies are given in Table 3. Goodness-of-fit tests show that the FF offspring distribution is on the borderline of fitting, $X^2 = 23.6$ compared to χ^2 with 14 d.f., while the SS offspring distribution does not fit well, $X^2 = 73.0$ compared to χ^2 with 9 d.f. However, the data sets are not independent, so the results must be interpreted cautiously.

Multiple Mating Model

In this model the following assumptions are made:

(i) The population is in Hardy-Weinberg equilibrium;

(ii) there is no mutation or selection operating;

(iii) the probability that a fertilized female has been mated k times is

γ_k, $k=1,2,\ldots, \gamma_1+\gamma_2+\ldots=1$;

(iv) given that there were k matings, the probability that a fertilized egg has been fertilized by the jth male is

$\pi_{jk}, j=1,\ldots,k. \quad \pi_{1k}+\ldots+\pi_{kk}=1.$

This assumption is to allow for sperm displacement.

(v) Once multiple mating is completed and sperm displacement has taken place, the eggs are fertilized independently from the sperm bank (Lefevre and Jonsson 1962).

It is not possible to tell from the offspring which of the probabilities corresponds to the jth male and the model is invariant under permutations of the parameters $\{\pi_{jk}\}$.

All the assumptions are necessarily simplistic, particularly (iv) as it seems likely that the amount of sperm displacement will depend on the times between fertilization.

When there are multiple offspring it is important to consider how the probability, P, of a male F gamete being contributed to an egg in a fertilized female varies depending on the different male crosses. A respresentation of this random variable is

$$P = \pi_{1K} X_1 + \pi_{2K} X_2 + \ldots + \pi_{KK} X_K,$$

where $X_1, X_2, \ldots$ are mutually independent, identically distributed random variables such that

$$\Pr(X=1) = p^2, \quad \Pr(X=\tfrac{1}{2}) = 2pq, \quad \Pr(X=0) = q^2$$

and K is a random index, independent of the X sequence such that

$$\Pr(K=k) = \gamma_k, \quad k = 1, 2, \ldots$$

The proportion of F gametes contributed to a female by the jth male in K matings is $\pi_{jK} X_j$. The values $X_j = 1$, ½, 0 correspond to the jth male being of genotype FF, FS or SS respectively. (A more general model where the male genotype frequencies in the population were not in Hardy-Weinberg equilibrium would replace p^2, 2 pq, q^2 by the respective male frequencies of the FF, FS, and SS genotypes.)

A simple notation used for double mating is

$$\pi_{12} = 1-\pi, \quad \pi_{22} = \pi, \quad \gamma_2 = \gamma, \quad \gamma_1 = 1-\gamma.$$

The model is symmetric in π and $1-\pi$.

Random mating implies that the expected gene frequency in the population remains at p and Hardy-Weinberg equilibrium is also maintained. The increased effective population size caused by multiple mating means that such a population is less likely to become fixed by fluctuations due to random drift than a population with single mating.

Effective Population Size

It is of interest to derive a formula for the effective population size. Denote N_M and N_F as the number of male and female flies in the population, held constant over generations, and $N = N_M + N_F$. The (inbreeding) effective population size is defined by $N_e^{-1} = 2 \times$ Probability that two genes chosen at random from the population have the same gene parent in the preceding generation (see, for example Ewans 1979)). The probability that two genes chosen are from the same female parent gene is $(N-1)/\{4(2N-1)N_F\}$ and the probability they are from the same male gene parent is

$$\frac{Q(N-1)}{4(2N-1)N_F} + \frac{(N_F-1)(N-1)}{4(2N-1)N_M N_F},$$

where $Q = \sum_{k \geqq 1} \gamma_k \sum_{j=1}^{k} \pi_{jk}^2$ is the probability that two offspring chosen from within a family have been sired by the same male. Therefore for large N_M, N_F,

$$N_e = \frac{4 N_M N_F}{N(1+\alpha Q)},$$

where $\alpha = N_M/N$.

The ratio of multiple mating effective population size to that with single mating is $(1+\alpha)/(1+\alpha Q)$. In the particular case of double mating, $Q = 1-\gamma+\gamma \times (\pi^2 + (1-\pi)^2)$. The effective population size increases with the amount of double mating and decreases as sperm displacement increases from 0.5 to 1.

Description of the Offspring Distributions

We suppose that data is available of the genotype of female parent together with the genotypes of n of her offspring, randomly chosen.

Let M denote the number of FF offspring, out of n from an FF female parent. (The number of FS offspring from an SS female parent has the same probability distribution.) The probability distribution of M is

$$\Pr(M=m) = \binom{n}{m} E\{P^m (1-P)^{n-m}\}, \quad m = 0, 1, \ldots, n. \tag{1}$$

E denotes expectation in the distribution of P.

In the offspring of heterozygous females denote

M = number of FF offspring in a family,
R = number of SS offspring in a family, and
U = number of FS offspring in a family.

$$\Pr(M=m, R=r) = \frac{n!}{m!\, r!\, (n-m-r)!\, 2^n} E(P^m (1-P)^r), \quad m + r \leq n. \tag{2}$$

The marginal probability distributions are

$$\Pr(M=m) = \binom{n}{m} E(\tfrac{1}{2}P)^m (1-\tfrac{1}{2}P)^{n-m}, \quad m = 0, 1, \ldots, n$$

$$\Pr(R=r) = \binom{n}{r} E(1-P)^r (1+P)^{n-r} (\tfrac{1}{2})^n, \quad r = 0, 1, \ldots, n$$

and

$$\Pr(U=u) = \binom{n}{u} (\tfrac{1}{2})^n, \quad u = 0, 1, \ldots, n.$$

The above distributions do not have a simple form but can be calculated numerically. Clearly there is no information about multiple mating in the distribution of U. The conditional distribution of M, given that there are k homozygotes in a family, is the same as the distribution of the number of FF offspring in a family of k from an FF female.

Double Mating Fitted to the Data

The observed distribution in Table 1 shows an indication of multiple mating, the number of families of one genotype being too few, and the distribution in the cells 1 to 21 being asymmetric.

A model of double mating was fitted to the data. In this case there are two parameters other than the gene frequency:

γ = probability that a fertilized female has been mated twice; and

π = probability that a fertilized egg has been fertilized by the 2nd male, given double mating.

Maximum likelihood estimates were $\hat{\gamma} = 0.21$, $\hat{\pi} = 0.83$ based on the distributions (1) and (2) and the observed frequencies in Tables 1 and 2. Unfortunately there are no simple analytic expressions for the estimates and they had to be found numerically by searching for the maximum likelihood over a grid of points $0 \leqq \gamma < 1$, $0.5 \leqq \pi < 1$ with a mesh size of 0.01×0.01. As the model is symmetric in π, $1 - \pi$ the estimate is really $\hat{\pi} = 0.83$ or $\hat{\pi} = 0.17$. The variance-covariance matrix was calculated from the information matrix and $\mathrm{var}(\hat{\gamma}) = 0.0013$, $\mathrm{var}(\hat{\pi}) = 0.0015$, correlation $(\hat{\gamma}, \hat{\pi}) = 0.16$. A 95% confidence region for the parameters is shown in Figure 1. Only the region for $\hat{\pi} = 0.83$ and not $\hat{\pi} = 0.17$ is shown.

The maximum likelihood estimate of γ is robust under different values of the parameter π; $\hat{\gamma}$ is between 0.2 and 0.3 for π between 0.5 and 0.9. When $\pi = 0.5$, then $\hat{\gamma} = 0.21$ also. Denote the likelihood as $L(\gamma, \pi)$. A likelihood ratio test of whether there is single or double mating is to compare $-2\{\log L(0, 0.5)/L(\hat{\gamma}, \hat{\pi})\} = 748.9$ with χ^2 with 2 d.f. and a test ot whether sperm displacement is random or not ($\pi = 0.5$) to compare $-2 \log\{L(\hat{\gamma}, 0.5)/L(\hat{\gamma}, \hat{\pi})\} = 167.4$ with χ^2 with 1 d.f. Both of these are clearly significant at the 5% level, so there is multiple mating, and sperm displacement is not random.

The effective population size with the parameter values $\hat{\pi}$, $\hat{\gamma}$ is 1.02 times the effective population size for single mating, assuming the proportion of males in the population is 0.5. Expected frequencies of the offspring distribution from homozygous females and marginal SS and FF offspring distributions from heterozygous females are given in Tables 1 and 3.

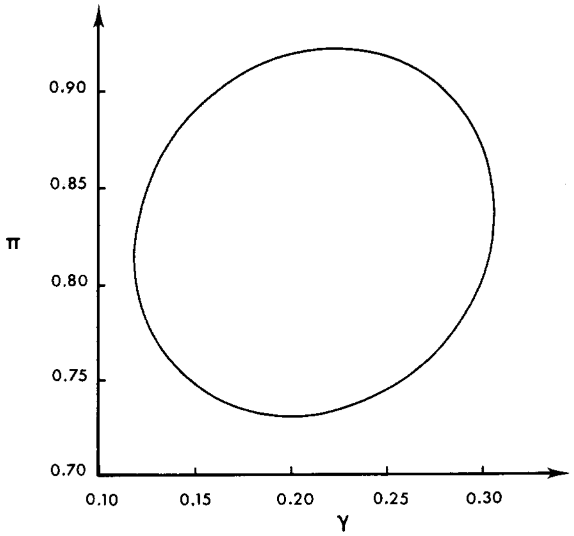

Fig. 1. 95% Confidence region for the estimates of γ, π

One overall criterion of whether the model fits is to consider the total X^2 from the offspring distribution of homozygous parents and SS marginal distribution from heterozygous parents. This gives $X^2 = 37.0$ which is not significant at the 1% level, but significant at the 5% level compared to χ^2 with 20 d.f. If the FF marginal distribution from heterozygous parents is used instead of the SS distribution, $X^2 = 38.5$, which is again not significant at the 1% level but significant at the 5% level, compared to χ^2 with 24 d.f. A double mating model is a large improvement over single mating where $X^2 = 195.4$ compared to χ^2 with 19 d.f. if the SS distribution is used, and $X^2 = 146.0$ compared to χ^2 with 24 d.f. if the FF distribution is used. In calculating the d.f. in the double mating model, two d.f. have been subtracted for estimation of (γ, π). As the estimate of p is based on a larger set of data and its variance small, a d.f. is not subtracted for its estimation.

A minimum χ^2 method was also tried to estimate (γ, π) using the offspring distribution from heterozygous females. This gave estimates $\hat{\gamma} = 0.3$ and $\hat{\pi} = 0.8$. A difficulty with this method here is that grouping may bias the estimates, and maximum likelihood seems a better technique.

Another method of estimating the frequency of multiple mating, carried out by Sassaman (1978) and by Zouros and Krimbas (1970) is to infer multiple mating by a significant lack of fit to Mendelian expectations in each family. This is a very ad hoc statistical procedure; it has the problems of cumulative type 1 error, a high type 2 error, and it is not clear that it gives an unbiased estimate of the frequency of multiple mating.

Discussion

Mother-offspring data collected for the *Adh* polymorphism of *D. melanogaster* in the cellar population at Tahbilk have been analyzed by comparison of the distributions of families among classes of progeny genotypes with those expected under models of single and various degrees of double mating. Random mating has been assumed. A single male fertilising each female does not provide an adequate description of the distributions. However, a model allowing 21% of females to be double mated, with an average of 83% sperm displacement in these matings, provided an acceptable fit.

One genetic consequence of multiple mating is to increase effective population size. The magnitude of this increase in the Tahbilk population has been estimated at 1.02 times the effective population size under single mating, assuming the above parameter values. Although this effect is small, it may be one of the factors contributing to stabilisation of the genetic variation at this locus in the cellar, especially during winter when numbers are low (McKenzie and McKechnie 1981; McKenzie 1980).

The level of multiple mating estimated here is at the lower end of the range of estimates for field studies of various other *Drosophila* species (Anderson 1974; Stalker 1976; Richmond 1976; Cobbs 1977). It is also lower than the only other estimate we know of for a natural population of *D. melanogaster* – a level of 47% (minimal level of concurrent multiple paternity) in a fruit market collection made by Milkman and Zeitler (1974). (Note, however, that Craddock and Johnson (1978) have reported 4% for *D. silvestris.*) Previous estimates have possibly been biased towards higher values due to the likelihood of multiple insemination occurring subsequent to capture (Richmond 1976). Such a difficulty has been avoided in this study by the collection technique used. Whether the lower estimate in the Tahbilk population of *D. melanogaster* is due to this effect, to different estimation techniques, or to differences in mating structures among *Drosophila* populations remains to be shown.

It is more difficult to compare our estimated level of sperm displacement with those in other populations since field data are rare (Cobbs 1977). Estimates from laboratory studies suggest that the amount of displacement may range from nothing to virtually complete displacement, although this is likely to be dependent upon the species, genotype or experimental design utilised (Lefevre and Jonsson 1962; Dobzhansky and Pavlovsky 1967; Fuerst et al. 1973; Stalker 1976; Gromko and Pyle 1978). Suffice it to say that an average value of 83% in a natural population of *D. melanogaster* does not seem unreasonable considering previously published laboratory data.

The models have been based on an assumption of random mating. Modification of the single mating model to include heterozygote male mating advantage could provide a better fit than random mating to some, but not all, characteristics of the data. In particular, the decrease in single genotype sibships from homozygous females accords with heterozygote advantage, but the asymmetry of the progeny distribution does not (Table 1). The general difficulty of deducing specific mating patterns from a comparison of maternal and offspring phenotypes when multiple insemination occurs in the population is emphasized by the model in this paper. Laboratory studies with *Adh* genotypes have shown male heterozygotes to mate more commonly with virgin females than do either of the homozygotes. Subsequent mating of the inseminated females was found to be random with respect to the genotype of the male (McKenzie and Fegent 1980). A mating advantage of this type could contribute to the maintenance of the *Adh* polymorphism (McKenzie and McKechnie 1981) but any contribution of mating behaviour must be placed in a context of its relation to overall fitness. This can only be assessed when the relative success of different genotypes at all stages of the life cycle is elucidated. Elegant laboratory experimentation has been directed towards this end with some success (Prout 1971; Bundgaard and Christiansen 1972). In appropriate ecological circumstances similar estimations may be attempted in natural populations (Christiansen and Fenchel 1977).

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