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EVIDENCE FOR PREFERENTIAL MATING IN *ADALIA BIPUNCTATA*

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SUMMARY

Non-random mating may be involved in the maintenance of colour polymorphism in *Adalia bipunctata*. Mating choice experiments have been carried out on a stock of *A. bipunctata* from Keele. Specific frequencies of the melanic *quadrimaculata* form and the non-melanic *typica* form were placed in population cages or mating chambers and allowed to mate. The frequencies of the forms amongst mating pairs were scored. The results gave a good fit to a model of mixed sexual selection and assortative mating showing a strongly frequency dependent, non-assorting preference for *quadrimaculata* males in the matings. Data from the wild Keele population showed a similar preference for *quadrimaculata* males, and an excess of males of a second melanic phenotype, *sexpustulata*. Observation of courting pairs indicated that female choice determined frequency of mating. Field data from Keele also gave evidence of assortative mating between *typica* and another non-melanic form, *annulata*.

1. INTRODUCTION

THE ladybird *Adalia bipunctata L.* is polymorphic for the colour and pattern of elytra and pronotum. There is a wide range of forms from all red to almost all black (Mader, 1926–37). The forms are controlled by a multiple allelic series comprising at least 11 alleles at a single locus. In general the more melanic forms are dominant to non-melanics (Lus, 1928, 1932). In England the commonest melanic forms are *quadrimaculata* and *sexpustulata* (black with four and six red spots respectively), and the most frequent non-melanics are *typica* (red with two round black spots) and a variable phenotype which is red with two irregular black patches or with two large black spots each having one or more small satellite spots. Following Lus (1928), this form will be referred to as *annulata*. There are a number of forms of *A. bipunctata* which are non-melanic but have more black markings than *typica*. These include *interpunctata*, *unifasciata*, *haneli*, and *annulata*, which are all described by Lus (1928) as homozygous forms of the *annulata* allele S^a. The genetic relationships between these forms and other similar forms are not clearly understood, and in this paper they will all be classed together under the name *annulata*.

Mating pairs are often seen in the field. Lusis (1961) presented data showing that the frequency of melanics amongst mating pairs from Riga and Moscow was greater than in the populations from which they came. He suggested that this excess occurred because melanics have greater activity in sunlight. This he related to data recorded by Timofeeff-Ressovsky (1940) in Berlin. Here the frequency of melanics increased during the summer months. However, Creed (1975) found no excess of melanics in summed data from several British populations or from Potsdam (data quoted by Meissner, 1907*a*, *b*, 1909). The method of analysis used by both

Lusis and Creed is unsatisfactory for two reasons. Firstly, an excess of one morph in matings relative to the frequency of that morph in the population as a whole, combined with non-random mating among the mating imagines, could lead to apparent random mating. Secondly the method of analysis used is not valid because the expected values are calculated from samples which are themselves subject to sampling errors.

Muggleton (1979), using Meissner's data (1c) and data collected in 1976 and 1977 in England, suggested that non-random mating in *A. bipunctata* is frequency dependent, melanics being in excess when at a frequency of less than 40 per cent of the population, and typicals in excess when melanics comprise over 50 per cent of the population. O'Donald and Muggleton (1979) fitted this data to a model of constant and variable mating preference developed by O'Donald (1978). They found that the frequencies in Potsdam matings gave rise to strongly frequency dependent sexual selection, while the frequency dependence from the English data was less pronounced. However, the model used takes no account of the different phenotypes in the main data classes, melanics and non-melanics.

Karlin and O'Donald (1978) have developed a mixed assortative mating and sexual selection model for a dominant trait. This paper describes experimental and field data on the relative mating frequencies of the *typica* and *quadrimaculata* forms of *A. bipunctata* and the fitting of these data to Karlin and O'Donald's model of sexual selection and assortative mating.

2. Materials and methods

Adalia bipunctata is widely distributed over the temperate regions of the northern hemisphere. In Britain it is widely distributed and common. It hibernates as an imago. Generally, hibernating adults become active in late April or May. The ladybirds then mate and lay eggs in batches on herbage infected by aphids. Females lay on average approximately 250 eggs, but there is considerable variation and totals of over a thousand have been recorded (Weir, pers. obs.). Females mate several times. Hodek (1973) notes that one copulation is sufficient for permanent fertility of females in most species of ladybird. However, Ellingsen (1969) questions this for *A. bipunctata* because the death of males was correlated with a reduction in overall viability. Furthermore, if females are kept isolated from males after the first mating, the fertility of the later eggs is greatly reduced (Weir, pers. obs.). Sem'yanov (1970) has shown that copulation stimulates oviposition in *A. bipunctata*; the maximum number of eggs is laid after a female's first mating; oviposition then decreases but increases after each further mating.

The duration of the immature stages is variable, depending primarily on climate and food availability. It may be as little as 21 days in the laboratory, although five to eight weeks is probably usual in the wild in Britain. Muggleton (1978) suggests that in England *A. bipunctata* is univoltine. He noted that in southern and eastern England there was apparently only one generation in 1976, no mating pairs being observed after the end of June. However, Creed (1975) assumed that there were up to three generations a year in England. Furthermore matings have been noted during August in Surrey in 1975 and 1977, Staffordshire in 1981, and Cambridge in 1981 (Majerus, pers. obs.). If *A. bipunctata* is multivoltine

in Britain the generations certainly overlap. Females will continue laying eggs for over three months during the summer, so that the hibernating populations may comprise imagines from two or more generations. In the wild both the larvae and adult ladybirds feed on various species of aphid, and adults will migrate from one plant species to another depending on aphid availability. They may be found on both herbaceous and arboreal species. They move to hibernation sites in late August and September.

The genetic relationships between some of the forms of *A. bipunctata* were established by Lus (1928, 1932) by breeding laboratory stocks of discrete families for up to five generations. Of the forms discussed in this paper, he notes that there is a straightforward dominance hierarchy from the top dominant *quadrimaculata* through *sexpustulata*, and *typica*, to *annulata*, the bottom recessive.

Hodek (1973) notes that inbreeding causes a very rapid decline in viability; this is because *A. bipunctata* has a number of recessive alleles which are lethal when homozygous and the frequency of these is high in all populations (Lusis, 1947*a*, *b*).

The stock being considered in this paper originally came from a population in the grounds of Keele University, Staffordshire (grid ref: SJ823441). Two samples of ladybirds comprising four phenotypes, *quadrimaculata*, *sexpustulata*, *typica* and *annulata*, were collected in May 1981.

The main stock was reared in population cages in a glasshouse. The cages were 2 m × 1 m × 1 m in size. They were constructed of perspex and supported by a dexion frame with wood and muslin doors at each end. Each cage contained four trays of sand in which six 125 mm pots of broad beans (*Vicia faba L.*) were growing, six plants per pot. The trays of beans were changed by rotation, the oldest tray being substituted by a new tray of two-week-old beans each week. Fresh beans were infected with the aphid *Acyrthosiphon pisum* Harris. When necessary supplementary heat and light were used to ensure a minimum temperature of 21.1°C and 16 hours light.

This stock has been used for two types of experiment to investigate mating preferences between phenotypes; population cage experiments and formal mating tests. Data were also collected from the Keele population in August 1981.

(i) *Population cage experiments*

Populations of 100 ladybirds of specific phenotypes were set up in the population cages and maintained as outlined above. Two phenotypes, *quadrimaculata* and *typica* were used. Three phenotypic ratios were used in separate experiments: 30 *quadrimaculata*, 70 *typica*; 50 *quadrimaculata*, 50 *typica*; 70 *quadrimaculata*, 30 *typica*. Two experiments were conducted at each ratio. In each population there were equal numbers of males and females. It is difficult to differentiate the sexes of *A. bipunctata* by sight. Males are generally smaller than females and have a more rounded abdomen; however, these characters are not absolutely reliable. Despite this we have found that with practice we can attain an accuracy exceeding 96 per cent when determining the sexes of our laboratory stocks, and 98 per cent when sexing ladybirds in the field where they seem to vary less in size.

The phenotypes *in copuli* were recorded by direct observation every hour. The duration of each experiment varied slightly; the end of each experiment being determined by the emergence of the first progeny which occurred generally about four weeks after the beginning of the experiment.

(ii) *Formal mating tests*

A sample of ladybirds was removed from the main stock. The sex of each individual was determined and males and females were kept separate for at least 48 hours prior to a test. Females laying eggs during this period were returned to the main stock. The procedure of keeping the sexes separate would also allow males which had recently mated to recover their normal levels of sexual activity. Equal numbers of males and females of each phenotype (*quadrimaculata* and *typica*) were introduced into a mating chamber. When matings occurred, pairs were removed from the chamber and replaced by a male and female of the same phenotype. The tests lasted approximately eight hours or until the replacement stocks had been exhausted. The phenotypes of the mating pairs were recorded.

(iii) *Keele population field data*

Data on mating preferences in the wild Keele population were collected to compare with the results of the population cage experiments and formal mating tests. To assess the frequency in the population as a whole, samples of mating and non-mating ladybirds were recorded during 3–5 August 1981, both the phenotype and sex of each individual being noted.

3. Results

(i) *Population cage experiments*

The data from the population cage experiments are given in table 1(i). Comparisons of the data from each pair of experiments at a particular frequency showed no significant heterogeneity. Chi-squared tests, the results of which are given in table 1(ii), showed significant deviations of the observed matings from the expectations based on the frequencies of the phenotypes used in the experiments. Analysis of chi-squared demonstrated that this was due to a significant excess of *quadrimaculata* males in all the experiments. This analysis also showed that there was no significant deviation from expected values amongst mating females, nor was the residual chi-squared, which is partly dependent upon assortment in the matings, significant. Direct tests of the significance of assortment, using a cross product ratio, also gave non-significant results at all frequencies.

Karlin and O'Donald (1978) described a model which combined both assorting and non-assorting preferences for two phenotypes. O'Donald (1980) fitted this model to data of matings of two mutant strains of the parasitic wasp *Mormoniella vitripennis*. The same method can be used to fit the model to the population cage data of the *typica* (T) and *quadrimaculata* (Q) ladybirds. Consider the two phenotypes T and Q at frequencies u and v in males and females. The following parameters describe the

TABLE 1

(i) *The phenotypes of mating pairs in the population cage experiments* (Q = quadrimaculata, T = typica)

Experiment number	Number of Ladybirds	Q×Q	Matings Q×T	Observed T×Q	(Males first) T×T	Total
5	30Q, 70T	13	21	12	27	73
12	30Q, 70T	16	27	14	34	91
9	50Q, 50T	24	20	9	9	62
10	50Q, 50T	18	13	7	7	45
8	70Q, 30T	71	24	15	8	118
11	70Q, 30T	60	27	12	7	106

(ii) *Analysis of chi-squared*

Component of variation	30Q:70T	50Q:50T	70Q:30T	Dfs
Deviation in males	22·4402	17·2804	13·5000	1
Deviation in females	0·9767	0·7570	0·0306	1
Residual	2·3928	0·7570	0·7711	1
Total	25·8097	18·7944	14·3017	3

"Dfs" refers to degrees of freedom for the tests of each pair of experiments at a given ratio of Q:T.

preferential mating:

	Preference for T	Preference for Q
Non-assorting preference	α	γ
Assorting preference	a	c

Preferential matings thus take place at the following frequencies:

	Male T	Male Q
Female T	$u(\alpha+a)$	γu
Female Q	αv	$v(\gamma+c)$

Since T and Q females mate at random with frequencies $u(1-\alpha-\gamma-a)$ and $v(1-\alpha-\gamma-c)$, the total frequencies of the matings, preferential and random, will then be:

$$P_T(\mathrm{T}\times\mathrm{T}) = u(\alpha+a)+u^2(1-\alpha-\gamma-a)$$
$$P_T(\mathrm{T}\times\mathrm{Q}) = \gamma u+uv(1-\alpha-\gamma-a)$$
$$P_T(\mathrm{Q}\times\mathrm{T}) = \alpha v+uv(1-\alpha-\gamma-c)$$
$$P_T(\mathrm{Q}\times\mathrm{Q}) = v(\gamma+c)+v^2(1-\alpha-\gamma-c)$$

When multiplied by the total number of matings observed at each ratio of males, these expressions then give the expectations of the numbers of matings observed. If, at a particular male ratio, the observed numbers are N(TT), N(TQ), N(QT) and N(QQ), then the log likelihood is given by the

equation

$$\log L = N(\mathrm{TT}) \log P_T(\mathrm{TT}) + N(\mathrm{TQ}) \log P_T(\mathrm{TQ}) + N(\mathrm{QT}) \log P_T(\mathrm{QT}) + N(\mathrm{QQ}) \log P_T(\mathrm{QQ})$$

where the summation is taken over each of the different experiments. The method of trial and error, described by O'Donald (1980, pp. 72–77) has been used to find the maximum likelihood estimates of the parameters. The expected numbers of the matings at maximum likelihood, corresponding to the observed numbers in table 1(i), were calculated from the values of $P_T(\mathrm{TT})$, $P_T(\mathrm{TQ})$, $P_T(\mathrm{QT})$ and $P_T(\mathrm{QQ})$, and hence the residual chi-squared after the fitting of the model. There are four classes of matings and three degrees of freedom after the total number of matings at each male ratio has been allowed for—nine degrees of freedom before any parameters have been fitted.

Table 2 gives the estimates of the parameters and analysis of chi-squared.

TABLE 2

Fitting data of matings of quadrimaculata *and* typica, *from the population cage experiments, to Karlin and O'Donald's model of sexual selection and assortative mating*

Parameters	M.L. estimates of parameters				Residual	Dfs
	$\hat{\alpha}$	$\hat{\gamma}$	$\hat{a}$	$\hat{c}$		
—	—	—	—	—	58·9059	9
α	0·0000	—	—	—	58·9060	8
γ	—	0·3046	—	—	7·0408	8
a	—	—	0·0000	—	58·9059	8
c	—	—	—	0·3924	19·4449	8
α, γ	0·0000	0·3050	—	—	7·0403	7
α, a	0·0000	—	0·0000	—	58·9059	7
α, c	0·0000	—	—	0·3939	19·4481	7
γ, a	—	0·3323	0·0677	—	6·1983	7
γ, c	—	0·2304	—	0·1619	3·5310	7
a, c	—	—	0·0000	0·3913	19·4435	7
α, γ, a	0·0000	0·3335	0·0694	—	6·1976	6
α, γ, c	0·0000	0·2300	—	0·1627	3·5315	6
α, a, c	0·0000	—	0·0000	0·3921	19·4445	6
γ, a, c	—	0·2304	0·0000	0·1620	3·5309	6
α, γ, a, c	0·0000	0·2312	0·0000	0·1597	3·5297	5

α is the non-assorting preference for *typica*.
γ is the non-assorting preference for *quadrimaculata*.
a is the assorting preference for *typica*.
c is the assorting preference for *quadrimaculata*.
When estimates are shown as 0·0000, this indicates that the estimate was less than 0·00005, but not zero.

Only γ and c remove significant variation, with maximum likelihood (M.L.) estimates

$$\hat{\gamma} = 0{\cdot}2304$$

$$\hat{c} = 0{\cdot}1619.$$

The results show that the assorting parameter c is not sufficient to provide a fit of the model for it leaves a significant chi-squared with

$p = 0{\cdot}0127$. However, the non-assorting parameter γ is sufficient to provide a fit of the model, for it leaves a non-significant chi-squared with $p = 0{\cdot}532$. If c is then fitted in addition to γ, the residual chi-squared is further reduced by 3·5098. This is an almost significant reduction, with $P = 0{\cdot}0610$. After fitting γ

$$\log_e L = -603{\cdot}335.$$

Then after fitting c as well

$$\log_e L = -601{\cdot}524.$$

So the extra parameter c has increased the log likelihood by 1·811. This is nearly, but not quite, a significant increase in log likelihood. So we conclude that the parameter γ is sufficient to fit the data with M.L. estimate

$$\hat{\gamma} = 0{\cdot}3046.$$

(ii) *Formal mating tests*

The results of the formal mating tests are given in table 3. The data show a significant deviation from expected. Analysis of chi-squared demonstrates this deviation to be due to a significant excess of *quadrimaculata* males in matings. Again there is no deviation from expected numbers of mating females, nor any significant assortment.

TABLE 3

Matings observed in formal mating tests using equal numbers of quadrimaculata (*Q*) *and* typica (*T*) (*male phenotype given first*)

Q×Q	Q×T	T×Q	T×T	Total
47	39	26	28	140

Analysis of chi-squared Component of Variation	Value of chi-squared	Dfs
Deviation from 1:1 in males	7·3143	1
Deviation from 1:1 in females	0·2571	1
Assortment	0·7143	1
Total	8·2857	3

The preference for *quadrimaculata* is easily estimated. The phenotypes were maintained with equal numbers of unmated *quadrimaculata* and *typica* males and females. If a proportion of γ females of each phenotype prefer *quadrimaculata* males, the matings should take place at frequencies

$$\gamma + \tfrac{1}{2}(1-\gamma) = \tfrac{1}{2} + \tfrac{1}{2}\gamma \quad \text{for } \textit{quadrimaculata}$$

and

$$\tfrac{1}{2} - \tfrac{1}{2}\gamma \quad \text{for } \textit{typica}.$$

If n_Q and n_T are the numbers of these matings, the maximum likelihood estimate of γ is given by

$$\hat{\gamma} = \frac{n_Q - n_T}{n_Q + n_T}$$
$$= 0{\cdot}229.$$

This estimate has variance

$$\text{var}\,(\hat{\gamma}) = \frac{1-\gamma^2}{n_Q + n_T}$$
$$= 0{\cdot}00677.$$

Thus

$$\hat{\gamma} \pm \text{S.E.}\,(\hat{\gamma}) = 0{\cdot}229 \pm 0{\cdot}082.$$

We may compare the estimates of the preference for *quadrimaculata* obtained from the results of each of the population cage experiments. In general if *quadrimaculata* is at a frequency v, the maximum likelihood estimate of the preference γ is given by

$$\gamma = \frac{n_Q(1-v) - n_T v}{n(1-v)}$$

where $n = n_Q + n_T$.

This estimate has the variance

$$\text{var}\,(\hat{\gamma}) = \frac{(1-\gamma)(v+\gamma-\gamma v)}{n(1-v)}.$$

Thus we obtain the following estimates with their standard errors.

(a) Cage experiments at 30 Q : 70 T

$$\hat{\gamma} = 0{\cdot}2422 \pm 0{\cdot}0557.$$

(b) Cage experiments at 50 Q : 50 T

$$\hat{\gamma} = 0{\cdot}4019 \pm 0{\cdot}0885.$$

(c) Cage experiments at 70 Q : 30 T

$$\hat{\gamma} = 0{\cdot}3750 \pm 0{\cdot}0869.$$

The weighted mean of these estimates is

$$\bar{\gamma} = \frac{\sum \hat{\gamma}/\text{var}\,(\hat{\gamma})}{\sum 1/\text{var}\,(\hat{\gamma})}$$
$$= 0{\cdot}3073.$$

This is very close to the overall maximum likelihood estimate of γ. The weighted mean of estimates for all population cage experiments and the formal mating tests is found to be

$$\bar{\gamma} = 0{\cdot}2914.$$

The heterogeneity in the four estimates is tested by

$$\chi^2_3 = \sum (\hat{\gamma} - \bar{\gamma})^2/\text{var}\,(\hat{\gamma})$$
$$= 3{\cdot}8475$$
$$P = 0{\cdot}278.$$

(iii) *Keele population data*

The data collected from the Keele population are presented in table 4(i), which gives the phenotypes of all the ladybirds sampled, including

TABLE 4

(i) *The phenotypes of* A. bipunctata *sampled at Keele, August 1981 (including mating pairs)*

	sub.	quad.	sexp.	typ.	ann.	Totals
Males	1	123	19	204	100	447
Females	3	111	14	197	84	409

(ii) *The phenotypes of mating pairs of* A. bipunctata *at Keele, August 1982*

	Females				
Males	quad.	sexp.	typ.	ann.	Totals
quad.	19	2	31	14	66
sexp.	3	1	6	2	12
typ.	20	2	28	8	58
ann.	11	1	9	12	33
Totals	53	6	74	36	169

sub. is *sublunata.*
quad. in *quadrimaculata.*
sexp. is *sexpustulata.*
typ. is *typica.*
ann. in *annulata.*

mating pairs, and table 4(ii), which gives the phenotypes of mating ladybirds. The frequencies of the phenotypes in mating pairs were compared with those in the non-mating sample, using a Brandt and Snedecor partitioned contingency chi-squared test, presented in table 5. As in the population cage experiments and formal mating tests, *quadrimaculata* males mate in excess of their frequency in the population. *Sexpustulata* males show a

TABLE 5

Comparison of mating and non-mating A. bipunctata *from the Keele sample, August 1981*

Males	mating	non-mating	Total	Value of chi-squared	Dfs
typ.	58	146	204	0·5957	1
ann.	33	67	100		
Nm	91	213	304	8·3947	1
sexp.	12	7	19		
Nm + sexp.	103	220	323	17·3491	1
quad.	66	58	124		
Grand Total	169	278	447		
Contingency $\chi^2_3 = 26{\cdot}3395$					
Females					
typ.	74	123	197	0·6806	1
ann.	36	48	84		
Nm	110	171	281	0·0758	1
sexp.	6	8	14		
Nm + sexp.	116	179	295	1·7430	1
quad.	53	61	114		
Grand Total	169	240	409		
Contingency $\chi^2_3 = 2{\cdot}4994$					

Nm represents both *typica* and *annulata*

similar excess in their relative mating frequency. But there is no significant difference in the numbers of mating and non-mating *typica* males when compared with *annulata* males, nor do any of the female phenotypes differ in relative frequency of mating.

The mating data were analysed using Kimball's formulae (1954) for chi-squared. These formulae give a strictly orthogonal and additive analysis of chi-squared. The standard formula for the 2×2 contingency table gives closely similar, but not exactly additive values. For the analysis of the mating data, the two melanic forms, *quadrimaculata* and *sexpustulata*, have been classed together. Only mating pairs have been used in the analysis, which compares the relative frequencies of matings among different classifications of the phenotypes. For example, when the numbers of *typica* and *annulata* females are compared to the *typica* and *annulata* males they

TABLE 6

Analysis of chi-squared of data on matings of A. bipunctata *sample from Keele, August 1981*

		Females			
		M	T	A	Totals
Males	M	25	37	16	78
	T	22	28	8	58
	A	12	9	12	33
	Totals	59	74	36	169

(i) *Difference in preference of T and A females for T and A males*

		Females		
		T	A	
Males	T	28	8	
	A	9	12	$\chi_1^2 = 7{\cdot}1410$

(ii) *Difference in preference of M and Nm females for T and A males*

		Females		
		M	Nm	
Males	T	22	36	
	A	12	16	$\chi_1^2 = 0{\cdot}0227$

(iii) *Difference in preference of T and A females for M and Nm males*

		Females		
		T	A	
Males	M	37	16	
	Nm	37	20	$\chi_1^2 = 0{\cdot}3008$

(iv) *Difference in preference of M and Nm females for M and Nm males*

		Females		
		M	Nm	
Males	M	25	53	
	Nm	34	57	$\chi_1^2 = 0{\cdot}5214$

Overall contingency $\chi_3^2 = 7{\cdot}9859$

T represents *typica*.
A represents *annulata*.
M represents *quadrimaculata* and *sexpustulata*.
Nm represents *typica* and *annulata*.

mated with, the only effect tested is the possible assortment or disassortment between *typica* and *annulata*. The analysis is presented in table 6. Since the frequencies of mating individuals are not being compared with the frequencies of non-mating individuals, only an assortative mating preference, not an overall mating advantage, can be detected. The analysis shows that there is a significant difference in the preference of *typica* and *annulata* females for *typica* and *annulata* males. Observation shows that this difference in the preference arises because the two phenotypes mate assortatively. The other components of the chi-squared show no assortative mating.

4. Discussion

The population cage experiments and the formal mating tests show that *quadrimaculata* males mate more often than the *typica* males. This mating advantage is most probably determined by female preference. In the population cage experiments, a parameter of a non-assorting preference for *quadrimaculata* is sufficient to provide a full fit of the data to the model with the estimate $\hat{\gamma} = 0{\cdot}3046$. This excellent fit of a model of female preference is obtained with the same estimate of preference at all three different sets of frequencies of *quadrimaculata* and *typica*. This level of preferential mating gives rise to precisely the level of frequency dependent advantage gained by *quadrimaculata* males. According to the model, at a frequency of 0·3, *quadrimaculata* males mate with 1·71 females compared to an overall average of one female for each male. At a frequency of 0·5, *quadrimaculata* males mate with 1·30 females. At a frequency of 0·7, *quadrimaculata* males mate with 1·13 females. The actual number of matings of *quadrimaculata* males per female were as follows: at a frequency of 0·3, 1·57 matings; at a frequency of 0·5, 1·4 matings; and at a frequency of 0·7, 1·16 matings. Thus the great frequency dependent advantage that *quadrimaculata* males gain at lower frequencies is close to that expected in the model of preferential mating. Models of male competition do not produce strong frequency dependence in the mating advantage of the males (O'Donald, 1980). Male competition can produce a frequency dependent advantage if one male phenotype is likely to displace copulating males of another phenotype. But, no such displacement has been observed in the matings in either the laboratory or the field. Indeed only rarely were two males seen in attendance on a female at one time. Females, however, were frequently seen to reject males. The mechanism of sexual selection thus appears to be female choice. Females reject *quadrimaculata* males less often than *typica* males.

The hypothesis of preferential mating by female choice is supported by the strong frequency dependent advantage gained by *quadrimaculata* males at the lower frequencies. This frequency dependent advantage is exactly what a model of female choice predicts. The frequency of melanics in the Keele population is 31·66 per cent. The results presented are therefore consistent with the general finding of Muggleton (1978) that there is an excess of melanics amongst mating pairs compared with their frequency in the population as a whole when melanics comprise less than 40 per cent of the population. However, this does not mean that we would expect to find preferences for *quadrimaculata* males in all populations where *quadrimaculata* males constitute less than 40 per cent of the population.

If female preference is genetically controlled, the level of preference will presumably differ in different populations in the same way as we should expect frequencies of alleles, which control any polymorphism, to vary. Models of the evolution of preference show that a gene giving rise to preference for a specific phenotype can not be selected to reach fixation in a population (O'Donald 1980). Therefore, if female preferences are genetically determined we should expect to find a polymorphism in their preferences: some females will show the preference and some will not. The level attained in any population will depend on several factors such as the frequency at which the genes for the preference were introduced into the population, as well as the frequency of the gene for the preferred phenotype. Consequently different populations may vary widely in the level of preference attained.

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