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A Study of Nonrandom Mating in a British Population of the Two-Spot Ladybird with a High Frequency of the Melanic Morph

I. P. M. Tomlinson[1,2]

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*In some studies of the two-spot ladybird (*Adalia bipunctata*), melanic males have been found in excess over the typical morph in matings. Data suggest that a genetic female mating preference is responsible. The mating advantage of melanic males may be important in maintaining a polymorphism between melanic and typical ladybirds in many populations in the United Kingdom (U.K.). It has been reported that preference frequency varies linearly with melanic frequency throughout most of the U.K. One particular population of* Adalia bipunctata *near Aberdare, South Wales, is noted for its high frequency of melanic individuals. It has been suggested that local environmental factors account for the high melanic frequency in this population. It is also possible, however, that a female mating preference may be at least partly responsible for the high frequency of melanics (as has been proposed for the rest of the U.K.). In this study, experiments have been performed to determine the level of female mating preference in the Aberdare population. No evidence was found for any mating advantage to melanic males. There was inconsistent and unexpected evidence that melanic females were overrepresented in matings, but the cause for this was unclear. Female mating preference does not appear, therefore, to be responsible for the high melanic frequency in the population of* Adalia bipunctata *near Aberdare. There is not a simple association between mating preference and melanic frequency in U.K. populations of the two-spot ladybird.*

KEY WORDS: two-spot ladybird; *Adalia bipunctata*; sexual selection; female choice; polymorphism; melanism.

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[1] Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, U.K.

[2] To whom correspondence should be addressed at Cancer Genetics Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

INTRODUCTION

The two-spot ladybird (*Adalia bipunctata*) is polymorphic for the color of elytra and pronotum. Most morphs can be classified as typical or melanic. Melanism is inherited as an autosomal dominant trait. A genetic female mating preference for melanic males was shown by the work of Majerus *et al.* (1982a,b). The data of Majerus *et al.* (1986) indicated that a single locus was responsible for the preference. Experiments by Kearns *et al.* (1990, 1992) failed to find good evidence of any female preference, but data consistent with the existence of such a preference have been reported by O'Donald and Majerus (1992). Although there are likely to be other factors acting differentially on the morphs of *Adalia bipunctata* (see, e.g., Brakefield, 1984, 1985; Brakefield and Lees, 1987), the mating advantage of melanic males may be important in maintaining a polymorphism between melanic and typical ladybirds in many United Kingdom (U.K.) populations (O'Donald *et al.*, 1984).

It has been reported that preference frequency varies linearly with melanic frequency in U.K. populations of *Adalia bipunctata* (O'Donald and Majerus, 1992). Hence, preference is at a high level in Glasgow, intermediate in the Midlands, and absent in Cambridge, mirroring changes in the frequency of melanics. Presumably, either the female preference causes the high melanic frequency or—owing to genetic correlation—the preference is at a higher frequency in areas where the preferred character (melanism) is advantageous.

One particular population of *Adalia bipunctata* near Aberdare, South Wales, U.K., is noted for its high frequency of melanic individuals (Creed, 1974). The population exists in a high-sided valley, with considerable—though now declining—air pollution from a nearby smokeless fuel plant. Owing to these factors, sunshine levels are relatively low compared with those in neighboring valleys and nearby coastal areas. Cardiff, which is on the coast about 20 mi south of Aberdare, has a melanic frequency of about 10%. It has been proposed that local environmental factors account for the high melanic frequency (Creed, 1974). It is also possible, however, that a female mating preference may be at least partly responsible for the high frequency of melanics, as has been suggested in the rest of the U.K.

This study uses laboratory tests for nonrandom mating in the two-spot ladybird to determine the level of female mating preference in the Aberdare population. The experiments may provide useful data for two purposes. First, they may help to tease apart the components of natural and sexual selection that cause frequent melanism in the two-spot ladybird near Aberdare. Second, they may suggest whether or not there is an association between melanism and mating preference *throughout* the U.K., with general importance for the theory of sexual selection by female choice.

METHODS AND RESULTS

Three hundred and six two-spot ladybirds were collected in July 1987 from hazel and sallow trees adjacent to the smokeless fuel works near Aberdare, Mid-Glamorgan, South Wales, U.K. Of these ladybirds, 34% were melanic, with no difference in melanic frequency between sexes (details not shown). Individuals were kept under continuous light in petri dishes (~5/dish) and fed daily on live aphids. Petri dishes were changed every 2–3 days. All ladybirds were sexed by putting many individuals together in a Perspex box [about 30 (l) × 15 (w) × 15 cm (h)] and removing mating pairs. Over 95% of ladybirds mated during sexing, with no bias toward either morph (data not shown). Sexed individuals were kept in petri dishes as above, but separated according to their sex and morph.

About 24 h after sexing, "formal mating tests" were performed according to the technique of Majerus *et al.* (1982a), because this method had been used to determine the level of female preference from other populations of *Adalia bipunctata* in the U.K. (Majerus *et al.*, 1982a,b, 1986; O'Donald and Majerus, 1992; O'Donald *et al.*, 1984; but for criticisms see Kearns *et al.*, 1990). Two melanic and three typical individuals of each sex were placed in a Perspex box [about 20 (l) × 10 (w) × 10 cm (h)]. These frequencies were used because they were similar to those in the wild. The density was chosen to limit the numbers of mating pairs that formed simultaneously. Mating tests were carried out using continuous replacement (Majerus *et al.*, 1982a). After about 10 to 15 matings had taken place, the test was stopped. As many further tests as possible were performed with the available stock. Each female mated only once in all the tests. Males, which were about 20% less common than females both in the wild sample and in succeeding generations (Hurst *et al.*, 1992), were used randomly up to two times (as in all previous laboratory studies of mating in *Adalia bipunctata*). The same males were rested for a minimum of 2 days after being used in each run.

As in previous studies (e.g., O'Donald *et al.*, 1984), the statistical methods used to analyze the results of the mating tests necessarily assume that all matings are independent of each other. This is not so in reality: even if each individual is only used once in the tests, those indiviuals which do not take part in a particular mating are usually left in the mating chamber and are potentially involved in the next mating. It is, however, impractical to sample and maintain sufficient ladybirds to allow all individuals that have been rejected in the mating tests to be discarded. In the absence of any practical alternative statistical test (P. O'Donald, personal communication), I suggest that the results given below should be interpreted with caution when they are borderline statistically significant. The interpretation of previous experiments requires at least the same, if not greater, caution.

Table I. Results of Mating Tests on a Wild Sample of Two-Spot Ladybirds from Aberdare (Parental Generation) and Three Succeeding Generations After Selection for Mating with Melanic Females[a]

Generation and line	Number of matings							
	$m \times m$	$m \times t$	$t \times m$	$t \times t$	χ^2f	χ^2m	χ^2t	χ^2i
Parental	15	32	14	29	5.7***	2.3	8.0***	0.0
sf1	7	13	8	9	3.1	0.0	3.6	0.5
sf2	21	32	19	33	4.8*	0.1	5.0*	0.1
sf3	7	20	9	14	4.3*	1.5	7.0***	1.2

[a] Females are shown first in the matings. *m* represents an individual of the melanic morph; *t* represents a nonmelanic (typical) ladybird. The *m*:*t* ratio in the tests is 2:3. χ^2f is the chi-square test for deviation from random mating in females, χ^2m is the corresponding figure for males, χ^2t is the total chi-square figure, and χ^2i is the chi-square test for assortative mating between the morphs. *Statistical significance at a level of 5% (one-tailed test), **at 2%, and ***at 1%. Unmarked χ^2 values are not statistically significant. All χ^2 values have a single degree of freedom, except for χ^2t, which has 3 df.

Table I shows the results of the "formal mating tests" on the wild sample. Male morphs mated at random. There was, however, a significant excess of melanic females in mating pairs. Although not previously described, a male mating preference for melanic females is theoretically possible in *Adalia bipunctata*. An attempt was therefore made to select for heritable variation in the tendency for a male to mate with a melanic female. Those males that had mated with melanic females were removed and their offspring reared separately. Once sufficient selected offspring had been obtained, the parental generation was recombined and allowed to mate to produce a control line.

The first filial selected (sf1) generation was tested for nonrandom mating using the same method and morph ratios as used for their parents. Matings contained an excess of melanic females (Table I), but this was not significant, possibly as a result of the smaller sample size used. Selection was then repeated to produce generations sf2 and sf3. Mating tests on these individuals (Table I) showed a significant excess of melanic females in mating pairs. The advantage to melanic females as measured by the cross-product ratio (CPR) (Muggleton, 1979) was very similar in the parental and all three selected generations and there was no significant heterogeneity in male or female mating success (Table II). There was, moreover, no significant difference between morph mating frequencies in the control line and those in the parental and three selected lines (Table III). In no generation did the mating of the male morphs differ significantly from random.

To help determine whether the mating advantage to melanic females was consistent and could be detected again in the Aberdare population, a further

Table II. Test for Heterogeneity in Female Nonrandom Mating Among the Parental, sf1, sf2, and sf3 Generations of the Aberdare Stock of the Two-Spot Ladybird[a]

Generation and line	Number of matings of female morphs			
	Melanic	Typical	CPF_f	χ^2
Parental	47	43	1.64	5.5
sf1	20	17	1.76	3.0
sf2	53	52	1.53	4.6
sf3	27	23	1.76	4.0
Total	147	135	1.63	17.3

[a] CPR_f is the cross-product ratio, a measure of the mating success of melanics females, given by [(No. of melanic females mating/No. of typical females mating) × ratio of typical females to melanic females used in mating tests (here 1.5)]. Heterogeneity $\chi^2 = 0.2$, df = 3, $P > 0.95$.

sample of 155 individuals was taken almost exactly 12 months later (thereby trying to control for seasonal factors). Thirty-seven percent of these were melanic, not significantly different from the samples of the previous year ($t = 0.78$, df $= 459$, $P > 0.40$). Mating tests on this sample used a slightly different method from the formal mating test described above (see Kearns *et al.*, 1990). With the new "singleton" method, one male was placed in a petri dish with two melanic and three typical females. The male morph was not recorded, there being no evidence for assortative mating. After a maximum of five matings, all the females were changed. This method was designed to reduce the influence of individuals unwilling or unable to mate, which may have accumulated in the formal mating test and biased the data.

The singleton tests showed that melanic females gained 24 of 51 observed matings, compared with 20.4 expected under random mating. This is a nonsignificant advantage ($P > 0.10$, exact binomial) and slightly smaller (CPR = 1.33) than that measured for the previous sample or any of the selected daughter

Table III. Mating Tests on the "Control" Line of the Aberdare Stock of the Two-Spot Ladybird[a]

Number of matings								
$m \times m$	$m \times t$	$t \times m$	$t \times t$	CPR_f	χ^2f	χ^2m	χ^2t	χ^2i
7	14	8	12	1.57	2.2	0.25	2.7	0.25

[a] Nomenclature is as given in Table I, footnote *a*. CPF_f is the cross-product ratio for mating female melanics as given in Table II; footnote *a*. χ^2 analysis shows no significant difference between the control line results and those from any of the parental or selected generations (details not given).

generations. Although the different experimental methods used for this and the original samples should be borne in mind, the difference in morph mating frequencies between the two samples taken from the wild is nonsignificant ($\chi^2 = 0.37$, df = 1). The results also provide evidence that the singleton and formal mating tests are consistent in this case.

DISCUSSION

At the time of sampling, melanics still occurred at a high frequency in the population of the two-spot ladybird near Aberdare, South Wales [although the melanic frequency appeared to be declining both at Aberdare and in other regions of the U.K. (Creed, 1974; Brakefield and Lees, 1987; Kearns *et al.*, 1992)]. This study found no evidence for any mating advantage to melanic males in the Aberdare population of *Adalia bipunctata*. Sexual selection by female choice is not, therefore, responsible for the high melanic frequency in this population. The Aberdare population of two-spots contrasts with the Glasgow population, which has a similarly high frequency of melanics, but also has a high frequency of preference (O'Donald and Majerus, 1992). It follows that, in general, a female mating preference is not necessary to produce a high frequency of melanic two-spots. Moreover, the previously noted association between high levels of female preference and melanic frequency does not hold throughout all of the U.K.

In several mating tests on the Aberdare population, melanic females were overrepresented in matings. This mating advantage was consistent between samples. It was not possible to select for a putative male mating preference for melanics through the progeny of males mating with melanic females. There is, moreover, no evidence from observing ladybird behavior that male two-spots choose females as mates. It seems unlikely, therefore, that a male mating preference equivalent to the female mating preference has been discovered. Since no other experiment on *Adalia bipunctata* has shown a mating advantage to melanic females *only*, it is possible only to speculate about its cause here. Possibilities include biological differences (true or spurious) between melanic and nonmelanic females sampled from the wild (for example, in size, activity, or reproductive status), some persistent but unknown bias in the treatment of melanic females prior to mating tests, and thermal or lighting conditions during mating tests that happened to favor melanic females.

Why is there no female preference for melanic males at Aberdare when one has been found at Glasgow? One possibility is as follows. Suppose that natural selection had initially caused melanism to evolve at Aberdare. It is quite plausible to assume that a female preference for melanic males subsequently arose in, or was introduced into, the population. Clearly, such a preference did not persist, perhaps for the following reasons. While the female preference might be advantageous in Aberdare, many females would disperse on the prevailing winds down the valley toward the South Wales coast. From the data on melanic frequencies in the area (Creed, 1974), there probably exists a steep selective gradient in melanic fitness. Dispersal of short distances would therefore take females into areas in which melanics were rare and at a natural selective disadvantage. Hence, a female carrying a preference gene on the coast, just 20 mi from Aberdare, would incur substantial costs of preference (since melanics are uncommon) and prefer a disadvantageous male trait. In effect, preference would not persist at Aberdare because its net loss via dispersal would outweigh any advantage to preference in the Aberdare population itself. In Glasgow, the selective gradient is probably much less steep, and preference for melanic males would persist.

This study illustrates the complex factors that may influence the mating frequency of the melanic morph of the two-spot ladybird. In addition to evidence for the female preference for melanic males (Majerus *et al.*, 1982a,b, 1986), other studies have found an excess of melanics of both sexes in mating pairs (reviewed by Kearns *et al.*, 1990). The results above show a mating advantage to melanic *females* only. It is not clear how these three types of mating advantage interact, if at all. This study does, however, provide evidence that female choice is not the driving force behind the evolution of melanism in *Adalia bipunctata*. Further circumstantial evidence for this is provided by the fact that melanism in ladybirds is not sex limited. It remains possible that in some populations of the two-spot, such as at Glasgow, conditions of natural selection that are different from Aberdare have permitted a female mating preference to evolve to reinforce a melanic advantage in natural selection (Tomlinson, 1988).

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