Nonrandom Mating in the Two-Spot Ladybird (*Adalia bipunctata*): The Influence of Weight on Mating Success

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In some populations of the two-spot ladybird, *Adalia bipunctata*, there is evidence that melanics of both sexes have a general mating advantage over the typical morph. There is also evidence that some female ladybirds possess a simple mating preference for melanic males. We have determined the influence of body weight on mating success in the two-spot ladybird and assessed whether weight differences might influence the mating success of the melanic morph. We found that the "formal mating tests" used in previous studies of the two-spot ladybird did not detect any influence of weight on mating success. Using more sensitive "singleton" tests, however, a significant mating advantage to heavier males was detected, irrespective of morph. There was also evidence in favor of a similar advantage to heavy females, but this was not present in all ladybird groups that were tested. Heavy individuals neither produced more eggs in matings nor showed higher activity rates. We suggest that some form of mate competition favors large individuals: for example, the larger an individual, the greater is the chance of an encounter with a potential mate. There is no evidence in this study that either sex chooses heavier mates. Effects of weight might contribute to the general melanic mating advantage found in some populations but cannot account for all the data in favor of a female mating preference for melanic males.

KEY WORDS: Two-spot ladybird; *Adalia bipunctata*; weight; nonrandom mating; mating success.

INTRODUCTION

Previous papers (Kearns *et al.*, 1990, 1992; O'Donald and Majerus, 1992) have analyzed the evidence for nonrandom mating in the two-spot ladybird, *Adalia bipunctata*, and presented new data on the same subject. Kearns *et al.* (1990, 1992) concluded that there was evidence that the melanic morph gained a general mating advantage over the

typical morph in some populations. In contrast, a separate advantage to melanic females only had been described by Majerus *et al.* (1982a, b, 1986). This was ascribed to a genetic female mating preference. Kearns *et al.* (1992) did not, however, confirm that female ladybirds could possess a simple mating preference for melanic males. O'Donald and Majerus (1992) subsequently presented further evidence that was consistent with a female preference for melanic males.

This paper reports the results of further investigations into nonrandom mating in *Adalia bipunctata*. In particular, we wished to determine the influence of body weight on mating success and to assess whether this might have any effect on the relative mating success of the typical and melanic morphs.

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Table I. The Stocks, the Groups Derived from Them According to Weight, and the Experiments in Which Each Group Was Used

Group name	Sex	Stock of origin	Individuals chosen[a]	Experiment
THML	♂	Keele (1987)	20 heaviest T, 20 lightest M	Formal mating test Singleton Activity rate
MHTL	♂	Keele (1987)	20 heaviest M, 20 lightest T	Formal mating test Singleton Activity rate
HL	♂	Keele (1988)	20 heaviest, 20 lightest	Singleton
HL	♂	Aberdare (1988)	20 heaviest, 20 lightest	Singleton
THML	♀	Aberdare (1987)	20 heaviest T, 20 lightest M	Singleton Activity rate
MHTL	♀	Aberdare (1987)	20 heaviest M, 20 lightest T	Singleton Activity rate
HL	♀	Keele (1987)	20 heaviest, 20 lightest	Singleton
HL	♀	Aberdare (1988)	20 heaviest, 20 lightest	Singleton
Egg No.	♂ + ♀	Aberdare (1987)	Unselected	Weight & egg No.

[a] T, typical; M, melanic.

Body size and weight are known to be important influences on mating success in a number of organisms. There are examples from many species of male mating success being increased by greater size. Larger *Drosophila melanogaster* males have greater mating success (Partridge *et al.*, 1987a, b); female tungara frogs, *Physalaemus pustulosus*, prefer to mate with larger males (Ryan, 1985); larger elephant seals, *Mirounga angustirostis*, compete more successfully for females; and in the North American bullfrog, *Rana catesbeiaria*, larger males hold bigger territories and are consequently preferred by females. There are few examples of size influencing mating success in females.

Size differences between the sexes often appear to be the result of sexual selection. In assessing the effects of size and weight, however, problems usually arise in determining whether selection on size is "natural" or "sexual" (see, e.g., Grafen, 1987). If larger males move around more quickly, encounter more females and gain more mates, is their advantage one of sexual or natural selection? The problem is compounded if some behavior indirectly related to mating, such as food-gathering, is also enhanced by faster movement and greater size. Consequently, it may be best to refer to the influence of size on "mating success" and defer from categorization as "sexual" or "natural" selection.

The possible benefits of size in competition for mates are clear. Size may also be the object of mate choice and may be chosen for at least four reasons. First, it might provide some immediate benefit, whether heritable or not, such as more sperm or eggs. Second, greater size might be associated with better parental care or provision of resources or territory. Third, size might provide a heritable benefit for offspring, directly or by association with heritable fitness. Fourth, size may be some arbitrary type of preferred character.

Below, we report the effect of size on both male and female mating success in the two-spot ladybird. We then investigate whether size differences might account (a) for a general melanic mating advantage and (b) for a female preference for melanic males in particular.

METHODS

The origins of the stocks used and the groups derived from them on the basis of weight are summarized in Table I. Different stocks were used for different experiments. The experiments in which each stock was used are also shown in Table I.

Sampling and Maintenance of Stocks

Stocks derived from four wild samples of *Adalia bipunctata* were used. Individuals from each stock were kept separately in petri dishes and fed on live aphids. Sexing was performed by putting many ladybirds from the same stock together in a

petri dish and removing mating pairs. This form of sexing avoids possible errors in identifying males and females by eye alone. It also ensures that the ladybirds used in the mating tests are sexually competent. In practice, over 95% of ladybirds do mate during sexing. The numbers remaining unsexed are generally too low to test formally for bias toward one or another morph, but no gross association has been detected between morph and failure to mate during sexing (Tomlinson, 1989).

The first stock of two-spot ladybirds was grown from 65 individuals sampled from a wild population at Keele, Staffordshire, UK, in August 1987. Originally, these animals were used in experiments (Kearns et al., 1992) to study the female mating preference for melanic males that had been proposed by Majerus et al. (1986). After that work had failed to support the hypothesis of a female preference, the remaining ladybirds were amalgamated into a single stock of two-spots. A second stock of 310 individuals was collected from Keele in August 1988. A third stock was sampled from Aberdare, Mid-Glamorgan, South Wales, UK. Three hundred individuals had been taken in summer 1987. This population (Creed, 1974) was known to show a very high frequency of melanics. Consequently, female ladybirds from this site might have been expected to show a strong preference for melanic males. Again, no such preference was detected in laboratory experiments (Tomlinson, 1989). The remaining individuals were then combined into a single stock. A fourth stock, of about 150 individuals, had been sampled from Aberdare in 1988.

The prime reason for using several different stocks of ladybirds was to provide sufficient numbers of insects for the mating tests. In doing this, however, the experiments also potentially supplied useful comparisons between different populations of Adalia bipunctata.

Subdivision of Ladybird Stocks into Groups on the Basis of Weight

After sexing, all ladybirds were weighed. Typically, the weights of males in all stocks ranged from 0.007 to 0.015 g, with a mean of about 0.010 g. Females were heavier. Their weight lay between 0.011 and 0.022 g, with a mean of about 0.016 g. For both males and females, there was no significant difference in weight among the four stocks

used here (t tests; details not shown). There was also no significant difference between the weights of the melanic and those of the typical morphs in each stock (t tests; details not shown).

Groups of ladybirds were then derived from each stock according to weight. Previous experiments (Tomlinson, 1989) had shown no overall association between morph and weight. Given the reports of nonrandom mating of the morphs of Adalia bipunctata, however, we decided to control for this factor in our initial experiments by selecting ladybirds according to morph as well as weight. Sufficient male ladybirds were available from the Keele (1987) stock to form two groups: the 20 heaviest male melanics and 20 lightest male typicals were picked out to form the MHTL group, and the 20 lightest male melanics and 20 heaviest male typicals were picked out to form the THML group. Similar MHTL and THML groups of females were derived from the Aberdare (1987) stock.

Insufficient ladybirds were available to derive groups based on morph and weight from the other stocks. A group of the 20 lightest and 20 heaviest males was picked out from the Keele (1988) stock by weight, irrespective of morph, to form a male HL group. A similar group of HL males was derived from the Aberdare (1988) stock. Females were also picked out from the Keele (1987) stock and Aberdare (1988) stocks by weight alone to form separate HL groups.

In all of the groups selected by weight, heavy females weighed more than 0.020 g and light females, less than 0.011 g. Heavy males weighed more than 0.014 g and light males, less than 0.008 g. For the sake of brevity, detailed data regarding the mean and variance of weight in each selected group are not shown. In each case, however, there was a significant difference in weight (by the t test) between heavy and light individuals derived from the same stock.

Mating Tests

Two types of test were used to determine the mating frequencies of heavy and light ladybirds.

Formal Mating Tests

Majerus et al. (1982a, b, 1986) first used these tests. A perspex box (15 × 7.5 × 7.5 cm) was used as a mating chamber. Five heavy and five light in-

dividuals were placed in the box, together with 10 unselected individuals of the other sex (from the same stock). Mating pairs were sampled every 30 min. Mating individuals were replaced by ladybirds of the same weight class, morph, and sex. After two to four 30-min "runs," the test was halted.

The "formal mating test" was used in the initial tests of the THML and MHTL male groups, because it was the standard technique for assessing nonrandom mating in ladybirds in the laboratory (Majerus et al., 1982a, b, 1986). Owing to potential problems with the formal mating test and concerns that it could produce spurious mating preferences (discussed in detail by Kearns et al., 1990), it was subsequently replaced by the "singleton" test. The experiments on the THML and MHTL male groups were repeated using the singleton test. This repeat experiment provided more evidence concerning the problems of experimental method when studying ladybird mating (see below).

Singleton Mating Tests

This method was devised to minimize the effect on the mating data of individuals that are unable or unwilling to mate in the formal mating test (see Kearns et al., 1990). Five heavy and five light individuals of one sex from a particular group (THML, MHTL, or HL) were placed in a mating chamber with a single, unweighed individual of the other sex, taken from the appropriate stock. If, for some reason, the "singleton" was unable or unwilling to mate (in practice, only about 1% of the time), no contribution was made to the mating data. If one ladybird of the sex in excess could not mate, several others of the same morph could do so and little bias was consequently introduced into the data. Mating pairs were always removed and replaced. Ideally, all those of the sex in excess should also have been replaced after each mating. In practice, a shortage of stock at any one time occasionally rendered this difficult. After about seven or eight mating pairs had formed and been removed, the tests were ended. This process was repeated several times, allowing the stocks to recover for 48 h between tests.

A Statistical Caveat

In practically all previous and current laboratory experiments on nonrandom mating in lady-

birds, individuals of both sexes have been used more than once in mating tests (and sometimes much more frequently). This difficulty was recognized and discussed by Kearns et al. (1990). They failed to find a solution to the problem, however, owing to constraints on the number of ladybirds that could be maintained in the laboratory (with about 1000 ladybirds being bred and maintained for the MHTL and THML groups alone in this study). The statistical tests used to analyze the data necessarily assume that matings are independent of each other. This is clearly not the case when ladybirds are used more than once in the tests, irrespective of whether or not they mate more than once. In the tests herein, singleton individuals were used only once. Other ladybirds were used up to four times at random. However, although matings are not independent in the tests, neither are they replicates, since individuals are used in different combinations when they are reused in the tests. In the absence of any practical alternative statistical test (P. O'Donald, personal communication), we have used the precedent of all previous experiments and employed statistical tests that assume independence. The results given below should therefore be interpreted in the light of this caveat, in particular when results are borderline statistically significant. The interpretation of previous experiments requires similar caution.

Egg Laying as a Component of Fitness

This experiment was used to test whether heavier individuals might have higher Darwinian fitness, thus providing a direct advantage to ladybirds choosing mates on the basis of weight. The number of eggs from each mating was used as a convenient, measurable component of fitness. Egg number is a trait that might easily be affected if, for example, larger individuals produce more ova or sperm. We measured the number of eggs laid by unselected females from the Aberdare (1987) stock after separate mating experiments (Tomlinson, 1989). Every male and female had been weighed beforehand. Each female was isolated for 4 days before and after mating in clean petri dishes and the number of eggs counted.

Activity Rates

Weight differences might affect any form of male and female "competition" for mates. Hence,

Table II. Mating Success of Heavy and Light Males from the THML and MHTL Groups in Formal Mating Tests[a]

	Male in mating pairs		
	Typical	Melanic	Total
THML	36	36	72
MHTL	36	36	72
Total	72	72	144

[a] THML is experiment with heavy typical and light melanic males. MHTL is experiment with heavy melanic and light typical males. χ^2 (association) = 0, df = 1, $p > .99$. No significant association between morph in matings and heavy (or light) morph in THML or MHTL group.

Table III. Mating Success of Heavy and Light Males from the THML and MHTL Groups in Singleton Tests[a]

	Male in mating pairs		
	Typical	Melanic	Total
THML	27	8	35
MHTL	11	26	37
Total	38	34	72

[a] THML is experiment with heavy typical and light melanic males. MHTL is experiment with heavy melanic and light typical males. χ^2 (association) = 16.1, df = 1, $p < .001$. Significant association between morph in matings and heavy morph in THML and MHTL group (i.e., significant advantage to heavy males). No evidence of nonrandom mating with respect to morph (χ^2 = 0.48, df = 1, $p > .8$).

Table IV. Test for Heterogeneity in the Singleton Experiments on Heavy and Light Males[a]

	Heavy males	Light males	Total
Keele (1987)	53	19	72
Keele (1988)	52	19	71
Aberdare (1988)	15	8	23
Total	120	46	166

[a] χ^2 (association) = 0.64, df = 2, $p > .6$. No evidence for heterogeneity among stocks.

the activity rates of a number of melanic and typical individuals of different sizes were measured. Single light or heavy ladybirds from the male and female THML/MHTL groups were placed in a petri dish divided into many 1-cm² squares. "Activity" was measured as the number of squares entered by each individual within a 1-min time interval.

Table V. Mating Success of Heavy and Light Females from the THML and MHTL Groups[a]

	Female in mating pairs		
	Typical	Melanic	Total
THML	19	7	26
MHTL	22	27	49
Total	41	34	75

[a] THML is experiment with heavy typical and light melanic females. MHTL is experiment with heavy melanic and light typical females. χ^2 (association) = 5.47, $.02 > p > .01$. Significant association between morph in matings and heavy morph in THML and MHTL group (i.e., significant advantage to heavy males), but note statistical caveat in Methods. No evidence of nonrandom mating with respect to morph (χ^2 = 2.24, df = 1, $p > .2$).

RESULTS

The Influence of Weight on Male Mating Success

"Formal mating tests" were carried out separately on THML and MHTL male stocks, with the results shown in Table II. No difference in mating success was observed between heavy and light males, irrespective of morph.

However, a repeat of the experiment using the "singleton" method gave very different results (Table III). There was a highly significant mating advantage to heavy males ($p < 0.001$), with no significant influence of morph. Repeating this experiment with the HL group of males from Keele (1988), 52 heavy males and only 19 light males mated, a significant excess ($p < 0.001$, exact binomial). The HL males from Aberdare (1988) showed a nonsignificant excess of heavy males in a smaller number of matings (15 heavy, 8 light; $p > 0.20$, exact binomial). There is no evidence of heterogeneity in the data (Table IV) and consistent evidence among populations for a mating advantage to heavier males.

The Influence of Weight on Female Mating Success

After the possible problems with the formal mating tests in initial tests on the male THML and MHTL groups, the singleton method was used for the female stocks. Table V shows the results of testing the THML and MHTL groups from the Aberdare (1987) stock. There is a significant associ-

Table VI. Test for Heterogeneity in the Singleton Experiments on Heavy and Light Females[a]

	Heavy females	Light females	Total
Aberdale (1987)	46	29	75
Keele (1987)	20	20	40
Aberdare (1988)	17	12	29
Total	83	61	144

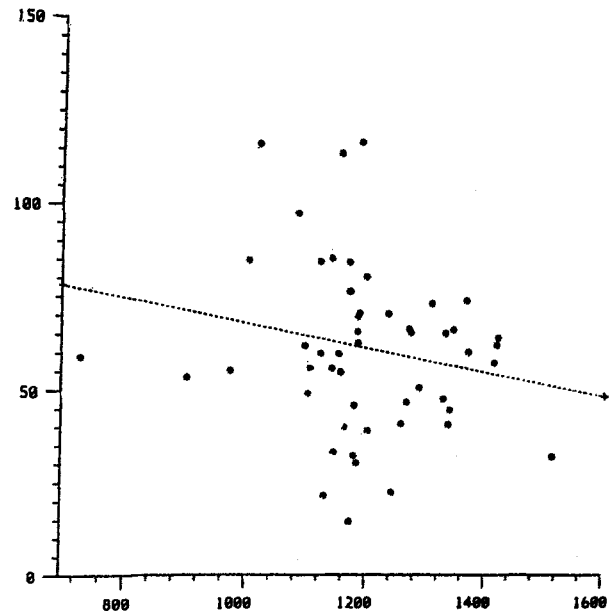[a] χ^2 (association) = 1.4, df = 2, $p > .3$. No evidence for heterogeneity among stocks.
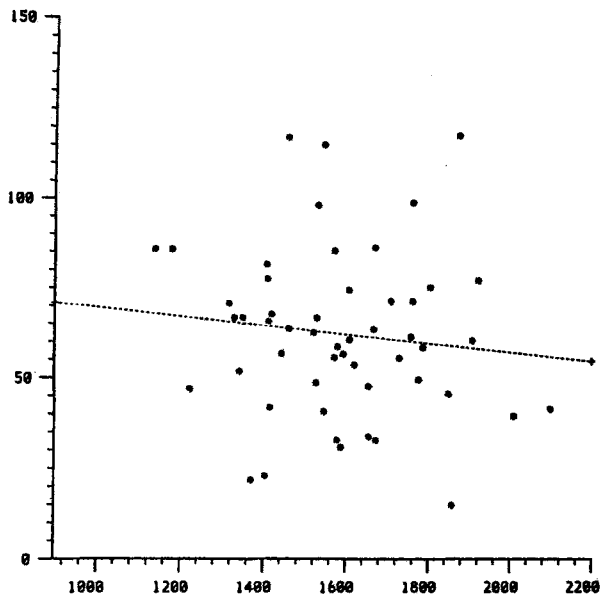
Fig. 1. Regression of egg number (y axis) on female weight (g $\times 10^{-5}$; x axis) in the two-spot ladybird. The regression ($y = 81.37 - 0.012x$) is not significant ($t = .76$, df = 51, .50 > $p > .40$).

Fig. 2. Regression of egg number (y axis) on male weight (g $\times 10^{-5}$; x axis) in the two-spot ladybird. The regression ($y = 99.98 - 0.031x$) is not significant ($t = 1.31$, df = 51, .20 > $p > .10$).

ation between greater weight and mating success ($p < 0.02$). The advantage to heavy, typical females is significant by itself ($p < 0.02$, exact binomial), but the smaller advantage to heavy melanic females is not ($p > 0.20$, exact binomial).

A further singleton experiment with light and heavy females was performed using the HL group from Keele (1987). Twenty heavy and 20 light females were represented in mating pairs. There is therefore no advantage to heavy females in these data ($p > 0.99$, exact binomial).

The singleton tests were used again to test the HL females from the Aberdare (1988) stock. Owing to a shortage of ladybirds, a single male was placed in a petri dish with only one heavy and one light female. The mating pair that formed was removed and the unmated female was discarded from the tests for the day. Of 29 matings, 17 were with heavy and 12 with light females. This is not a significant difference ($p > 0.20$, exact binomial). The results do, however, leave open the possibility that heavier females gain a mating advantage and there is no evidence of heterogeneity in the data from the three singleton tests on females (Table VI).

Weight and a Component of Fitness

Using (unselected) Aberdare (1987) stock (Table I), the number of eggs produced depended on neither female weight (Fig. 1) nor male weight (Fig. 2). There was no significant difference in the mean number of eggs laid by melanic (60.97) vs. typical (59.30) females ($t = 1.15$, df = 66, .30 > $p > .20$). Similarly, there was no significant difference in the mean number of eggs from matings of melanic (61.60) vs. typical (59.76) males ($t = 0.57$, df = 74, .60 > $p > .50$).

Weight and "Competition" for Mates

On measuring activity (Table VII), no significant difference was found in the activity rates of

Table VII. Activity Rates of Heavy and Light Males and Females Derived from the THML and MHTL Male and Female Groups

Ladybird sex and morph	Activity rate (squares/min)	
	Mean	Variance
Heavy female	22.79	190.74
Light female	21.50	262.39
Heavy male	12.06	95.05
Light male	14.31	296.70

heavy vs. light males ($t = 0.463$, df $= 29$, $p > .60$) or between those of heavy and light females ($t = 0.058$, df $= 26$, $p > .90$). Nor was there any difference between melanic and typical individuals for males ($t = 0.811$, df $= 29$, $p > .40$) or females ($t = 1.135$, df $= 26$, $p > .20$) (not shown in Table VII). Females, however, moved around significantly faster than males ($t = 2.440$, df $= 57$, $p < .02$).

DISCUSSION AND CONCLUSION

Results show that heavy male two-spot ladybirds may possess a mating advantage over light males. The data suggest that the mating advantage to large males does not depend on the origin of the ladybird stock. As expected, the Keele (1987) and Keele (1988) stocks are consistent with each other. The results from the Aberdare (1987) stock are consistent with the Keele (1987 and 1988) results. However, it was only possible to test a small sample from the Aberdare (1987) stock.

There is evidence for a smaller mating advantage to heavy females. However, there is no significant heterogeneity among the mating data from different ladybird stocks, and in most stocks mating did not differ significantly from random. Only in the Aberdare (1987) stock was there significant deviation from nonrandom mating ($p < .02$). Taking into account the statistical *caveat* above, these data from Aberdare (1987) are also consistent with random mating with respect to female size. There is, moreover, no evidence of assortative mating for weight in the Keele (1987) stock (Tomlinson, 1989), consistent with (although not proving) the absence of an advantage to heavy females from Keele in the above tests. It is not known whether females from Aberdare mate assortatively for

weight. The possible mating advantage to heavy females must, in summary, remain unproven.

The reason for the overrepresentation of heavy individuals in matings is not clear: it may result directly from size or weight or reflect the effect of some associated variable. It does not appear to be the result of higher activity rates, nor do heavier individuals produce more eggs following mating. Whatever the source of the advantage, we suspect that it derives from some form of competition for mates rather than choice: we observed no preferential rejection by females of light males and there is no evidence that males can choose heavier females. Perhaps the explanation is simply that the larger an individual, the greater is the chance of an encounter with a potential mate. Locally acting pheromones might also be produced in greater quantities by larger ladybirds. We can also speculate that heavy males are better able to cling on to females that are trying to reject them or to remain *in copuli* for longer.

The above experiments do not consider the mating success of intermediate-sized males. Such an experiment would be necessary were an attempt to be made to determine the roles of directional and stabilizing selection on size in mating. The data here are consistent either with directional selection favoring large males or with asymmetric stabilizing selection.

The heritability of weight in *Adalia bipunctata* is not known (either from the wild or the laboratory). The stocks used here were maintained in a near-constant environment and varied little in weight. Observations of other laboratory stocks (Veltman, Kearns, and Tomlinson, unpublished results) suggest, however, that weight is strongly affected by changes in environment. Nonheritable factors are therefore likely to play a significant part in determining mating success.

These results show how widespread is the effect of size on mating success in animals. We must also, however, consider the importance of these results for (a) the observed general mating advantage of melanic two-spots and (b) the female mating preference for melanic males. Given an association between morph and weight, size differences alone could explain the general mating advantage to melanics that has been found in some populations. There is no evidence that such an association occurs consistently in ladybird populations (Tomlinson, 1989). We have, however, found one or

another morph to be significantly heavier in some laboratory stocks not used in these experiments (Kearns, Veltman, and Tomlinson, unpublished data) and suspect that the different habitats of wild populations might sometimes produce an association between weight and morph.

Can size differences explain a female mating preference for melanic males? It is possible that an association between weight and morph in laboratory stocks reared under different conditions could produce such an effect; indeed, we have provided evidence that this mechanism may work (Kearns et al., 1992). Nevertheless, it is difficult to explain selection for preference (Majerus et al., 1982b) unless there is assortative mating for weight. We have not detected such assortment. Weight differences cannot, moreover, explain the proposed control of the female preference by a single locus (Majerus et al., 1986).

An incidental finding of this study has been the apparent failure of the "formal mating test" to detect the mating advantage of heavy males. This has importance for the study of sexual selection by female choice in Adalia bipunctata. In previous papers (Kearns et al., 1990, 1992), we suggested that the "formal mating test" could produce spurious mating advantages. Now we have found that the test may fail to detect what we believe is a true mating advantage. Defects in the formal mating test cannot refute all the qualitative data in favor of a female preference for melanic males [see, e.g., O'Donald and Majerus (1992, Table 2), in which some stocks show a near-100% preference]. It would be interesting, however, to determine whether the singleton and formal mating tests gave similar quantitative estimates of female preference. Quantitation of female preference was integral to the evidence for the control of the female preference by a single locus (Majerus et al., 1986).

We conclude that weight may be an important determinant of mating success in Adalia bipunctata. There is no evidence that this advantage results from mate choice and we suspect that some form of competition for mates is responsible. It is not clear how nonrandom mating with respect to

weight interacts with nonrandom mating with respect to morph in this species. It is possible in the wild that weight is sometimes associated differentially with the melanic and typical morphs: nonrandom mating of the morphs may then result. Weight effects cannot, however, account for all the data in favor of a female mating preference for melanic males. Mating success in Adalia bipunctata may therefore depend on (a) weight, (b) a general melanic mating advantage, and (c) a female preference for melanic males. We speculate that several other factors that influence mating success in this beetle remain to be discovered.

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