Functional Ecology 1998
12, 757–761

Colour-associated mating success in a polymorphic Ladybird Beetle, *Harmonia axyridis*

H. UENO,* Y. SATO* and K. TSUCHIDA
**Laboratory of Biology, Faculty of Education, Niigata University, 8050 Ikarashi-Ni-No-Cho, Niigata 950–21, Japan, and Laboratory of Entomology, Faculty of Agriculture, Gifu University, Yanagido, Gifu 501–11, Japan*

Summary

1. Body size is often an important character in mating success, but has been only infrequently mentioned in regard to colour polymorphism. In this study, mating success was investigated in a colour polymorphic Ladybird Beetle, *Harmonia axyridis*, with reference both to colour morph and to body size.
2. In the non-melanic males the mating individuals were significantly larger than solitary individuals, while in melanic males there was no significant difference.
3. The mating pattern was close to random mating with respect to colour morph and there was no significant deviation.
4. The results suggest both body size and colour morph affect the male mating success and males of different body size obtain mating advantage according to the colour morph. Colour polymorphism in this species is controlled by alleles on a single locus. Thus, the alleles on that locus significantly influence the effect of selection on the quantitative character.

Key-words: Body size, colour polymorphism, morph frequency

Functional Ecology (1998) **12,** 757–761

Introduction

Colour polymorphism in coccinellid beetles has attracted the attention of researchers regarding the maintenance of equilibrium polymorphism (Dobzhansky 1933; Komai 1956). In *Adalia bipunctata* Linnaeus, widespread in Europe, extensive research has been focused on postwinter copulation, and evidence has been obtained that the polymorphism is influenced by non-random mating involving a larger contribution by melanic phenotypes to the mating group (Muggleton 1979; Majerus, O'Donald & Wier 1982; Brakefield 1984c; O'Donald *et al*. 1984).

A factor that seems of major importance is the more efficient absorption of solar energy by melanics, probably leading to relatively greater activity (Benham, Lonsdale & Muggleton 1974; Creed 1975; Muggleton, Lonsdale & Benham 1975; Brakefield 1984a,b; Brakefield & Willmer 1985; Steward & Dixon 1989). On the other hand, the mate choice for melanic morphs is also supposed to cause non-random mating (O'Donald & Muggleton 1979; Majerus *et al*. 1982; O'Donald *et al*. 1984).

For the present species, *Harmonia axyridis* Pallas, Komai (1956) reported a geographical cline in morph frequencies and suggested the presence of climatic factors affecting colour polymorphism. Recently, Osawa & Nishida (1992) revealed non-random mating in a natural population and female mating preference for non-melanic males.

While most of the previous studies have focused on the comparison of the colour morph frequencies of mating individuals with those of solitary individuals (Creed 1975; Muggleton 1979; Majerus *et al*. 1982; Brakefield 1984c; O'Donald *et al*. 1984), factors other than the colour morph were relatively disregarded. Body size is an important character affecting mating success (Osawa & Nishida 1992), but has not been mentioned in regard to colour polymorphism. Because thermal absorption rate depends not only on the colour morph but also on the body size of the individuals (Brakefield & Willmer 1985; Stewart & Dixon 1989), if the thermal factor is involved in mating success, selection on body size may be affected by the difference in thermal absorption. The present study focused on the mating pattern with reference to body size as well as to colour morph and therefore estimated selection on both quantitative and qualitative characters. The particular aspects are (1) whether body size is associated with mating success and, if so, how, and (2) whether non-random mating is observed, thus whether there is mating advantage with respect to colour morph.

Materials and methods

A study area was chosen in the campus of Gifu University, near Gifu City, central Japan. Beetles overwinter at the adult stage and copulate in early

May of the next spring in the studied population. Reproduction occurs near the place of copulation. Most of the beetles prey upon aphids on the plants *Vicia sativa* and *Pittosporum tobira*. First generation offspring emerge and emigrate before copulation unlike those of the Kyoto population where Osawa & Nishida (1992) reported copulation before emigration.

Adult beetles of the overwintered generation were collected in May 1992 and 1993, regardless of their mating status. Beetles were measured for body length under the microscope to 0·01 mm. Differences in body size between mating and solitary individuals were scored as standardized selection differentials by the regression, assigning 1 for mating and 0 for solitary individuals (Lande & Arnold 1983). Because the binary distribution of mating status values does not allow parametric testing, Spearman's rank correlation was used for the test of statistical significance.

The relationships between mating success and body size were further visualized by the cubic spline method (Schluter 1988). Fitness functions with standard errors (based on 1000 bootstraps) were estimated using a FORTRAN 77 computer routine provided by D. Schluter (GLMS, ver. 3).

For testing the deviation from random mating, Manly's mating preference coefficient (β) was calculated as follows:

$$\beta_{ij} = \frac{d_{ij}/a_i b_j}{\sum\sum d_{rs}/(a_r b_s)}$$

where a_i and b_j are numbers of non-mating adults of colour types i and j, respectively; and d_{ij} is the number of mating pairs of i type male and j type female (Manly 1985).

Results

Totals of 590 and 914 beetles were collected and, of them, 105 and 155 pairs mated in 1992 and 1993, respectively. Table 1 shows measurements of body length for mating and solitary individuals for each sex. Body size measurement was transformed to natural logarithms prior to analysis. ANOVA, where sex, morph, year and mating status were set as independent variables, indicated that the main effect of sex and the interactions of sex × mating status, morph × mating status and sex × morph × mating status were significant (Table 2). Females were larger than males in this species (Tables 1 and 2).

When two sexes were separately analysed with morph, year and mating status as independent variables, in males ANOVA detected significant effects of mating status (F = 13·976, df = 1, 687, P < 0·001) and morph × mating status interaction (F = 18·295, df = 1, 687, P < 0·001), while no significant effect was detected in any variables in females. Variance of body length of mating males in 1992 was significantly smaller than that of solitary males (F = 1·399, df = 180, 104, P = 0·030; one tailed test for stabilizing direction). In the other cases, although mating individuals indicated smaller variances, the differences were not statistically significant (males in 1993: F = 1·129, df = 253, 154, P = 0·206; females in 1992: F = 1·158, df = 189, 104, P = 0·204; females in 1993: F = 1·041, df = 349, 154, P = 0·393).

Two further ANOVAS indicated that in the non-melanic males, the mating individuals were significantly larger than solitary individuals (F = 20·313, df = 1, 102, P < 0·001), while in melanic males there was no significant difference between mating and solitary individuals (F = 0·460, df = 1, 585, P = 0·498). Smaller variances of body length were suggested for mating individuals in all cases. However, any differences were not statistically significant (non-melanic males in 1992: F = 2·042, df = 35, 18, P = 0·054; in 1993: F = 1·061, df = 31, 18, P = 0·459; melanic males in 1992: F = 1·318, df = 144, 85, P = 0·082; in 1993: F = 1·116, df = 221, 135, P = 0·244).

The differences in male mating success are summarized in the terms of the standardized coefficients of the regression in Table 3. For non-melanic males, directional selection differentials were about half of one unit of standard deviation of body size

Table 1. Measurement of body length (mm) for melanic and non-melanic individuals in 1992 and 1993. Sample size and standard error are in parentheses

Sex	Melanic			Non-melanic		
1992						
Male	Mating (86)	Solitary (145)	Total (231)	Mating (19)	Solitary (36)	Total (55)
	6·37 (0·04)	6·44 (0·03)	6·41 (0·03)	6·60 (0·07)	6·23 (0·07)	6·36 (0·06)
Female	Mating (96)	Solitary (172)	Total (268)	Mating (9)	Solitary (18)	Total (27)
	6·96 (0·05)	7·05 (0·04)	7·02 (0·03)	6·88 (0·18)	7·13 (0·08)	7·05 (0·08)
1993						
Male	Mating (136)	Solitary (222)	Total (358)	Mating (19)	Solitary (32)	Total (51)
	6·39 (0·04)	6·38 (0·03)	6·39 (0·02)	6·54 (0·10)	6·15 (0·07)	6·30 (0·06)
Female	Mating (141)	Solitary (308)	Total (449)	Mating (14)	Solitary (42)	Total (56)
	6·87 (0·04)	6·93 (0·03)	6·91 (0·02)	7·06 (0·15)	7·02 (0·08)	7·03 (0·07)

distribution for 2 years, and the values were statistically significant when tested by Spearman's rank correlation. For melanic males no deviations were detected. Significant reduction in variance was indicated for males in total in 1992.

Univariate fitness functions were presented for melanic and non-melanic males, and males in total (Fig. 1). In general, spline analyses confirmed the results based on selection coefficients. Larger male advantages were indicated for non-melanic males, while no significant trends were suggested for melanic males. For total males in 1992, the selection surface was slightly concave, whereas no selection was indicated in 1993.

Table 4 shows Manly's mating coefficient (β) with the standard error in parentheses. The values were close to the random mating expectation ($\beta = 0{\cdot}250$) both in 1992 and 1993, and there were no significant deviations from random mating. Frequencies of melanic and non-melanic morphs among mating individuals were not significantly different from those of solitary individuals either in 1992 (for males: $G = 0{\cdot}14$, df = 1, $P = 0{\cdot}710$; for females $G = 0{\cdot}47$, df = 1, $P = 0{\cdot}491$) or in 1993 (for males: $G = 0{\cdot}010$, df = 1, $P = 0{\cdot}919$; for females: $G = 0{\cdot}99$, df = 1, $P = 0{\cdot}319$). No significance was detected when males and females were pooled (in 1992: $G = 0{\cdot}41$, df = 1, $P = 0{\cdot}522$; in 1993: $G = 0{\cdot}52$, df = 1, $P = 0{\cdot}472$).

Table 2. Analysis of variance for the body length setting sex, morph, year and mating-status as independent variables

Source	df	SS	*F*
Sex	1	109·525	216·034***
Morph	1	0·291	0·575
Mating status	1	0·940	1·855
Year	1	1·171	2·310
Sex × morph	1	0·754	1·487
Sex × mating status	1	5·472	10·793**
Sex × year	1	0·610	1·203
Morph × year	1	0·009	0·017
Morph × mating status	1	3·414	6·733*
Mating status × year	1	0·475	0·937
Sex × morph × mating status	1	3·633	7·166*
Error	1483	751·851	

*$P < 0{\cdot}01$, **$P < 0{\cdot}005$, ***$P < 0{\cdot}001$.

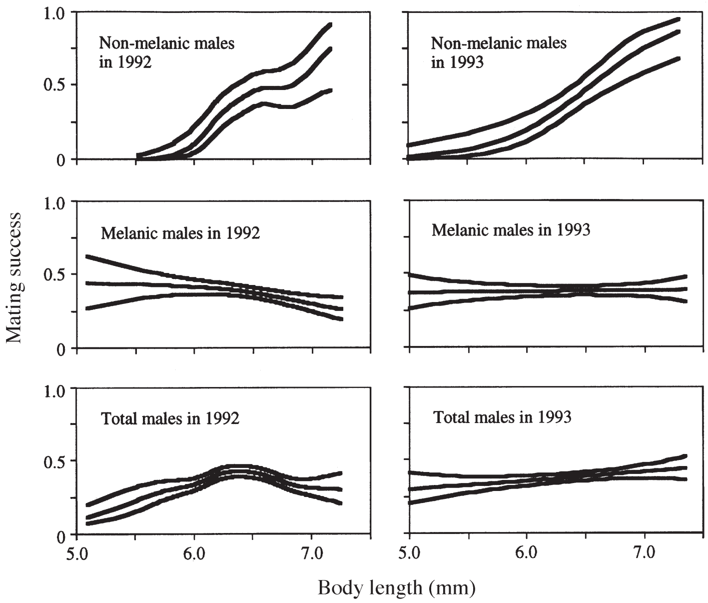

Fig. 1. Fitness functions and bootstrap SE based on cubic spline method (Schluter 1988), for male body length in 1992 and 1993.

Table 3. Standardized directional and variance selection coefficient of male body length in 1992 and 1993 with sample size in parentheses

	Directional	Variance
1992		
Melanic (231)	– 0·103	– 0·147
Non-melanic (55)	0·578**	– 0·007
Total (286)	0·032	– 0·141*
1993		
Melanic (358)	0·009	– 0·044
Non-melanic (51)	0·540**	0·295
Total (409)	0·073	– 0·023

*$P < 0{\cdot}05$; **$P < 0{\cdot}005$.

Table 4. Estimation of Manly's mating preference coefficient (β) for melanic and non-melanic individuals in 1992 and 1993, with standard errors in the parentheses. No estimation was significantly different from random mating expectation ($\beta = 0{\cdot}250$)

Male morph / Female morph	Melanic	Non-melanic	Total
1992			
Melanic	0·294 (0·076)	0·210 (0·086)	0·504
Non-melanic	0·255 (0·084)	0·241 (0·142)	0·496
Total	0·549	0·451	
1993			
Melanic	0·287 (0·065)	0·204 (0·068)	0·491
Non-melanic	0·273 (0·080)	0·236 (0·137)	0·509
Total	0·560	0·440	

Discussion

Body size is often an important character in mating success in insects (Thornhill & Alcock 1983), but has been only infrequently mentioned in regard to colour polymorphism. In the studies on colour polymorphic coccinellids, attention has been drawn to the difference in relative mating frequencies among colour morphs (Creed 1975; Muggleton 1979; Majerus *et al*. 1982; Brakefield 1984c; O'Donald *et al*. 1984). For the present species, larger male advantage in postwinter copulation was reported in the Kyoto population (Osawa & Nishida 1992), but no reference has been made to colour polymorphism. In the present study, both body size and colour morph were concerned. When body size of mating and solitary individuals was compared, in the non-melanic males mating individuals were significantly larger than solitary individuals, while in melanic males there was no significant difference. The results suggest that both body size and colour morph affect male mating success and that males of different body size obtain mating advantage according to the colour morph. Colour polymorphism in this species is controlled by alleles on a single locus (Tan & Li 1934). Thus, the present results indicate that the alleles on that locus significantly influence the effect of selection on the quantitative character.

In the experimental studies of coccinellid beetles, a higher rate of thermal absorption was indicated in melanic than in non-melanic phenotypes and also in larger body size individuals (Brakefield & Willmer 1985; Stewart & Dixon 1989). Some studies indicated that the difference in thermal absorption between melanic and non-melanic *A. bipunctata* indeed resulted in higher activity in melanics (Benham *et al*. 1974; Muggleton *et al*. 1975). These studies may support the idea that some thermal factor is involved in the present results and larger non-melanic phenotypes have as large thermal absorption as melanic morph individuals, which results in the differential activity.

For the present species, mate choice for non-melanic individuals was indicated in the Kyoto population, and the preference was supposed to cause the observed excess of mating frequency of non-melanic male and non-melanic female combination (Osawa & Nishida 1992). In the present study, mating frequency was close to random and was inconsistent with that observed in the Kyoto population. However, the presence of sexual preference would not necessarily exclude involvement of environmental factors. Even in the Kyoto population, no preference for non-melanics was detected in summer, and larger male advantage indicated in post-winter copulation was not observed in summer copulation (Osawa & Nishida 1992). These variations may suggest that the mating pattern of the present species could be mediated by seasonal differences in environmental factors regardless of the presence of sexual preference, and factors responsible for a population may be less relevant to other populations.

Because this study did not follow individuals longitudinally, sequential change was undetectable. If mating frequency and larger body size advantage were varying within a mating season, the present results could only represent an average attribute. In *A. bipunctata* investigated in the Netherlands, melanic phenotypes were reported to start postwinter reproduction and emerge from pupa to adult in the offspring generation earlier than non-melanic individuals (Brakefield 1984b). Sequential changes should be involved in the future studies on the mating system in this species.

Acknowledgements

We would like to express our sincere thanks to S. Higashi and K. Tsuji for discussion on the earlier draft. We are also indebted to B. Manly and D. Schluter for instruction in calculations of mating preference coefficient and univariate fitness function.

References

Benham, B.R., Lonsdale, D. & Muggleton, J. (1974) Is polymorphism in two-spot ladybirds an example of non industrial melanism? *Nature* **249,** 179–180.

Brakefield, P.M. (1984a) Ecological studies on the polymorphic ladybird *Adalia bipunctata* in the Netherlands. I. Population biology and geographical variation of melanism. *Journal of Animal Ecology* **53,** 761–774.

Brakefield, P.M. (1984b) Ecological studies on the polymorphic ladybird *Adalia bipunctata* in the Netherlands. II. Population dynamics, differential timing of reproduction and thermal melanism. *Journal of Animal Ecology* **53,** 775–790.

Brakefield, P.M. (1984c) Selection along clines in the ladybird *Adalia bipunctata* in the Netherlands: A general mating advantage to melanics and its consequences. *Heredity* **53,** 37–49.

Brakefield, P.M. & Willmer, P.G. (1985) The basis of thermal melanism in the ladybird *Adalia bipunctata*: differences in reflectance and thermal properties between the morphs. *Heredity* **54,** 273– 277.

Creed, E.R. (1975) Melanism in the two-spot ladybird: the nature and intensity of selection. *Proceedings of the Royal Society of London B* **190,** 135–148.

Dobzhansky, T.G. (1933) Geographical variation in ladybeetles. *American Naturalist* **67,** 97–126.

Komai, T. (1956) Genetics of ladybeetles. *Advances in Genetics* **8,** 155–188.

Lande, R. & Arnold, S.J. (1983) The measurement of selection on correlated characters. *Evolution* **37,** 1210–1226.

Majerus, M.E.N., O'Donald, P. & Weir, J. (1982) Evidence for preferential mating in *Adalia bipunctata. Heredity* **49,** 37–49.

Manly, B.F.J. (1985) *The Statistics of Natural Selection.* Chapman and Hall, New York.

Muggleton, J. (1979) Non-random mating in wild populations of polymorphic *Adalia bipunctata. Heredity* **42,** 57–65.

Muggleton, J., Lonsdale, D. & Benham, B.R. (1975) Melanism in *Adalia bipunctata* L. (Col., Coccinellidae) and its relationship to atmospheric pollution. *Journal of Applied Ecology* **12,** 451–464.

O'Donald, P. & Muggleton, J. (1979) Melanic polymorphism in ladybirds maintained by sexual selection. *Heredity* **43,** 143–148.

O'Donald, P., Derrick, M., Majerus, M. & Weir, J. (1984) Population genetic theory of the assortative mating, sexual selection and natural selection of the two-spot ladybird, *Adalia bipunctata. Heredity* **52,** 43–61.

Osawa, N. & Nishida, T. (1992) Seasonal variation in elytral colour polymorphism in *Harmonia axyridis* (the ladybird beetle): the role of non-random mating.

Heredity **69,** 297–307.

Schluter, D. (1988) Estimating the form of natural selection on a quantitative trait. *Evolution* **42,** 849–861.

Stewart, L.A. & Dixon, A.F.G. (1989) Why big species of ladybird beetles are not melanic. *Functional Ecology* **3,** 165–177.

Tan, C.C. & Li, J.C. (1934) Inheritance of the elytral colour patterns in the lady-bird beetle, *Harmonia axyridis. American Naturalist* **68,** 252–265.

Thornhill, R. & Alcock, J. (1983) *The Evolution of Insect Mating Systems*. Harvard University Press, Cambridge.

Received 1 September 1997; revised 12 January 1998; accepted 23 January 1998