Seasonal variation in elytral colour polymorphism in *Harmonia axyridis* (the ladybird beetle): the role of non-random mating

NAOYA OSAWA† & TAKAYOSHI NISHIDA

Laboratory of Entomology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

Seasonal variation in the frequency of elytral colour polymorphism in the ladybird beetle, *Harmonia axyridis*, was analysed through the combination of field observations and laboratory mate-choice experiments. Field observations involving daily mark-recapture of a local population revealed an overall preponderance of melanic morphs in the population, but the proportion of non-melanics notably increased from spring to summer due to their higher frequency of mating among different morphs of the spring generation. In the laboratory experiments, both melanic and non-melanic females preferentially chose non-melanic males in spring, but non-melanics were less successful at mating than melanics in summer. Therefore, the relative frequencies of melanics and non-melanics within a population vary throughout the seasons, with non-melanics increasing in proportion towards summer and decreasing thereafter. Laboratory experiments revealed that non-random mating among morphs is the basis of this variation, with females and, to a lesser degree, males being involved in the process of mate selection. The results of an artificial colour-change experiment indicate that male elytral colour is an important factor in female mate selection but other factors such as behaviour and chemical cues must also be involved.

Keywords: elytral colour polymorphism, *Harmonia axyridis*, ladybird beetle, mating preference, non-random mating, seasonal variation.

Introduction

Attention has been drawn to elytral colour polymorphism in coccinellids as an ideal subject of study in ecological genetics (Dobzhansky, 1933; Komai, 1956; Hodek, 1973). In *Adalia bipunctata* LINNÉ, which is widely distributed in Europe, melanic and non-melanic morphs have been recognized and interpreted as an adaptation to varying thermal environments within the geographical distribution of the species ('thermal melanism', Lusis, 1961; Benham *et al.*, 1974; Muggleton *et al.*, 1975; Creed, 1975; Brakefield, 1984a,b; Brakefield & Willmer, 1985; Brakefield & Lees, 1987; Stewart & Dixon, 1989) or as a mechanism to reduce the non-melanic frequency by toxic substances of the polluted air in industrial areas ('industrial melanism', Creed, 1966, 1971a,b, 1974; Lees *et al.*, 1973; Bishop *et al.*, 1978). In north-eastern Asia, *Harmonia axyridis* PALLAS is known to have four elytral

colour morphs, three of them being regarded as melanic and one as non-melanic (Hosino, 1936; Komai, 1956; Sasaji, 1971). Interestingly, the proportion of melanic morphs of this species increases towards southern districts, which Komai (1956) suggested as another form of climatic adaptation.

Past studies have considered colour polymorphism in coccinellids in terms of geographical clines (e.g. Komai, 1956; Scali & Creed, 1975), whilst a few investigated temporal variations in morph frequency within a regional population (Timoféeff-Ressovsky, 1940; Tan, 1949). Nevertheless, note that geographical and temporal morph variation does not always correspond to what is expected from thermal and industrial adaptation theories (Bengtson & Hagen, 1975, 1977; Honek, 1975; Muggleton, 1978). Furthermore, no previous investigation has revealed morph-related selective death in polymorphic ladybird beetles. It seems unlikely that thermal and/or industrial melanism hypotheses can fully explain the patterns of elytral colour polymorphism in coccinellids.

†Correspondence.

Apart from external factors, such as thermal and industrial stresses, which can effect polymorphic characters, non-random mating among different morphs constitutes an important mechanism affecting the maintenance of polymorphism in a population, with different patterns of mating being associated with different fitness consequences (Partridge & Halliday, 1984). In *A. bipunctata*, non-random mating as well as behaviour-based mate choice have been observed (Muggleton, 1978, 1979; Majerus *et al.*, 1982a,b, 1986; O'Donald & Muggleton, 1979; O'Donald *et al.*, 1984; Brakefield, 1984c; Kearns *et al.*, 1990). Very few studies, however, have demonstrated fitness consequences of non-random mating in field populations of polymorphic coccinellids, mainly due to the difficulty of quantitatively observing mating in a highly mobile and dispersive species. The present study combines intensive field observations and laboratory mate choice experiments to elucidate the temporal patterns of polymorphism in *H. axyridis* in central Japan. The main focus of analysis is the role of non-random mating in relation to seasonal variation rather than a fixed trend through the year.

Materials and methods

The ladybird beetle

H. axyridis typically has four elytral colour morphs: *succinea* [*h*, orange with some or no black spot(s)], *axyridis* (h^X, black with some red spots), *spectabilis* (h^S, black with four red spots), and *conspicua* (h^C black with two red spots) (see Komai, 1956). The genes that control colour morphs form multiple-alletic series, with the dominance order of $h < h^X < h^S < h^C$ (Hosino, 1933, 1936; Tan & Li, 1934). Following previous works (Hosino, 1936; Komai, 1956; Sasaji, 1971) beetles with black elytra (*axyridis*, *spectabilis*, and *conspicua*) were regarded as melanic and those with orange elytra (*succinea*) as non-melanic in this study.

The ladybird beetle has two or three generations in a year (Okada *et al.*, 1973; Osawa, 1991). In the study area, adults of the overwintering generation terminate hibernation in late March and mate and lay eggs in April and May. Adults of the first generation emerge in mid May to June. In mid summer, the beetles seem to aestivate, although no detailed information is available. Adults of the second and/or third generations reappear in autumn and gradually aggregate at hibernation sites in early November (Obata, 1986). In this paper, the overwintering generation is referred to as the spring generation and the first generation as the summer generation.

Field observations

All observations were done in the Botanical Garden (~ 10,000 m²) of Kyoto University (35°02′N 135°47′W), central Japan. Every day from early April to early August in 1985, 1987, and 1988, adults were captured and individually marked by four dots with quick drying paint and also by drilling tiny holes to prevent the paint from falling off. Newly marked individuals were released at the site where they were caught after recording body size (total body length to the nearest 0.01 mm, recorded in 1987 and 1988), sex, elytral colour, whether mating or not at the time of capture, code of a mate and site of capture. For all recaptured beetles, individual code, sex, whether mating or not at the time of recapture, code of a mate and the recapture site were recorded. A total of 2,572 individuals were thus observed.

Individuals of the summer generation were distinguished from those of the spring generation on the basis of pale and soft elytra of newly emerged adults (cf. Brakefield, 1984a).

Endler's (1986) selection coefficient, *S*, was used to assess the relative frequencies of morphs in the spring and summer generations. Mating preference in the field population was analysed using Manly's (1985) mating preference coefficient (β), which measures deviation from random mating among morphs in a finite population (cf. Spencer, 1988). When a random sample of non-mating individuals contains a total of a_i males and b_i females of morph type i and a random sample of mating individuals contains d_{ij} pairs of morph i male−morph j female (i, $j = 1-K$), the preference coefficient for $i − j$ pairing is given as

$$\hat{\beta} = [d_{ij}/(a_i b_i)] \left/ \left[\sum_{r=1}^{K} \sum_{s=1}^{K} d_{rs}/(a_r b_s) \right] \right.$$

Laboratory experiments of mate choice

Four different experiments were carried out in the laboratory at 25°C and 16L–8D, using beetles collected from the field as adults (spring generation, immediately after hibernation) and as pupae (summer generation), and maintained as mass culture in unisexual groups of two to five individuals. Beetles were reared on aphids *Aphis spiraecola* VAN DER GOOT and *Myzus varians* DAVIDSON. The experimental arena was a Petri dish (9 cm in diameter and 2 cm in height) to which different combinations of male and female morphs were introduced. An experimental session consisted of a 30-min continuous observation of mating activities, counting all successful and unsuccessful mating attempts; this was replicated 18–60 times

using different individuals from the stock culture. In all the sessions, copulating pairs were artificially separated after 2 min of copulation and released again in the same experimental arena. Beetles which continued genital contact for 2 min were regarded as having completed copulation; in a separate observation 78 per cent of matings lasting 2 min reached the 'body shaking' stage, which signals sperm transfer in this species (Obata, 1987). Melanic and non-melanic morphs used in the experiments (Experiment 2, 3, and 4) were *conspicua* and *succinea*, respectively.

Experiment 1. Free mate choice experiment. Four individuals (male and female, one individual per morph per sex) were introduced. A total of 60 replicated sessions were performed.

Experiment 2. Female choice experiment. One female (melanic or non-melanic) and two males (one melanic and one non-melanic) were kept together. Eighteen replications.

Experiment 3. Male choice experiment. One male (melanic or non-melanic) and two females (one melanic and one non-melanic) were kept together. Twenty replications.

Experiment 4. Artificial colour-change experiment. One female (melanic or non-melanic) and two males (one melanic and one non-melanic) painted with quick drying paint (reverse-coloured pair — one melanic male painted orange and non-melanic painted black with red spots, or duplicate-coloured pair — one melanic male painted black with red spots and non-melanic painted orange) were observed. The paints used were Magic Ink® No. 500 for black and Mitsubishi Paint Marker®PX-20 for orange and red. The use of these paints did not appear to affect the beetles adversely (see Results). Beetles were kept for 4–5 min in Petri dishes, to dry the paints completely, before being used in the experiment. Twenty replications.

Results

Morph frequencies and mating patterns

Figure 1 shows the frequencies of four morphs in terms of phenotype and allele in the spring and summer generation. Whilst *conspicua* was dominant in all the cases, there was a notable trend for non-melanic *succinea* to increase in frequency from spring to summer in each year, which is also confirmed by an analysis of the selection coefficient, S, of non-melanics against melanics in phenotype and allele (Table 1).
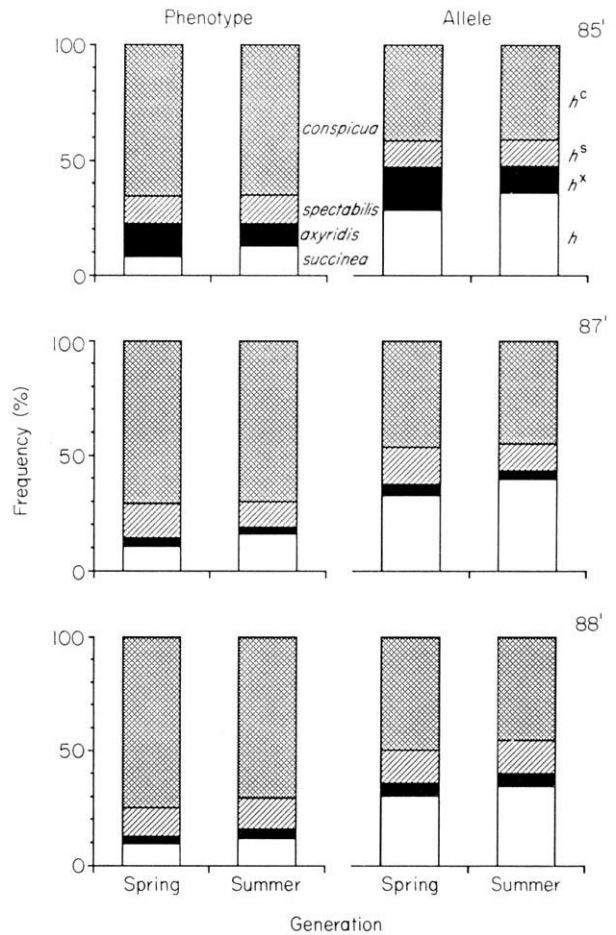

Fig. 1 Morph frequencies in phenotype and allele in spring and summer generation.

Table 1 Selection coefficient of non-melanics against melanics from spring to summer generation, with respect to phenotype and allele. Significance is indicated by probability values

Year	Phenotype	Allele
1985	$0.410\ (P < 0.05)$	$0.295\ (P < 0.05)$
1987	$0.375\ (P < 0.001)$	$0.272\ (P < 0.001)$
1988	$0.251\ (0.05 < P < 0.10)$	$0.174\ (P < 0.05)$
Total	$0.366\ (P < 0.001)$	$0.262\ (P < 0.001)$

One crucial factor, which determines morph frequencies in one generation, is the pattern of mating in the previous generation. Morph frequencies among non-mating and mating individuals observed in the field are shown in Fig. 2. In the spring generation, frequencies of four morphs among mating individuals (males and females combined) were significantly different ($\chi^2 = 16.9$, d.f. = 3, $P < 0.001$) from those of non-mating individuals. When sexes were treated
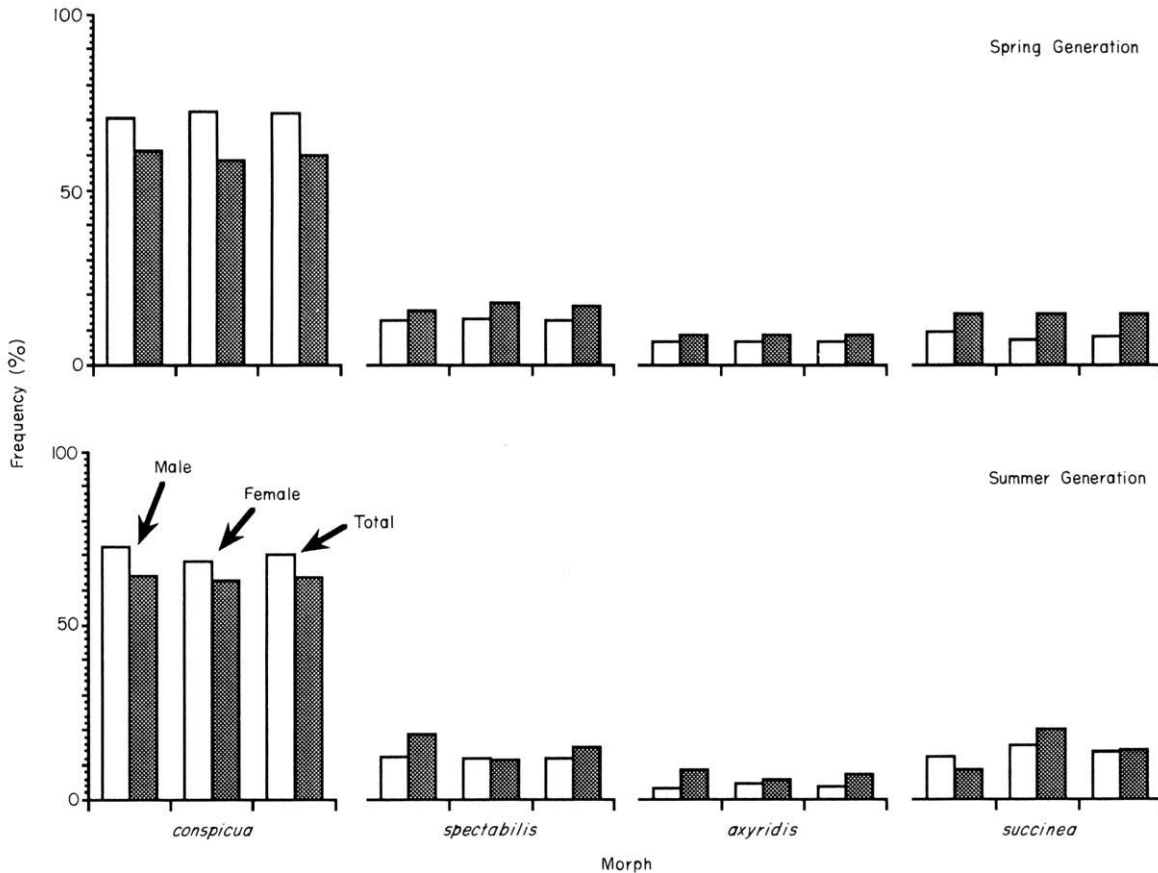
Fig. 2 Frequencies of different morphs among non-mating (□) and mating (▨) individuals.

separately, the difference in morph frequency between mating and non-mating individuals was more pronounced in females ($\chi^2 = 11.7$, d.f. = 3, $P < 0.01$) than in males ($\chi^2 = 5.67$, d.f. = 3, $0.10 < P < 0.20$). Nevertheless, in both males and females the difference was largely due to a significantly smaller proportion of *conspicua* being represented among mating as against non-mating individuals, while the reverse was true for *succinea*. Thus, *succinea* demonstrated a disproportionately large frequency of mating in the spring generation.

A different pattern of mating was observed in the summer generation, where males but not females showed a significant difference in morph frequency between mating and non-mating individuals (males, $\chi^2 = 8.05$, d.f. = 3, $P < 0.05$; females, $\chi^2 = 1.41$, d.f. = 3, $0.70 < P < 0.80$). In the case of males, the difference was due to a high proportion of *spectabilis* and a low proportion of *conspicua* among mating individuals. When males and females were combined, the difference in morph frequency was only marginally significant ($\chi^2 = 5.28$, d.f. = 3, $0.10 < P < 0.20$). Notably males and females of *succinea* showed neither signifi-

cantly increased nor reduced frequency of mating compared with three melanic morphs in this generation.

Mating preferences among morphs were further analysed by Manly's preference coefficient, β (Table 2), with twice the value of S.E. being used as a criterion for judging a departure from random mating at ~ 5 per cent level of significance (Manly, 1985). Of 16 possible pairs among four morphs, three pairs, all involving *conspicua*, had a significantly small value of β, whilst none involving *succinea* did so in the spring generation. In the summer generation, only one pair (*conspicua–conspicua*) had a significantly small value. Thus melanics may be expected to decrease in frequency among the offspring of the spring generation (i.e. summer generation), while the effect of the low frequency of *conspicua–conspicua* pairing in the summer generation may be obscured by intra-melanic pairings.

Adult body size and mating

Body size constitutes an important trait which could influence behaviour, competitive capacity, fecundity and the longevity of organisms. It is therefore worth-

Table 2 Estimates of the preference coefficient β

Male morph	Female morph								Total β
	conspicua		*spectabilis*		*axyridis*		*succinea*		
	β	S.E.	β	S.E.	β	S.E.	β	S.E.	
Spring generation									
conspicua	0.025*	0.001	0.052	0.007	0.026*	0.008	0.077	0.013	0.180
spectabilis	0.038*	0.007	0.070	0.035	0.174	0.086	0.085	0.066	0.367
axyridis	0.054	0.012	0.038	0.041	0.070	0.096	0	—	0.162
succinea	0.051	0.008	0.051	0.033	0.048	0.057	0.140	0.086	0.290
Total β	0.168		0.211		0.318		0.302		
Summer generation									
conspicua	0.029*	0.008	0.038	0.024	0.054	0.052	0.050	0.024	0.171
spectabilis	0.053	0.029	0.077	0.096	0	—	0.089	0.087	0.219
axyridis	0.130	0.098	0	—	0.417	0.829	0	—	0.547
succinea	0.033	0.023	0	—	0	—	0.030	0.051	0.063
Total β	0.245		0.115		0.471		0.169		

*Significantly different from the non-selection value of $1/16 = 0.0625$ at approximately the 5 per cent level of significance.

Table 3 Body size (total body length in millimetre) of different morphs

Morph	Female	Male
	Mean ± S.E. (n)	Mean ± S.E. (n)
Spring generation		
conspicua	6.63 ± 0.04 (179)	6.17 ± 0.04 (130)
spectabilis	6.77 ± 0.08 (31)	6.05 ± 0.08 (22)
axyridis	6.75 ± 0.16 (11)	6.06 ± 0.33 (5)
succinea	6.86 ± 0.10 (33)	6.00 ± 0.10 (20)
Summer generation		
conspicua	6.75 ± 0.04 (241)	6.14 ± 0.03 (208)
spectabilis	6.76 ± 0.09 (43)	6.18 ± 0.07 (41)
axyridis	6.78 ± 0.11 (13)	6.32 ± 0.15 (12)
succinea	6.66 ± 0.07 (56)	6.22 ± 0.08 (29)

while to analyse the variation in body size among four morphs of *H. axyridis* under study. Table 3 shows the mean body size of each morph for males and females separately. Among females of the spring generation, a difference in body size was marginally significant (ANOVA: d.f. = 3, $SS = 1.80$, $F = 2.15$, $0.05 < P < 0.10$) with *succinea* being the largest and *conspicua* the smallest. Males of the spring generation did not show a significant difference in size between morphs. In the summer generation, neither males nor females demonstrated a significant difference, though female *succinea* appeared to be slightly smaller than three melanic morphs and male *axyridis* slightly larger than other morphs.

When body size was compared between non-mating and mating individuals (Table 4), only males of the

Table 4 Comparison of body size of non-mating and mating beetles

	Body size, mean ± S.E. (n) (mm)		
	Non-mating	Mating	P (t-test)
Spring generation			
Female	6.70 ± 0.04 (180)	6.66 ± 0.06 (74)	> 0.30
Male	6.08 ± 0.04 (22)	6.25 ± 0.06 (55)	< 0.05
Summer generation			
Female	6.74 ± 0.03 (291)	6.72 ± 0.06 (62)	> 0.30
Male	6.17 ± 0.03 (231)	6.12 ± 0.05 (59)	> 0.30

Table 5 ANOVA of minimal adult life span

Source	d.f.	SS	F	P
Body size (B)	1	20.9	0.67	0.41
Morph (M)	3	76.2	0.81	0.49
Sex (S)	1	10.3	0.33	0.57
B*M	3	72.2	0.77	0.51
B*S	1	13.3	0.42	0.51
M*S	3	42.1	0.45	0.72
B*M*S	3	34.3	0.36	0.78

spring generation demonstrated a significant difference; mating individuals were larger than non-mating ones. On the other hand, the fact that females did not show a significant difference in spring may at first sight seem contradictory to the results of Table 3; note here that *succinea* was less abundant than *conspicua*, thus the effect of a slightly larger body of *succinea* was damped by smaller but more abundant *conspicua* in the calculation of mean body size.

An observed high frequency of mating of a particular morph may simply result from that morph being longer-lived than others, rather than from it being more abundant in absolute numbers. In the present study there was no significant difference in minimal adult life span (i.e. time between first capture of an individual and its last observation in the field) in terms of body size, morph, sex and their interactions (Table 5).

Mate choice experiments

Table 6 summarizes the results of a multiple mate choice experiment (Experiment 1) involving four males and four females of different morphs. In the spring generation, males of non-melanic *succinea* achieved a significantly higher frequency of mating than other melanic males ($\chi^2 = 79.8$, d.f. = 3, $P < 0.001$). Conversely, male *conspicua* had the lowest mating frequency coupled with a high refusal rate (37.9 per cent of all mating attempts). Amongst females, melanic *spectabilis* had a significantly low frequency of mating compared with others ($\chi^2 = 18.3$, d.f. = 3, $P < 0.001$). All these phenomena potentially contribute to increasing the proportional abundance of non-melanic *succinea* against that of melanic morphs in a subsequent generation. In contrast to the spring generation, males of the summer generation did not demonstrate a statistically significant difference in mating frequency among different morphs ($\chi^2 = 2.53$, d.f. = 3, $0.30 < P < 0.50$), although the highest incidence recorded for *spectabilis* may agree with the field data of Fig. 2, where they were observed with a disproportionately high frequency of mating. Among females of the summer generation, the difference in the frequency of successful mating among morphs was marginally significant ($\chi^2 = 7.37$, d.f. = 3, $0.05 < P < 0.10$), with *conspicua* being associated with the highest frequency and the lowest refusal rate (10.6 per cent). Thus, a general trend in the summer generation is towards a slight

Table 6 Total number of successful and unsuccessful (refused by females, in parenthesis) mating attempts observed for different morph combinations in the free mate choice experiment with multiple males and females (Experiment 1)

		Female morph				
		Melanic			Non-melanic	
		conspicua	*spectabilis*	*axyridis*	*succinea*	Total
Spring generation						
Male morph	*conspicua*	12 (4)	6 (6)	8 (7)	10 (5)	36 (22)
Melanic	*spectabilis*	13 (5)	6 (5)	21 (9)	13 (3)	53 (22)
	axyridis	12 (1)	7 (7)	10 (1)	11 (6)	42 (15)
Non-melanic	*succinea*	38 (4)	15 (15)	34 (13)	38 (10)	125 (42)
Total		75 (14)	34 (33)	73 (30)	72 (24)	
Summer generation						
Male morph	*conspicua*	17 (4)	11 (4)	17 (2)	17 (5)	62 (15)
Melanic	*spectabilis*	22 (1)	19 (5)	21 (3)	11 (3)	73 (12)
	axyridis	14 (2)	17 (10)	18 (7)	11 (10)	60 (29)
Non-melanic	*succinea*	23 (2)	9 (8)	14 (2)	10 (3)	56 (15)
Total		76 (9)	56 (27)	70 (14)	49 (21)	

advantage for melanics at the expense of non-melanic *succinea*.

In the female choice experiment (Experiment 2, Table 7), where a female was offered a melanic (*conspicua*) and a non-melanic (*succinea*) male, non-melanic males were chosen more frequently than melanics by both melanic and non-melanic females (melanic females, $\chi^2 = 4.00$, d.f. = 1, $P < 0.05$; non-melanic females, $\chi^2 = 13.5$, d.f. = 1, $P < 0.001$) in the spring generation. In contrast, no preference towards either melanic or non-melanic male was demonstrated by females in the summer generation. On the other hand, note that females of melanic *conspicua* tended to mate more frequently with a lower rate of refusal (11.1 per cent, melanic and non-melanic males combined) than females of non-melanic *succinea* (28.1 per cent) in summer.

Males put in a position to choose either a melanic or non-melanic female (Experiment 3, Table 8) showed no significant difference in the frequency of mating with a melanic versus non-melanic female in the spring generation (melanic, $\chi^2 = 0.10$, d.f. = 1, $P > 0.90$; non-melanic, $\chi^2 = 0.68$, d.f. = 1, $0.30 < P < 0.50$). However, in the summer generation both melanic and non-melanic males seemed to mate more frequently with melanic females ($\chi^2 = 3.00$, d.f. = 1, $0.05 < P < 0.10$, melanic and non-melanic males combined); this is in agreement with the tendency observed in Experiment 2.

Finally, the results of the elytral colour-change experiment (Experiment 4) are summarized in Table 9, where information from Table 7 is also incorporated for comparison. In the spring generation, whilst unpainted male *conspicua* (melanic) are clearly less successful at mating than unpainted *succinea* (non-melanic), the former's chance of mating was substantially improved when reverse-painted (i.e. in the same colour as non-melanic *succinea*) ($\chi^2 = 4.59$, d.f. = 1, $0.01 < P < 0.05$), this trend being particularly notable with female *succinea* ($\chi^2 = 30.9$, d.f. = 1, $P < 0.001$); on

Table 7 Total number of successful and unsuccessful mating attempts in the female choice experiment (Experiment 2)

	Female morph			
	Melanic (*conspicua*)		Non-melanic (*succinea*)	
Male morph	Mated	Refused	Mated	Refused
Spring generation				
Melanic (*conspicua*)	12	15	3	9
Non-melanic (*succinea*)	24	10	21	8
Summer generation				
Melanic (*conspicua*)	16	1	12	5
Non-melanic (*succinea*)	16	3	11	4

Table 8 Total number of successful and unsuccessful mating attempts in the male choice experiment (Experiment 3)

	Male morph			
	Melanic (*conspicua*)		Non-melanic (*succinea*)	
Female morph	Mated	Refused	Mated	Refused
Spring generation				
Melanic (*conspicua*)	19	13	21	2
Non-melanic (*succinea*)	21	15	16	2
Summer generation				
Melanic (*conspicua*)	12	2	18	1
Non-melanic (*succinea*)	7	2	11	1

Table 9 Total number of successful and unsuccessful (in parenthesis) mating attempts observed in the artificial colour-change experiment (Experiment 4)

Female morph	Male morph and treatment					
	Unpainted		Reverse-coloured		Duplicate-coloured	
	conspicua	*succinea*	*conspicua*	*succinea*	*conspicua*	*succinea*
Spring generation						
conspicua	12 (15)	24 (10)	23 (18)	23 (6)	3 (6)	11 (0)
succinea	3 (9)	21 (8)	32 (6)	15 (8)	5 (21)	13 (1)
Summer generation						
conspicua	16 (1)	16 (3)	20 (3)	28 (3)	19 (3)	12 (2)
succinea	12 (5)	11 (4)	31 (1)	27 (2)	21 (0)	30 (4)

the other hand, the reverse-painted male *succinea* (i.e. coloured as melanic *conspicua*) demonstrated little change in mating success. When males of *conspicua* and *succinea* were painted with their original colours ('duplicate-colouring'), the pattern of mating was basically the same as in the unpainted control ($\chi^2 = 0.04$, d.f. = 1, $0.80 < P < 0.90$, with female *conspicua*; $\chi^2 = 0.41$, d.f. = 1, $0.50 < P < 0.70$, with female *succinea*); *succinea* males mated more frequently than *conspicua* with both melanic and non-melanic females. In the summer generation, neither reverse- nor duplicate-colouring seemed to alter the pattern of mating; males of *conspicua* and *succinea* had a roughly comparable mating success.

Discussion

Geographical variation in morph frequency has extensively been studied in the European coccinellid *Adalia bipunctata*, with physiological background to support the thermal melanism hypothesis (e.g. Brakefield & Willmer, 1985; Stewart & Dixon, 1989). In contrast, seasonal variation in morph frequency has received less attention and virtually no study has integrated field and laboratory investigations. Timoféeff-Ressovsky (1940) observed an increase in the frequency of melanics as against non-melanics from spring to autumn within a population of *A. bipunctata* in Germany, and suggested that melanics had a higher mortality in winter and were more active in spring. In *Harmonia axyridis*, Tan (1949) suggested that spring and summer climate were more favourable to *conspicua* (melanic) while the autumn climate was more favourable to *succinea* (non-melanic). Despite these earlier works, no detailed study has been undertaken to relate field observations on seasonal morph variation to mating behaviour, although the phenomenon of non-

random mating is well known in *A. bipunctata* (cf. Muggleton, 1978, 1979; Kearns *et al.*, 1990). Because morph variations can be subtle, a clear pattern may not emerge from either field or laboratory investigations alone; a combination of the two is most desirable in this respect.

The present study has demonstrated that an increase in the frequency of non-melanic morph (*succinea*) from the spring to the summer generation is related to non-random mating among different morphs. Evidence to this effect is that, in spring (i) both males and females of non-melanic *succinea* mate more frequently than melanic *conspicua* in the field, the latter being associated with a low selection coefficient, *S*; (ii) male *succinea* are more successful at mating than other morphs, particularly *conspicua*, in laboratory Experiment 1 and 2; (iii) female *spectabilis* (melanic) are less successful at mating than other morphs in Experiment 1. Note that, while the advantage of male *succinea* is evident in both field and laboratory results, the success of female *succinea* is confirmed only in the field. Similarly, the disadvantage of female *succinea* is confirmed only in the field. Similarly, the disadvantage of female *spectabilis*, which apparently occurs in the laboratory but is not evident in the field. These point to the subtleties of observed phenomena which are subjected to sampling biases under field or laboratory conditions. Nevertheless, all these observations concur with the general pattern of increasing non-melanic *succinea* from spring to summer, thus giving support to the view that non-random mating forms a basis of seasonal morph frequency variations. In addition to mating patterns, the fact that female *succinea* of the spring generation are larger in body size than other morphs, particularly *conspicua*, could contribute to their increase in the subsequent generation, probably through their higher fecundity and/or better survival of eggs and hatched larvae.

Different mating patterns are seen in the summer generation in comparison with the spring generation. Male *spectabilis* (melanic) mate more frequently than other morphs in the field and in Experiment 1, although the trend is only marginally significant in the latter. Similarly, while female *conspicua* (melanic) engaged in mating more frequently than female *succinea* (non-melanic) (Experiments 2 and 3), the former's advantage is more modest among four morphs (Experiment 1). Note that male *succinea*, which shows an overwhelming mating advantage in spring over other morphs in both field and laboratory investigations, demonstrates no such trend in summer. All these observations of the summer generation suggest an overall increase in melanics against non-melanics, thus reversing the trend seen in spring.

One of the most interesting results of the present study concerns the varying effects of elytral colour-change (Experiment 4) on mating success. The fact that male *conspicua* substantially improve their mating success when painted as non-melanic *succinea* suggests that (i) colour serves as a strong cue to induce mating, particularly among females, and (ii) females respond positively to the colouring of *succinea*, at least in spring. On the other hand, elytral colour cannot be the only factor involved in male mating success because the frequency of mating of non-melanic *succinea*, even when painted as disadvantaged melanic *conspicua*, shows no perceptible decline. Therefore, non-melanic *succinea* must also have chemically or physiologically based attractiveness which is independent of elytral colours. This dual interaction of elytral colour and other non-colour factor(s) in attracting a mate has never been demonstrated for coccinellid species. Furthermore, the present study also shows that the attractiveness of the traits varies temporally, as is evident in the changing patterns of mating from the spring to the summer generation. Thus, frequencies of morphs change as patterns of non-random mating change on a seasonal basis.

It has been suggested for *A. bipunctata*, that non-random mating is closely linked to mate choice by females (Muggleton, 1979; Majerus *et al.*, 1982a,b; Kearns *et al.*, 1990). In the present study, female mate choice is clearly implicated in the spring results of Experiments 1 and 2, where males of non-melanic *succinea* achieved a higher frequency of mating than other morphs. Similarly, the role of female mate choice appears decisive in the artificial colour-change experiment, as mentioned above. On the other hand, the possibility of male mating preference cannot be negated because in the summer generation females of melanic *conspicua* were apparently more successful at mating than females of *succinea* in Experiments 2 and

3, which implies some kind of choice on the part of males. In addition, field observations reveal that mating males were larger than non-mating ones in the spring generation, which suggests that size-related male–male competition must also be involved. At the same time, differential attractiveness or receptiveness of female morphs to males may also play a part in male mate choice. Thus, several different factors rather than a single factor seem to be concurrently influencing mating patterns in this species.

That the proportion of non-melanic individuals of *H. axyridis* increases towards the summer may be interpreted to conform to the thermal melanism hypothesis, in that non-melanics suffer less from overheating than melanics under strong irradiation in spring–summer (cf. Brakefield & Willmer, 1985; Stewart & Dixon, 1989). However, the fact that melanic *conspicua* is dominant in terms of numbers in both spring and summer, despite an increased proportion of non-melanic *succinea*, and that there is no significant difference in the adult minimum life span among morphs, suggests that thermal melanism is a weak, if not totally insignificant, factor in the seasonal variation of colour polymorphism in *H. axyridis*. Komai's (1956) observation that melanic morphs of this species increase in frequency towards lower latitudes also contradicts the thermal melanism hypothesis. Thus, the maintenance of polymorphic characters in *H. axyridis* needs to be sought in other mechanisms.

The behavioural basis of non-random mating in *H. axyridis* appears to be fairly complex. Although females seem to have more potential for being choosy, particularly by refusing to mate with a certain male (Obata, 1987, 1988), the present study suggests that males could also exert their choosiness by mating preferentially with certain females (cf. Experiment 3). Whether such choosiness is freely expressed under natural conditions, however, remained to be explored. Nevertheless, it is notable that a change in mating preference from one generation to another corresponds to a cyclic variation in morph frequency as observed in the field population. Further investigation is necessary to unravel the adaptive significance of this phenomenon.

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