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Differences in biological and behavioural characteristics of *Harmonia axyridis* (Coleoptera: Coccinellidae) according to colour patterns of elytra

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Abstract

Samples of overwintering populations of *Harmonia axyridis* were classified into five colour patterns, *succinea 1, succinea 2, conspicua, spectabilis* and *axyridis*. Generally, the *succinea 1* colour pattern was dominant at six collecting sites in Daejon and Chungnam provinces of Korea and their occurrence proportion was over 50%. Non-melanic forms such as *succinea 1* and *2* were noted in about 90% of collected individuals. On the other hand, *conspicua* and *spectabilis* as melanic forms were dominant in the laboratory. One hypothesis is that the difference between field and laboratory populations is related to non-random mating and environmental conditions. Practically, in mate-choice experiments, most colour pattern ladybirds seemed to mate with the melanic form, especially the *conspicua* form. The body size (length) of *H. axyridis* in colour patterns was slightly different, but in females, there was no significant difference among colour patterns. However, both lengths of males were significantly different among colour patterns. In reproductive ability (fecundity), there was no difference among colour patterns.

Introduction

The role of predaceous insects in the development of biological pest control has rendered the Coccinellidae of great practical and scientific interest. About 90% of the approximately 4200 coccinellid species are considered beneficial because of their activity, mainly against homopteran pests and mites. Beginning with the introduction of *Rodolia cardinalis* from Australia into California during the 1880s to control the cottony cushion scale (*Icerya purchasi*) on citrus crops, ladybird beetles have played a significant role in biological control strategies. In fact, the former case of *R. cardinalis* is considered by the founding event in classical biological control. While ladybird species that prey upon stationary pests, scale insects or mealybugs have often been successful as biological control agents, aphidophagous species, which migrate over long distances between feeding sites and hibernating shelters, have yielded less satisfying results (Iperti 1999). However, it is a fact that aphidophagous (and to a lesser extent coccidophagous) coccinellids from hibernating sites during dormancy can be very useful in biological control programs by providing a supply of reproductively active predators that can be mass-released against aphid populations (Davidson 1924; Zaslavskii and Sem'yanov 1983).

The multicoloured asian ladybird beetle, *Harmonia axyridis*, is a potential agent for biological control of aphids because of its overwintering behaviour. *Harmonia axyridis* originating from Korea, Japan and Russia tend to aggregate in overwintering sites from late autumn to late winter. Thus, we can collect the large number of overwintering populations that can then be used in aphid control.

In the course of sampling the overwintering populations, we observed that there are considerably variable colour patterns on elytra. Komai (1956) reported that *H. axyridis* had four colour patterns, *succinea, conspicua, spectabilis* and *axyridis*. In this study, we divided the non-melanic, succinea form into two groups by the number of spots on the elytra. Individuals with 19 black spots on elytra were classified as *succinea* 1 and those with 0–18 spots were *succinea* 2. Among individuals in succinea 2 groups, their variation of elytra patterns was fairly different due to the numerical difference of spots

In this paper, *H. axyridis* was classified according to five colour patterns and respective frequency of occurrence was investigated. The difference in occurrence frequency between field and laboratory populations was also compared, and because there were considerable differences, we conducted mate-choice experiments in the laboratory. In their genetic composition and in characters, there are many practical differences between natural and laboratory populations. Therefore, the importance of variation between laboratory-reared and wild type individuals has been widely recognized. Mackauer (1976) pointed out the significance of the origin and size of samples collected in the field for establishment of preservation of genetic variation in laboratory populations.

Apart from external factors, such as thermal and industrial stresses, which can affect polymorphic characters, non-random mating of 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 and Halliday 1984). The present study carried out mating-choice experiments in the laboratory to explain the temporal patterns of polymorphism in *H. axyridis* and so investigated whether colour patterns can influence mating behaviour.

Materials and Methods

Field collections of *H. axyridis* overwintering populations

Overwintering adults of *H. axyridis* were collected from six aggregation sites (table 1) in the Chungnam and Taejon area in October to late-December from 2000 to 2002. After collection, they were brought to the laboratory. The sex ratio of samples was recorded. All ladybird stages were maintained at a temperature of 25 ± 2°C and 65 ± 5% relative humidity. They were then placed in 15 cm diameter plastic Petri dishes and reared with an artificial diet

Table 1 Proportion of different colour patterns of *H. axyridis* collected from overwintering sites from 2000–2002

Collection site	Year	No. of collected insects	Colour pattern (%)				
			succinea 1	*succinea 2*	*conspicua*	*spectabilis*	*axyridis*
Songgang-dong	2000	859	64.49	25.20	4.49	3.28	2.54
Yusung-gu	2001	17,775	52.67	41.30	3.49	2.45	0.10
Daejeon	2002	4,024	52.31	40.23	4.10	3.08	0.27
	Total	22,658	56.49	35.58	4.03	2.94	0.97
Gung-dong	2001	585	45.61	47.05	4.85	2.50	0.00
Yusung-gu	2002	711	53.73	36.99	5.49	3.80	0.00
Daejeon	Total	1,296	49.67	42.02	5.17	3.15	0.00
Gao-dong	2000	5,773	54.30	38.07	4.65	2.73	0.26
Dong-gu	2001	1,576	60.28	30.52	4.76	4.19	0.25
Daejeon	2002	344	50.58	41.28	5.23	2.91	0.00
	Total	7,693	55.05	36.62	4.88	3.28	0.17
Noeun-dong	2002	2,139	51.99	40.25	4.91	2.66	0.19
Yusung-gu							
Daejeon							
Geumsan	2001	1,633	53.32	37.87	4.92	3.67	0.22
Chungnam Prov.	2002	152	65.79	26.97	4.61	2.63	0.00
	Total	1,785	55.56	32.42	4.77	3.15	0.11
Non-san	2001	162	46.20	47.12	4.63	2.08	0.00
Chungnam Prov.							

composed of ground chicken liver containing 10% sucrose. Some natural populations were in a wood or Styrofoam box and were then stored in an incubator at 10°C for 3–5 months. Some individuals among overwintering populations were maintained in acryl cages and fed with the cotton aphid (*Aphis gossypii*), the greenpeach aphid (*Myzus persicae*) and the turnip aphid (*Lipaphis erysimi*) as prey.

Classification of *H. axyridis* adults according to colour pattern of elytra and pronotum

There are five main components of the colour patterns: ground colour, colour of markings, number of spots, strength of spots and fusions between the spots. *Harmonia axyridis* typically has four elytral colour patterns: *succinea* [orange with some or no black spots(s)], *axyridis* (black with some red spots or patches), *conspicua* (black with two red spots) and *spectabilis* (black with four red spots) (Komai 1956). Succinea were divided into two groups, succinea 1 and succinea 2 according to the number of spots on the elytra and their occurrence frequency. Individuals with 19 black spots on elytra were classified as succinea 1 and those with 0–18 spots were succinea 2 (fig. 1). Following previous work (Komai 1956), we regarded ladybird beetles with black elytra (*axyridis*, *conspicua* and *spectabilis*) as melanic and those with orange or yellow elytra (*succinea* groups) as non-melanic.

Frequencies of the five colour patterns of the laboratory-reared *H. axyridis* population

To obtain eggs, several males and females were randomly selected among the first generation of progeny produced from the field collected individuals, and then reared with the cotton aphid (*A. gossypii*) as prey in an acryl rearing cage (30 cm × 30 cm × 60 cm). Newly laid eggs were separated from mated females every day and placed into new Petri dishes until they hatched and sufficient aphids were supplied to promote development through four larval stages, and then through the pupal stage, adults emerged and could be obtained within 15 to 20 days from the first larval stage. After hardening and body colour pigmentation of adults had completely finished, colour patterns were classified by the method described above.

Measurement of body length of *H. axyridis*

Body size (length) of females and males were investigated according to collection sites and colour patterns. The five collecting sites were Gao-dong, Noeun-dong, Gung-dong and Songgang-dong in

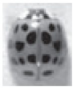
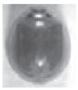
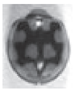
Pattern name	Characteristics	Figure
Succinea 1	19 black spots on yellow or red elytra with M-shaped marking on the pronotum	
Succinea 2	0–18 black spot(s) on yellow or red elytra with M-shaped marking on the pronotum	
Conspicua	2 red spots on black elytra without M-shaped marking on the pronotum	
Spectabilis	4 red spots on black elytra without M-shaped marking on the pronotum	
Axyridis	Irregular and rare patterns	

Fig. 1 The colour patterns of five forms of the multicoloured asian ladybird beetle, *H. axyridis*, which were collected in aggregation sites. Beside this pattern, there are several patterns in *axyridis* patterns.

Taejon city and Majeon-ri in Chungnam province. Body length was measured from the pronotum to rear margin of the elytra with a Digimatic caliper (CD-15cp, Mitutoyo Co., Kanagawa 213-8533, Japan). Each sample was selected randomly, and then the sample number was recorded.

Comparison of reproductive ability of different colour patterns

Reproductive ability of the ladybirds fed on different aphids as prey was investigated according to their different five colour patterns. There were two types of experimental conditions. One was a male and female of *H. axyridis* kept together in a plastic Petri dish. The other was a mated female alone. In this case, the male was removed from the plastic Petri dish after matings. The 30-day fecundity (total number of eggs laid by each female for 30 days) was recorded, and all of the eggs were observed to examine egg viability. We measured the fecundity and hatchability of the different colour pattern morphs. All adults of *H. axyridis* used were newly emerged in the laboratory within the previous 5 days, so they had not yet had a chance to mate.

Laboratory mate-choice experiments

The experimental arena was an acryl cage (30 cm × 30 cm × 60 cm); five morphological combinations of 25 males and three virgin females of the same colour pattern were used. Five different patterns of 25 males were first put in the acrylic cage and then three virgin females were introduced after 1 h. There was no food in the cage. In the reverse situation, five different patterns of 25 virgin females(five colour patterns × 5 individuals) were firstly released, and then were introduced to three males after 1 h. Mating observations were carried out for 1 h after introducing males or females. In all experiments, copulating pairs were taken out from the cage after 2 min of copulation. All male and female ladybird beetles were randomly chosen from the stock cultures and their developmental stages were the same, males and females were used 10 days after emergence from pupa. Six replicates were used in the mating-choice experiments.

Statistical analysis

All data were analysed by completely randomized one-way ANOVA at $P < 0.05$ with Tukey's-*post hoc* test HSD in SPSS software (SPSS, Chicago 60606, IL, USA).

Results

Field collection of *H. axyridis* overwintering population and classification of adults according to the colour pattern of elytra and pronotum

From 2000 to 2002, collections of overwintering populations of *H. axyridis* were carried out weekly at six aggregation sites. Varying degrees of spot fusion were also found (Chapin and Brou 1991; Tedders and Schaefer 1994; Kidd et al. 1995). Spots varied in size and shape, and insects assigned to a certain spot number category did not necessarily have the same spot location pattern. As a result of sampling of field populations, colour patterns of *H. axyridis* were classified into five colour patterns: *succinea 1* and *2*, *conspicua, spectabilis* and *axyridis*. Table 1 shows the frequencies of five types collected at six aggregation sites for 3 years. Generally, individuals of the *succinea 1* were dominant in all collecting sites, and represented more than 50% of beetles. Non-melanic forms such as *succinea 1* and *succinea 2* comprised about 90% of collected individuals. Notably, the *axyridis* type was rarely found in collecting sites, its frequency being less than 1%. The *conspicua* and *spectabilis* types collected were about 5% and 3%, respectively.

Table 2 shows the differences in occurrence frequencies and the appearance of females in field and laboratory populations. Six collected groups in the field and three laboratory groups were randomly selected and investigated. In the field groups, occurrence of colour patterns was significantly different among collected females ($F = 65.944$; $P = 0.000$), but there was no significant difference in the laboratory-reared groups ($F = 1279$; $P = 0.342$). Like females from the field collected group, males collected in the field showed a significant difference in colour patterns ($F = 63.528$; $P = 0.000$). In field collected groups, more *succinea 1* type females were collected than any other type. However, in the case of males, the *succinea 2* type was more dominant. There was no significant difference among *axyridis, conspicua* and *spectabilis*. In the laboratory-reared groups, there was no difference in occurrence of males' colour patterns ($F = 0.798$; $P = 0.553$). When the ratio of female occurrence was examined in the field, a difference in colour pattern frequency between males and females was observed ($F = 4.211$; $P = 0.007$). The percentage of females collected in overwintering sites ranged from 47.1% to 83.3%. Ratios of females in *axyridis, spectabilis* and *succinea 1* were 83.3%, 74.5% and 74.0%. Female frequencies were generally more than males except for the *succinea 2*

Table 2 Number of individuals in five colour patterns collected from overwintering sites and laboratory rearing

Site	Colour pattern	Collected individuals Females	 Males	Ratio of females (%)
Field collected groups	*succinea 1*	178.3 ± 46.43a	64.8 ± 27.04b	74.0 ± 6.28a
	succinea 2	85.6 ± 36.06b	89.5 ± 18.97a	47.1 ± 8.54b
	conspicua	11.4 ± 4.78c	4.4 ± 1.69c	70.9 ± 8.27ab
	spectabilis	9.3 ± 3.01c	3.3 ± 2.05c	74.5 ± 17.33a
	axyridis	1.1 ± 0.64c	0.3 ± 0.46c	83.3 ± 35.63a
Laboratory-reared groups	*succinea 1*	6.3 ± 3.51a	2.0 ± 3.46a	87.5 ± 21.65a
	succinea 2	11.7 ± 10.60a	13.0 ± 9.00a	42.6 ± 8.92a
	conspicua	20.3 ± 17.62a	10.7 ± 15.01a	74.0 ± 14.73a
	spectabilis	10.7 ± 9.85a	5.3 ± 4.16a	69.1 ± 19.09a
	axyridis	4.33 ± 3.21a	6.3 ± 4.93a	42.2 ± 23.41a

Numbers are means ± SD (eight for the field and three replicates for laboratory). Letters within columns indicate significant differences in different colour patterns at P < 0.05 with Tukey's HSD.

subtype group. In the case of the laboratory-reared group, there was no difference in proportion of males and females according to the colour pattern. There were no significant differences among types ($F = 3.586$; $P = 0.046$). Although *succinea 1* appeared more often any other type, this was not statistically significant.

Body length of *H. axyridis* according to colour patterns in overwintering populations

Body size (length) constitutes an important trait that could influence behaviour, competitive capacity, fecundity and the longevity of organisms (Osawa and Nishida 1992). It is therefore worthwhile to analyse the variation in body size among four colour patterns of *H. axyridis* under study. Because the *axyridis* type of *H. axyridis* collected at overwintering sites was very rare, their body length is not described, as they were not sufficiently frequent for statistical analysis.

Like other coccinellids, *H. axyridis* adults are sexually dimorphic in body size (Smith 1966). Adults of *H. axyridis* were collected from five different sites in 2002. Table 3 shows the mean body length of females and males separately for each collection site. Males and females had significantly different body lengths ($F = 2.589$; $t = 19.088$; $P = 0.000$). Average body length of 620 females and 452 males was 6.62 ± 0.46 and 6.11 ± 0.41 mm, respectively. However, there were no significant differences in the body length of females and males among the collection sites ($F = 0.973$, $P = 0.421$ for females; $F = 1.475$, $P = 0.209$ for males using a *t*-test).

Table 3 Body length of female and male *H. axyridis* in random samples taken from different overwintering sites in the Daejon area in 2002

Collecting site	Females Number of samplings	 Body length (mean ± SD)	Males Number of samplings	 Body length (mean ± SD)
Gao-dong	115	6.61 ± 0.46	78	6.07 ± 0.44
Majeon-ri	99	6.61 ± 0.48	64	6.06 ± 0.38
Noeun-dong	136	6.57 ± 0.45	112	6.08 ± 0.43
Gung-dong	130	6.66 ± 0.43	100	6.18 ± 0.45
Songgang-dong	140	6.66 ± 0.45	98	6.15 ± 0.35
Total	620	6.62 ± 0.46	452	6.11 ± 0.41

Table 4 shows the mean body length of female and male *H. axyridis* from overwintering sites according to four colour patterns. Body size of *H. axyridis* was slightly different between colour patterns, but in females there was no significant difference in body size between colour patterns ($F = 0.275$; $P = 0.843$). However, the body length of males was significantly different between colour patterns ($F = 10.902$; $P = 0.000$). In particular, the average body length of males in *spectabilis* type was larger (6.27 ± 0.31 mm) than any other type, and males of the *succinea 1* subtype were smaller (5.97 ± 0.45 mm) than any other type.

Reproductive ability (fecundity) of different colour patterns

When the greenpeach aphid was supplied as prey, fecundities were significantly different between the

Table 4 Body length of female and male *H. axyridis* depending on four colour patterns from overwintering sites and laboratory rearing

	Females		Males	
Colour pattern	Number of samplings	Body length (mean ± SD)	Number of samplings	Body length (mean ± SD)
succinea 1	241	6.63 ± 0.44a	160	5.97 ± 0.45b
succinea 2	248	6.62 ± 0.47a	239	6.19 ± 0.38ab
conspicua	75	6.61 ± 0.47a	34	6.18 ± 0.37ab
spectabilis	56	6.58 ± 0.48a	19	6.27 ± 0.31a
Total	620	6.62 ± 0.46	452	6.11 ± 0.41

Letters within columns indicate significant differences in different colour patterns at $P < 0.05$ with Tukey's HSD.

five colour patterns in the condition (a male and female of *H. axyridis* were placed in a Petri dish) ($F = 5.806$; $P = 0.011$). In both mating conditions, number of eggs laid by the *succinea 2* and *axyridis* types were 149.7 ± 30.55 and 151.7 ± 27.15, respectively, and were significantly less than those laid by *succinea 1, conspicua* and *spectabilis* types. ($F = 19.234$; $P = 0.000$).

On the other hand, when cotton aphids were supplied as prey, there was a significant difference in the experimental condition (a female and male were placed together in a dish until the experiment was finished) ($F = 6.829$; $P = 0.006$). Only the *axyridis* type laid fewer eggs (136.3 ± 25.66) than any other type. However, this was very similar to conditions in which greenpeach aphids were supplied (51.7 ± 27.15). In the condition where the female is alone after the male was removed after mating, a significant difference was found between colour patterns ($F = 6.148$; $P = 0.009$). For example, *conspicua* type laid about 370 eggs. This was the highest number of eggs laid among all tested conditions.

Where *M. persicae* were supplied as prey, there was a significant difference between *succinea 2* type and others in the condition where the male and female were placed together ($F = 22.775$; $P < 0.001$). However, there was no difference in hatchability where females were alone after mating once. Where *A. gossypii* were supplied as prey and females were alone after mating once, hatchability in *spectabilis* was significantly higher than in any other colour pattern ($F = 4.719$; $P = 0.021$). However, in the case where a male and female were placed together and maintained with *A. gossypii*, there was no significant difference between them ($F = 1.499$; $P = 0.275$). In the case where *M. persicae* were supplied to females who were alone after mating, no significant difference among five colour patterns was shown ($F = 0.394$; $P = 0.808$) (table 5).

Mating-choice laboratory experiments

In the female input (choice) experiment, three females of the same type were offered to five males per each type; it was shown that *conspicua* type males were predominantly selected for mating by many females. The colour pattern order according to mating frequency were *conspicua* > *axyridis* > *spectabilis, succinea 1* > *succinea 2*. Except for the *succinea 2* pattern, other male patterns chosen were nearly the same (table 6). In the male input (choice)

Table 5 Number of eggs laid and hatchability by the five colour patterns according to the different rearing conditions and prey

		Condition 1 Female & male with mating		Condition 2 Female alone after one mating	
Aphids as prey	Colour pattern	No. of eggs	% of eggs hatched	No. of eggs	% of eggs hatched
M. persicae	*succinea 1*	255.0 ± 31.61a	73.4 ± 0.67a	277.3 ± 38.55a	72.8 ± 9.55a
	succinea 2	149.7 ± 30.55b	26.4 ± 5.80b	238.7 ± 16.50a	65.9 ± 4.05a
	conspicua	204.7 ± 35.95ab	65.4 ± 10.33a	223.3 ± 34.85a	76.8 ± 8.02a
	spectabilis	249.3 ± 48.79a	68.1 ± 7.14a	105.7 ± 18.72b	73.3 ± 20.39a
	axyridis	151.7 ± 27.15b	61.9 ± 6.24a	119.0 ± 34.70b	69.9 ± 6.95a
	Subtotal	201.1 ± 54.87	59.0 ± 18.25	192.8 ± 74.98	71.7 ± 10.25
A. gossypii	*succinea 1*	311.7 ± 22.90a	52.6 ± 8.45a	239.7 ± 26.16b	55.0 ± 8.92ab
	succinea 2	250.7 ± 38.21ab	56.3 ± 9.85a	255.3 ± 27.47ab	56.7 ± 10.59ab
	conspicua	265.0 ± 45.21a	68.4 ± 7.03a	370.7 ± 29.87a	66.7 ± 4.97ab
	spectabilis	271.0 ± 69.54a	46.1 ± 16.18a	255.0 ± 30.27b	76.9 ± 10.68a
	axyridis	136.3 ± 25.66b	65.7 ± 19.38a	290.0 ± 22.61ab	51.6 ± 2.94b
	Subtotal	246.9 ± 71.25	57.8 ± 13.98	288.8 ± 55.61	61.4 ± 11.83

Numbers are means ± SD (three replicates for laboratory). Letters within columns indicate significant differences in different colour patterns at $P < 0.05$ with Tukey's HSD.

experiment, when three males having same colour pattern were placed with each of five non-melanic and melanic females, *conspicua* colour pattern females were more selected than any other colour pattern. Except *conspicua* pattern, there was no significant difference in number of successful matings of beetles with four colour patterns, as shown in tables 6 and 7. Males and females put in a position to choose either a melanic or non-melanic mate showed no difference in the frequency of mating with melanic or non-melanic. The mating frequency among colour patterns was in the order *conspicua* > *succinea 2* > *axyridis* > *succinea 1* and *spectabilis*. However, both males and females of non-melanic and melanic forms seemed to mate more frequently with melanic forms. In particular, in both experiments, *succinea 2* individuals preferred to mate with *conspicua* form individuals.

Table 6 Number of successful matings accomplished by three females (same colour pattern) with total 25 males (five individuals per each colour pattern) with six replicates in the acrylic cage (30 cm × 30 cm × 50 cm) for 1 h

Colour pattern	Females					Total
	Non-melanic type		Melanic type			
	succinea 1	*succinea 2*	*conspicua*	*spectabilis*	*axyridis*	
Males						
succinea 1	4	0	4	2	2	12
succinea 2	0	0	0	0	6	6
conspicua	4	10	2	4	2	22
spectabilis	0	2	4	4	2	12
axyridis	4	2	4	2	4	16

Table 7 Number of successful matings accomplished by three males in opposition to 25 females, respectively (five individuals per each colour pattern) in an acryl cage (30 cm × 30 cm × 50 cm) for 1 h

Colour pattern	Males					Total
	Non-melanic type		Melanic type			
	Succinea 1	*succinea 2*	*conspicua*	*spectabilis*	*axyridis*	
Females						
succinea 1	4	2	0	0	2	8
succinea 2	2	6	2	0	3	13
conspicua	4	10	2	4	2	22
spectabilis	4	0	0	2	2	8
axyridis	2	4	2	1	2	11

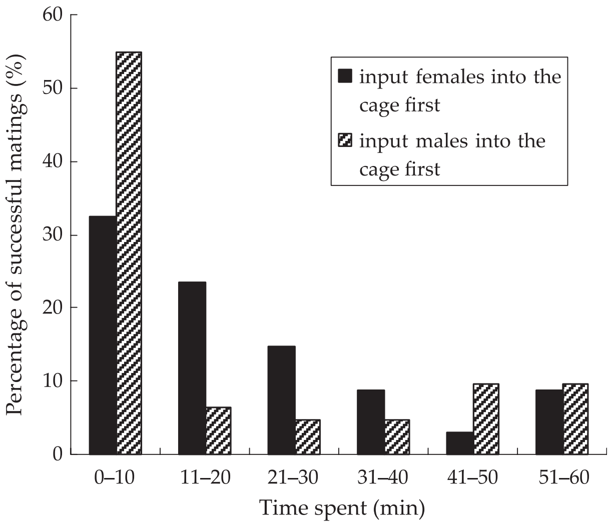


Fig. 2 Percentage of successful matings accomplished by females or males according to the time spent in an acrylic cage (30 cm × 30 cm × 50 cm) for 1 h.

The time necessary for successful mating is shown in fig. 2. When females were put into a cage first, the necessary time for successful mating ranged from 1 min to 1 h. On the other hand, when males were placed to the cage later, mating trial time was faster than when females were put in the cage. The time required for mating was mainly distributed within a 10-min-period (54.8%).

Discussion

We were able to collect about 18 000 individuals at one aggregation site in a year. Significantly, it was observed that the colour pattern within a population was variable. Geographic variation in morph frequency has been studied in the European coccinellid *Adalia bipunctata* with physiological background to support the thermal melanism hypothesis (e.g. Blakefield and Willmer 1985; Stewart and Dixon 1989). In contrast, the genetic basis for colour polymorphism has had less attention in *H. axyridis*. Individual variation is a conspicuous phenomenon, whenever organisms of the same species are carefully examined. The most obvious features of ladybirds are their colours and patterns. Many species are extremely variable, having a great range of different forms. In the case of *H. axyridis*, it is suggested that their variation in colour pattern is the largest among coccinellids. Variation among individuals may be the outcome of differences in their genetic constitutions, differences in the environments to which they have been exposed, or a combination of both. In many cases, it is not easy to discern whether a particular type of variation has a genetic or an environmental basis (Majerus 1994). Among the five colour patterns, individuals of *succinea 1*, as the non-melanic form, were the most frequently occurring in natural populations, while all five colour patterns occurred in the laboratory, where *conspicua* and *spectabilis* as melanic forms were dominant. It might be concluded that the difference between field and laboratory populations was related to non-random mating behaviour and environmental conditions. In laboratory mating experiments, it seemed that most colour pattern ladybirds preferred to mate with the melanic forms, especially the *conspicua* form. Although there is evidence that *A. bipunctata* in some populations prefer to mate with melanic males, nothing is known of the cues used by ladybirds in mate recognition (Majerus et al. 1982; O'Donald and Majerus 1998).

In *A. bipunctata*, non-random mating as well as behaviour-based mate-choice has been observed (Muggleton 1978; Majerus et al. 1982; Kearns et al. 1990). Very few studies, however, have demonstrated the fitness consequences of non-random mating in field populations of polymorphic coccinellids. This colour polymorphism is mainly due to the difficulty of quantitatively observing mating in a highly mobile and dispersive species.

Among the most obvious factors of adult size are those that affect larval development: food, temperature and population density. Although no detailed study on the mechanism of determination of adult size in coccinellids has been published, several authors demonstrated a correlation between environmental factors and final pupal or adult size (Hodek and Honek 1996).

Like other coccinellids (Smith 1966), adult *H. axyridis* were sexually dimorphic in body size. Polymorphism in this species is based on a series of multiple alleles and is known to vary with prey, season and geographic location (Dobzhansky 1933; Komai 1956); non-random mating also plays a role (Osawa and Nishida 1992).

Body size did not appear to be influenced by collection site or colour morph. On the other hand, for different colour patterns, body size was slightly different; in particular, the melanic form (for example, *conspicua* and *spectabilis* type) was larger than non-melanic form, *succinea 1* and *2* type. In contrast to many other species, effects of temperature on adult size of Coccinellidae are often small. For example, in case of *A. bipunctata* reared at constant 15–25°C, the variation (difference between minimum and maximum) of adult size was 12% of median size in males, and only 3% in females (Mills 1981). In *Coleomegilla maculata*, reared at 19–27.3°C, the variation (both sexes combined) was 5% of the median size (Wright and Laing 1978). In *Hippodamia quinquesignata,* the differences were 13% in males and 20% in females (Kaddou 1960). In the case of *H. axyridis* collected in fields, the variation of adult size was significantly different for each colour pattern and sex. In *succinea 1* type, the variation of size was 30.3% of median size in males, and 35.2% in females. In *succinea 2* type, the differences were 20.6% in males and 23.8% in females. In case of *conspicua* type, the size variation was 21.7% in males and 28.5% in females. In *spectabilis* type, the differences were 15.5% in males and 25.2% in females. Generally, the melanic forms, such as *conspicua* and *spectabilis* types, were more different than the non-melanic forms, *succinea 1* and *succinea 2* type, in variation of adult size.

Body size might also be affected by food quality. Variation in body size caused by feeding unfavourable prey cannot be explained by decreased consumption only. In *A. bipunctata*, *Propylea quatuordecimpunctata* and *Coccinella septempunctata* (Olszak 1986, 1988) feeding on non-preferred aphids decreased adult size and increased mortality. Prey consumption, however, increased when food is not favourable, due to prolonged development time. In this case, the effect may be due to poor ingestion and conversion of the food, rather than to starvation caused by prey rejection.

Our studies showed that reproductive ability (number of eggs laid and hatch rates) for the five colour patterns was different. That of melanic forms such as *conspicua, spectabilis* and *axyridis* was superior to non-melanic forms. In our mating experiments, it was confirmed that most colour patterns of *H. axyridis* preferred to mate with melanic-forms. It was considered that the two experimental results discussed above were correlated, and it is probable that body size is related with reproductive ability and mating preference.

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