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Non-random mating activity of colour morphs of ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Abstract. Ladybird beetles are highly sexually active as they mate often and for a long time. In some of the species that are polymorphic in terms of their elytral colouration there is a high proportion of melanic males in mating pairs. We recorded the number of individuals of *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) of both sexes and of all colour morphs (light *succinea*, melanic *spectabilis* and *conspicua*), and the numbers of the different morphs mating in May and June on ornamental shrubs in the Czech Republic. There were only 29% males among the individuals; 11% of individuals were melanic. About 17% of the individuals were recorded in copula, which means that 30% of males were mating at any time. The composition of pairs indicated that melanic individuals, especially those of morph *conspicua*, mated more frequently than light coloured individuals and more frequently with another melanic individual. Both the choice of partner and increased activity due to the higher body temperature of sun-basking melanics might have determined these trends.

Keywords. Sexual selection, female choice, colour polymorphism, Coccinellidae, equicolor.

INTRODUCTION

Ladybird beetles (Coleoptera: Coccinellidae) are generally very active sexually and mate for long periods (Hodek & Ceryngier 2000, Nedvěd & Honěk 2012). In some polymorphic species, differential or assortative mating among colour morphs is reported. In Latvian and Russian populations of *Adalia bipunctata*, Lusis (1961) records a higher proportion of melanic individuals in mating pairs than expected, which he hypothesized is a consequence of their being darker in colour and absorbing more solar radiation, which results in their having a higher body temperature and greater activity than paler coloured individuals. DeJong et al. (1996) were the first to show that melanic individuals of *A. bipunctata* have higher body temperatures and are more active than pale coloured individuals.

However, Creed (1975) does not report assortative mating in Britain and Western Europe. Muggleton (1979) proposes that mating is frequency dependent with the rarer morph being the preferred mate. Majerus et al. (1982) report that melanic males, but not females, are preferred and that an inherited female choice is responsible for this bias. Since ladybird vision is believed to be poor, it is suggested they use olfactory cues to recognize the different morphs. Brakefield's (1984) analysis of a large dataset of mating frequencies of *A. bipunctata* in the Netherlands revealed no evidence for assortative mating. Haddrill et al. (2013) confirm that the patterns of sexual selection and reproductive behaviour differ in different populations of *A. bipunctata*.

Although changes in the frequency of different colour morphs are reported for some polymorphic ladybirds, Honěk et al. (2005) record little variation in central European populations of *A. bipunctata* and *A. decempunctata* and only small long-term changes in *Hippodamia variegata* (Honěk et al. 2012).

Another polymorphic ladybird species, *Harmonia axyridis*, shows seasonal variation in the frequency of its elytral colour pattern (Tan 1949). The proportion of the non-melanic *succinea* morph increases from spring to summer because they mate more frequently than the melanic morphs in spring (Osawa & Nishida 1992). In the laboratory, non-melanic males are preferred in spring and melanic males are more successful at mating in summer. Males were active to a lesser degree in this mate selection. Although the male elytral colour pattern is important in female mate selection, behaviour and chemical cues must also be involved.

In China females of *H. axyridis* also prefer light *succinea* morph males to melanic ones in spring (Wang et al. 2009, Su et al. 2013). Melanic individuals make up 15–20% of the population in autumn and about 50% in spring, which indicates that melanism is advantageous in winter and costly in summer (Wang et al. 2009).

Refusal (rejection) of non-preferred males by females is probably involved in mate choice (Obata 1988, Wang et al. 2009). Retention of eggs before laying and fertilizing occurred after mating with non-preferred males (Wang et al. 2009, Su et al. 2013). Melanic females wait longer than light coloured females. Melanic males mate for longer with light than melanic females, which indicates that both sexes are actively involved in the choice of a partner.

As *H. axyridis* has now spread world-wide (Brown et al. 2011) the reproductive behaviour of invasive populations needs to be compared with that of this species in its native range to determine whether it differs and could have determined its invasive success. In the laboratory, males from invasive populations sire more offspring than males from native populations (Laugier et al. 2013).

Evolutionary changes in reproductive strategy and associated traits during the spread of a species may also have involved colour morph mating preferences in *H. axyridis*. We investigated the mating behaviour of individuals in an invasive population of this ladybird in the field in Central Europe in order to determine whether mate choice is similar to that reported in earlier studies. We also tried to determine whether the differences in mating frequency was a result of only mate choice or the higher activity of melanic individuals when exposed to bright sunlight.

MATERIAL AND METHODS

We visually observed and recorded all individuals of all species of ladybirds from 8 May to 3 June 2012. Observations were made in the morning, approximately from 8 to 10 o‘clock, and in the afternoon, from 13 to 15, each day, except when raining. We inspected five plots of ornamental shrubs in the town of České Budějovice, 48° 59’ N, 14° 27’ E, mostly of *Philadelphus coronarius* infested with *Aphis cirsiiacanthoidis* and *Spiraea* x *vanhouttei* with *Aphis spiraecola* and *Aphis spiraephaga*. The records do not equal the number of individuals since we may have observed some individuals several times. In addition to the species, we recorded the sex, mating activity, colour morph and composition of mating pairs in the case of *H. axyridis*.

Online QuickCalcs (GraphPad Software 2014) was used to calculate χ^2 values for frequency tables. Expected frequencies of a certain colour morph combination of a male and female pair; and of one of the sexes being of a particular colour morph were calculated based on the recorded frequencies of both mating and non-mating males and females of a particular morph collected over the course of the entire study.

When there was a difference between the observed and expected numbers of pairs or individuals of certain colour morphs in the table, we used stepwise complement χ^2 tests (SCCST) to evaluate whether the differences are significant. That is, a 2×2 frequency table was calculated for each category, one row including observed and expected numbers of that combination and another row including sum of all other categories. Probability level was adjusted to 0.05/number of categories.

RESULTS

During this study, we recorded 2,196 adult *Harmonia axyridis*. There were 634 records of males (29%) and 1,562 records of females (71%), which is very strongly biased in favour of females ($\chi^2(1)=392$, $p<0.0001$). In terms of the different colour morphs 89% were of the morph *succinea*, 8% of the morph *spectabilis* and 3% of the morph *conspicua*. The percentages of males and females of the different morphs were similar. Moreover, among these individuals, one male was of the rare form *equicolor* (heterozygous with *succinea* allele).

Seventeen per cent of the individuals were recorded copulating. This means that 30% of the males were in copula. All nine possible combinations of males and females of the three main colour morphs were recorded (Table 1).

There were fewer pairs of the combination *succinea* male and *spectabilis* female than expected, whereas both the combination *spectabilis* with *conspicua* and monomorphic *conspicua* pairs were more frequently observed than expected.

The analysis of males and females of the three different morphs recorded mating revealed a lack of both males and females of the *succinea* morph and excess of both males and females of the *conspicua* morph, and more *spectabilis* males and fewer *spectabilis* females than expected (Table 2). However, the only significant difference was the excess of *conspicua* females.

To support or falsify the thermal melanism hypothesis as an explanation of the differences in recorded frequencies, the records made in the morning (8–10 hours), when air temperature was low and sun-basking was more important, were analyzed separately. There were 787 individual records in the morning of which 29% were for males and of which 91% were of the morph *succinea*, 7% of the morph *spectabilis* and 2% of the morph *conspicua*. Nineteen per cent of them were recorded copulating. This means that 32% of the males were in copula at any given time during the day.

Eight of nine possible combinations of males and females of the three main colour morphs were recorded. There were significantly more *spectabilis* male with *conspicua* female matings than expected. Generally, there were fewer monomorphic *succinea* pairs than expected but this difference was not significant. Melanic individuals mated with individuals of all of the other morphs.

When the results for the males and females were analyzed separately, there were significantly more *conspicua* females than expected. Generally, there were fewer individuals of the light *succinea* morph and more of the melanic *conspicua* morph, although not significantly so. In addition, there were more *spectabilis* males and fewer *spectabilis* females recorded. Differences were somewhat weaker than those recorded in the afternoon.

Table 1. Frequency of pairs of all combinations of colour morphs recorded in the present study. Expected numbers of pairs were estimated based on the frequency of all adults (mating and non-mating) recorded in this study. The observed and expected numbers in the table differ significantly ($\chi^2(8)=297$, $p<0.0001$). Differences for individual combinations based on the SCCST method are indicated in rows χ^2 and P, and in bold if significant at an adjusted level of 0.006

male female	*succinea* *succinea*	*succinea* *spectabilis*	*succinea* *conspicua*	*spectabilis* *succinea*	*spectabilis* *spectabilis*	*spectabilis* *conspicua*	*conspicua* *succinea*	*conspicua* *spectabilis*	*conspicua* *conspicua*
observed	304	12	8	26	4	12	8	4	6
expected	305	26	8	30	3	1	9	1	0
difference	−1.2	−14.1	−0.5	−4.5	1.4	11.2	−1.3	3.2	5.7
ratio	1.00	0.46	0.94	0.85	1.54	14.17	0.87	5.06	23.34
χ^2	0.023	8.17	0.013	0.71	0.76	146	0.028	13.07	127
P	0.88	**0.004**	0.94	0.4	0.38	**$<10^{-4}$**	0.84	**0.0003**	**$<10^{-4}$**

Table 2. Frequency of males and females of the three colour morphs recorded in mating pairs. Expected numbers of individuals were based on the frequency of all adults (mating and non-mating) recorded in this study. The observed and expected numbers in the table differ significantly ($\chi^2(8)= 40.29$, $p<0.0001$). Differences for individual combinations based on the SCCST method are indicated in rows χ^2 and P, and in bold if significant at an adjusted level of 0.008

sex morph	male *succinea*	male *spectabilis*	male *conspicua*	female *succinea*	female *spectabilis*	female *conspicua*
observed	324	42	18	338	20	26
expected	339.8	33.9	10.3	344.9	29.5	9.6
difference	–15.79	8.08	7.70	–6.91	–9.50	16.41
ratio	0.95	1.24	1.75	0.98	0.68	2.71
χ^2	1.32	2.03	5.84	0.25	3.18	28.37
P	0.25	0.15	0.016	0.62	0.075	**$<10^{-4}$**

DISCUSSION

The very low percentage of males recorded in spring (29%) might seem surprising. It is, however, in accordance with the observation that there is a high incidence of mating in autumn (46% females fertilized, Nedvědová et al. 2013). In this way males avoid the consequences of high winter mortality and sire a substantial number of progeny. In autumn, 1571 of the *H. axyridis* collected during their migration to overwintering sites 845 were females and 726 males (46%), (Nedvědová et al. 2013). Sperm can survive to the following spring in fertilized females as they are able to produce viable progeny without further mating (Awad et al. 2013). In addition, half of the females sampled during the autumn migration have been fertilized by on average 2.8 males (Awad et al. 2015). The sex ratio of the adults at emergence did not deviate from 0.5 in the field, in laboratory stocks (personal observations) or in commercial biocontrol stocks (Heimpel & Lundgren 2000).

Deviations in the numbers of mating pairs made up of the different morphs from that expected if mating occurred at random were probably caused by partner choice. Another explanation is that it is due to differences in the body temperatures of the different morphs with the melanic morphs both warmer and more active. It is not easy to distinguish between partner choice and thermal melanism and if the former whether it is the male or the female that chooses its partner on the basis of its colour. The over-representation of both *spectabilis* + *conspicua* pairs and monomorphic *conspicua* pairs indicates this might be due to these strongly melanic individuals being more active. Under-representation of records of *succinea* males mating *spectabilis* females indicates choice of partner.

A separate analysis of the frequencies with which males and females of the different morphs were recorded mating revealed an under-representation of both males and females of the light coloured *succinea* morph and over-representation of both males and females of the melanic *conspicua* morph, which supports the thermal melanism hypothesis. But more *spectabilis* males and fewer *spectabilis* females were recorded mating than expected, which indicates some mate choice is involved.

The fewer than expected morning records of *succinea* individuals and more *conspicua* individuals (regardless mating status) also support the thermal melanism hypothesis. However, unexpectedly the frequency of mating was not lower and the frequency of melanic morphs in pairs was not higher on cold mornings. In addition, the differences in the frequency were weaker in the morning.

In conclusion, there is weak support for the thermal hypothesis. The opposite trends recorded for *spectabilis* males and females support specific mate choice or difference in the activity of this colour morph not due to higher body temperature resulting from basking in the sun.

Another possible explanation of these differences is that they include the different durations for which pairs made up of different morphs mated and hence false differences in the frequencies recorded. Mating in the laboratory tends to be shorter between similar morphs (Wang et al. 2009). This is not supported by a laboratory experiment (Awad et al., unpublished) in which males of three different morphs each mated with *succinea* females for similar period of time.

The percentages of the different colour morphs recorded in this study in spring were similar to those previously recorded in the autumn migration (Nedvědová et al. 2013): *succinea* 89 vs. 87%, *spectabilis* 8 vs. 10%, *conspicua* 3 vs.3.6%. Similar percentages (10.5% of both melanic morphs together) are also reported in Slovakia in mid-summer (Panigaj et al. 2014). In contrast to Japan and China (Osawa & Nishida 1992, Wang et al. 2009) there is no strong fluctuation in the percentage of melanic individuals during the course of a year. Hence, the small and ambiguous differences recorded for the invasive population in this study probably have little effect on the reproductive success of *H. axyridis* in Europe.

Similar percentages of melanic individuals to those recorded in invasive European populations are reported also in a native population in Korea (Seo et al. 2008). Of all the different colour morphs, *conspicua* was preferred for mating, as in the other populations. This morph tends to dominate laboratory strains and some biocontrol stocks. The difference between field and laboratory populations in the percentages of melanics is related on the one hand to a non-random mating preference for melanics and on the other hand selection against melanics by environmental conditions. Similarly, the reproductive success of the melanic morph of *C. septempunctata* in India is greater than that of typical individuals (Srivastava & Omkar 2005), whereas Stewart & Dixon (1989) show there are costs to being a large black ladybird.

The relatively high percentage of individuals of *H. axyridis* recorded mating confirms a generally high sexual activity in Coccinellidae. The incidence of mating reported for *A. bipunctata* in Britain is similar (20% of the individuals in copula; Haddrill et al. 2008). However, during this study the incidence of mating recorded for other species of ladybirds (*Coccinella septempunctata*, *Propylea quatuordecimpunctata*) was lower. In addition, the virility of males of two species of *Coccinella* decreases progressively from virgin to five times mated (Michaud et al. 2013) and the progeny of virgin fathers perform better. Similarly, temporal isolation of males enhanced subsequent mating frequency in *Coleomegilla maculata* (Harmon et al. 2008).

In the present study, we used direct observation of ladybird beetles in the field. We recorded individuals on each occasion (individual days, morning, afternoon) independently and summed all the records. Thus, it is highly likely the number of records of adult ladybirds was greater than the number of individuals present in the plots studied. Although adult ladybirds move in and out of plots, our impression is that they stayed in the plots longer than the usual interval between two observations. We did not set out to estimate the population density, only the relative percentages of the males and females of individual colour morphs. We thus assumed that dispersal or residence of individuals in plots was similar for both sexes and all colour morphs, and for mating and single individuals, and they were present in the same percentages as in the surrounding landscape.

We did not mark the observed individuals. Durable marking of ladybirds is difficult because of their oily surface (Hagler & Jones 2010). In previous years, we failed to recapture ladybirds marked in various ways 24 hours after release. Another reason why marking was not used is that manipulated individuals subsequently are more active and more likely to leave the release site. In addition, mating individuals would stop mating and thus their future mating activity might be distorted.

This study also includes the report of the first record of the morph *equicolor* in the Czech Republic. This was a single male among tens of thousands of individuals of *H. axyridis* recorded from 2008 to 2012. This colour morph is similarly very rare elsewhere in Europe with six individuals recorded in the Netherlands from 2006 to 2014 (waarneming.nl). It is unclear whether the allele controlling the *equicolor* phenotype was present in few ladybirds that arrived from another continent or it originated by mutation of another allele. Currently, we have a homozygous strain of *equicolor* in laboratory.

CONCLUSION

Colour polymorphism in *H. axyridis* including invasive European populations is probably maintained by both sexual and environmental selection. The percentage of melanic individuals is rather stable spatially and temporally. The small deviations in the frequency of individuals of the three main colour morphs in mating pairs from those expected if mating were random can be attributed to both partner choice and thermal melanism.

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