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Size-related non-random mating in a natural population of the ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Abstract. We analyzed the occurrence of non-random mating in relation to body size in a natural population of the ladybird *Harmonia axyridis* (Pallas, 1773). Mating males were significantly larger than non-mating males in the spring generation. There was no significant difference in the size of mating and non-mating females in the spring generation or among members of either gender in the summer generation. Large males mated more frequently than medium-sized or small males only in the spring generation. Notably, large females mated less frequently than those in other size classes in the summer generation, although the difference was marginally significant, but not in the spring generation. The ratios of the gender size difference in pairs of mating individuals were significantly different among size classes of females and males in both generations. Ratio values may be indicative of the degree to which the mating success of males and/or females depends on mating behaviour (female repulsion vs. male compulsion). Large ratio values may indicate a low degree of mating success because females often refuse males and are more successful in repulsing them when the males that attempt to mate are relatively small. Conversely, when the ratio is small, male mating behaviour may be better able to overcome female refusal, thereby enhancing the degree of mating success. The behaviour that overcomes the differences in the sizes of the different sexes in mating pairs may drive size variability in both genders in natural populations. Mating success also depends on female mating preference, and the condition and activity level of males and females.

Key words. Body size, non-random mating, seasonal variation, sex, sexual selection.

INTRODUCTION

Female mate choice and male–male competition are two major elements in the concept of sexual selection proposed by Darwin (1871) and subsequently extended by Fisher (1930). Female mate choice occurs in the non-random mating of polymorphic ladybird beetles *Adalia bipunctata* (Majerus et al. 1982, O'Donald & Majerus 1984, 1985, Hodek & Ceryngier 2000) and *Harmonia axyridis* (Osawa & Nishida 1992). Morph- and size-related non-random mating also occurs in the Coccinellidae (Osawa & Nishida 1992, Ueno et al. 1998, Wang et al. 2009). The female is generally larger than the male in this group of insects (Dixon 2000, Yasuda & Dixon 2002, Nedvěd & Honěk 2012); female body size is positively related to fecundity (Thornhill & Alcock 1983), but male body size is not directly related to male fitness per se. The energy and nutrient demands for egg production exceed the demands for sperm production (Nedvěd & Honěk 2012).

Notably, intense male–male competition during mating is not recorded in ladybird beetles. Thus, selection pressure for large body size is probably stronger in females than in males, which may explain the size differential between sexes. In both genders, body size is also influenced by temperature, feeding rates, food quality and quantity (e.g. Hodek & Honěk 1996, Yasuda & Dixon 2002) and cannibalism at hatching (Osawa 2002).

Male behaviour in *H. axyridis* prior to copulation can be divided into five sequential steps: approach, watch, examine, mount and copulation (Obata 1987). Females often refuse to mate and flee courting males and/or shake them from their bodies (Obata 1987). Hence, males that are able to overcome female refusal may successfully mate. This is likely to depend on male size and strength because successful copulation depends on the strength of a male's grip on a female's elytra during mounting. Other factors are also important in male mating success, e.g. physiological condition of the female (which is nutrition-dependent), mating experience, duration of time since the previous mating and age (Hodek & Honěk 1996). All of these factors influence the strength of a female's resistance to mating. Under laboratory conditions, this is independent of male size in *A. bipunctata* (Perry et al. 2009). However, when female resistance is low, large males successfully complete copulation more frequently than small males, which is suggested as a driver of selection for increased male body size (Perry et al. 2009). This form of directional selection is expected to reduce variability in male body size. Nevertheless, the body sizes of males and females are extremely variable in natural populations of ladybirds. In this study, we analyzed mating performance success in a natural population of *H. axyridis* in relation to variability in the differences in the body sizes of the males and females in mating pairs.

MATERIAL AND METHODS

Field observations

Our study site was located in the Botanical Garden of Kyoto University (35° 02' N, 135° 47' E) (Osawa 2000), where non-random mating was observed previously by Osawa & Nishida (1992). *H. axyridis* adults were captured and individually marked in the period extending from April to July in 1987 and 1988. The marked beetles were released at the sites at which they were captured after their sex, whether they had been mating and their body size (total body length to the nearest 0.01 mm) was recorded. Individuals of the summer generation (i.e. the first generation) were distinguished from those of the spring generation (i.e. the overwintering generation) based on the colour hue of the elytra (Brakefield 1984, Osawa & Nishida 1992). We analyzed the data collected from the beetles at first capture and a later recapture (n=1919), males (n=107) and females (n=112) mating individuals and mating pairs (body size of paired males and females were analyzed; n=128: 63 females and 65 males).

Body size categories

Beetle body sizes were divided into three categories within each gender (females: (i) large, ≥7.10 mm, 25% of animals; (ii) medium, ≥6.40 mm, <7.10 mm, 50% of animals; (iii) small, <6.40 mm, 25% of animals; males: (i) large, ≥6.45 mm, 25% of animals; (ii) medium, ≥5.85 mm to <6.45 mm, 50% of animals; (iii) small, <5.85 mm, 25% of animals).

Gender size difference ratio

We calculated a gender size difference ratio using the following formula: (female partner body size – male partner body size)/individual body size (female or male).

This ratio was usually greater than zero because females were larger than males. Ratio values may be indicative of the degree to which mating success (for males and/or females) depends on mating behaviour. Large values indicate a low degree of mating success because females often refuse males and are more successful in repulsing them when the males attempting to mate are relatively small. Conversely, when the ratio is small, male compulsion may be better able to overcome female refusal, thereby enhancing the degree of mating success.

Statistical analysis

We used JMP Discovery Software (SAS Institute 2013) to conduct *t*-test for analysing female and male body sizes, Fisher's exact test for the analysis of sex ratios and one-way ANOVA followed by a Tukey-Kramer HSD multiple comparison test to analyse the size difference ratio. We also used the extended Fisher's exact test routine in R software (R Core Team 2013) to analyse numbers of mating and non-mating male and female individuals in the spring and summer generations by size class.

RESULTS

Females were significantly larger than males in the spring and summer generations [spring generation: *t*-test: $t=-19.66$, $P<0.0001$; female: 6.71±0.02 mm (mean±SE) (n=676), male:

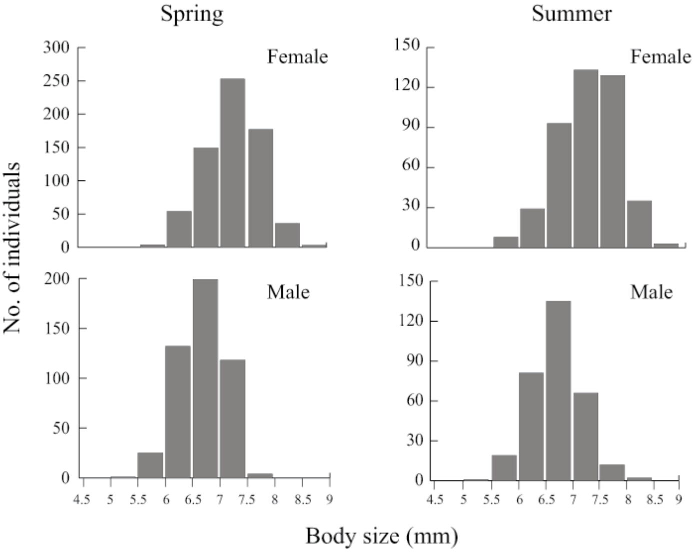

Fig. 1. Frequency distributions of the body sizes of *Harmonia axyridis* in the spring and summer generations.

6.15±0.02 mm (n=479); summer generation: *t*=–16.14, *P*<0.0001; female: 6.75±0.02 mm (n=430), male: 6.16±0.03 mm (n=334)] (Fig. 1). Size was not significantly different between generations (female: *t*=1.39, *P*>0.16; male: *t*=0.18, *P*>0.85) (Fig. 1). Sex ratio was female-biased in both

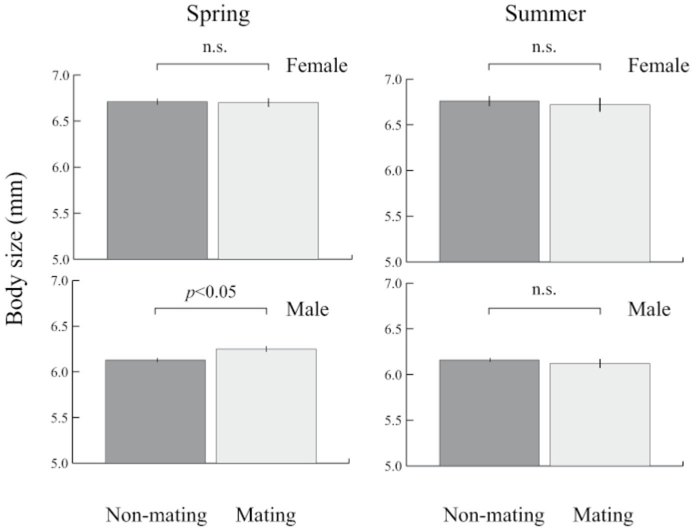

Fig. 2. Differences in the body sizes of non-mating and mating females and males of *Harmonia axyridis* in the spring and summer generations. Vertical lines indicate ±1 standard error and n.s. indicates non-significance.

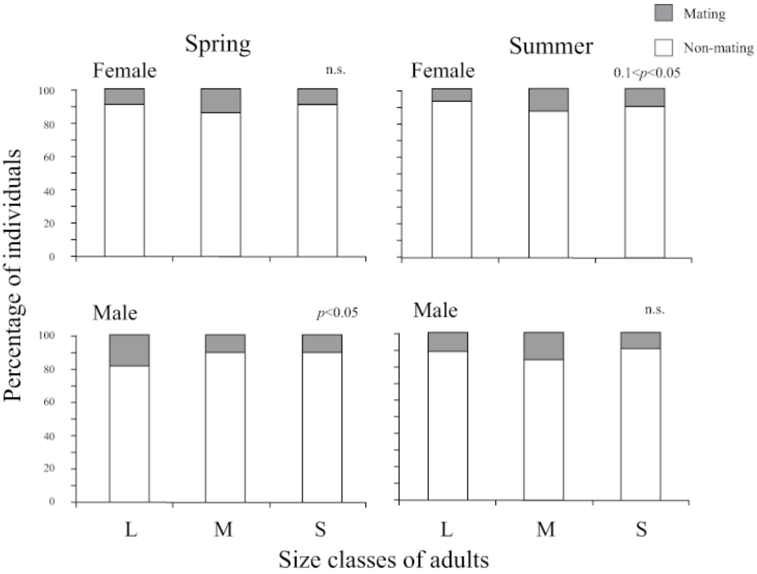

Fig. 3. Percentages (%) of non-mating (uncoloured parts) and mating (grey parts) individuals (both genders) of *Harmonia axyridis* in large (L), medium (M) and small (S) size classes in the spring and summer generations. n.s. indicates non--significance.

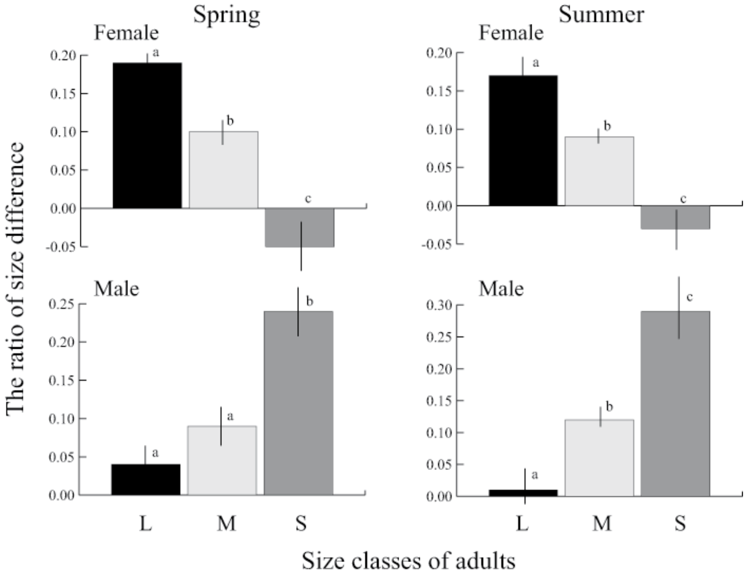

Fig. 4. Ratios of gender size difference in large (L), medium (M) and small (S) size classes of *Harmonia axyridis* in the spring and summer generations. Vertical lines indicate +1 standard error. Different letters indicate significant differences based on Tukey-Kramer HSD tests ($P<0.05$).

generations (Fisher's exact test for no. of individuals; spring: $P<0.0001$; summer: $P<0.05$), but the degree of bias was not different between generations: the percentages of males were equal in both generations ($P=0.33$). Mating males were significantly larger than non-mating males in the spring generation (*t*-test: $t=2.04$, $P<0.05$; mating males: 6.25±0.05 mm, n=64; non-mating: 6.14±0.02 mm, n=415) (Fig. 2). Body size was not significantly different between non-mating and mating females in the spring generation ($t=–0.04$, $P>0.96$; mating females: 6.71±0.02 mm, n=603; non-mating: 6.71±0.06, n=73) or between mating and non-mating individuals of either gender in the summer generation (females: $t=–0.46$, $P>0.64$; non-mating females: 6.76±0.03 mm, n=391; mating females: 6.72±0.09 mm, n=39; males: $t=–0.55$, $P>0.58$; non-mating males: 6.17±0.03 mm, n=291; mating males: 6.13±0.07, n=43) (Fig. 2).

In the spring generation, large males mated more frequently than males in the smaller size classes (extended Fisher's exact test for no. of individuals: $P<0.05$), but this was not the case in the summer generation ($P=0.23$) (Fig. 3). Notably, large females in the summer generation tended to mate less frequently than those in the smaller size classes, although the difference was not significant ($0.05<P<0.10$) (Fig. 3). A significant effect of size was not recorded in the spring generation ($P=0.24$) (Fig. 3).

Among the mating pairs, the size difference ratio was significantly different among size classes of both genders (Fig. 4). Thus, the ratio values for large females were significantly larger than those of medium and small females in both generations (one-way ANOVA; spring generation: $F_{2,30}=15.51$, $P<0.0001$; summer generation: $F_{2,33}=18.22$, $P<0.0001$) (Fig. 4). The opposite trend was recorded for males (Fig. 4). The ratio values of small males were significantly larger than those of medium and large males in both generations (one-way ANOVA; spring generation: $F_{2,29}=7.43$, $P<0.01$; summer generation: $F_{2,36}=17.82$, $P<0.0001$) (Fig. 4).

DISCUSSION

In both the spring and summer generations, the sex ratios were female-biased. Theoretically, differences in reproductive success among males are diminished by both the strongly biased sex ratio and the adjusted sex ratio to an extent dependent on the intensity of sexual competition among males (Thornhill & Alcock 1983). Male-killer has been recorded in this population (Osawa, pers. obs.), although the infection rate of *H. axyridis* is generally low (ca. 8%; Noriyuki et al. 2014). Furthermore, sex- and morph-related (i.e. melanic vs. non-melanic) differences in cold tolerance during overwintering may affect variations in the body sizes and sex ratios of the spring generation in this species (Osawa 2001). These effects may promote a female bias in the population sex ratio, which in turn may influence variability in male mating success in the population, although we emphasize that the sex ratio (male/female) was not strongly skewed (0.71 in spring; 0.78 in summer). Furthermore, we can not rule out the possibility that male–male competition may play a role in the variability in male mating success, even though intense male–male competition during mating is uncommon in ladybird beetles.

Mating males were larger than non-mating males in the spring generation (Fig. 2); moreover, large males mated more frequently than small males (only in this generation) (Fig. 3). Furthermore, the ratios of the gender difference in size for small males were significantly larger than those of males in other size classes (Fig. 4). Thus, for small males, the difference in size when mating is very large, which is not the case for large males. Large males were chosen more frequently by females than small males, although we do not know whether this was due to female sexual activity.

However, the degree of size-related non-random mating of males in the summer generation was less (size differences between mating and non-mating males were not significant in summer) (Fig. 3). This seasonal variation in mating preference by size may have been caused by the size-related mating tactics of males. Smaller males may have compensated for the large size differential they encounter during mating by engaging in more frequent mating attempts. Yasuda & Dixon (2002) clearly demonstrate under laboratory conditions that small males of the ladybird beetle *A. bipunctata* are more active in mating than large males when food is limited. Furthermore, small males of the yellow-spotted longicorn beetle *Psacothea hilaris* are more sensitive to female sex pheromones and more active in searching for mates and precopulatory behaviour than large males (Fukaya 2004).

The enhanced mating success of large males and reduced mating activity of large females that we detected (Fig. 3) also indicate that mating behaviour has a role in overcoming the relative gender size difference (but not the absolute body size) of individuals in mating pairs. Thus, mating behaviour related to the ratio of the gender difference in size in copulating pairs may have importantly affected size-related non-random mating that we recorded in this population, even when mating attempts were random and strongly influenced by female physical condition and male sexual activity. The behaviour that overcomes size differences in mating pairs may drive size variability in both genders in natural populations (Fig. 1). Thus, the relative size difference between individuals in mating pairs, which is an indicator of compensating behaviour, may influence fitness in natural populations. This effect operates synergistically with female mating preference, female condition and the level of beetle activity.

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