

## Do Males and Females of the Two-Spot Ladybird, *Adalia bipunctata* (L.), Differ in When They Mature Sexually?

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*After emergence from pupae males and females of *Adalia bipunctata* reared under the same constant conditions showed similar refractory periods in their mating behavior. The statistically significant slight protogyny might indicate that it is advantageous for females to accept mating and store sperm for a short period prior to becoming sexually mature, whereas males need to become sexually mature in order to mate. As the two-spot ladybird has overlapping generations, theory predicts that the most effective strategy for both sexes is to have similar sexual maturation periods. The results of this study support this prediction. The actual lengths of the maturation periods, however, will depend largely on the prevailing temperatures and the quality and quantity of food available to the beetle.*

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**KEY WORDS:** *Adalia bipunctata*; Coccinellidae; sexual maturity; protandry; protogyny.

### INTRODUCTION

Ladybirds show marked sexual size dimorphism, with the males on average 0.82 the size of the females (Dixon, 2000). This is a feature common to many poikilothermic animals (Wicklund and Karlsson, 1988) that has attracted

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a lot of attention. Four main hypotheses have been proposed to account for this sexual dimorphism. First, in mating systems dominated by scramble competition, where male reproductive success is a function of encountering females, small males may be favored when food is limiting because they require lower absolute amounts of food and can therefore spend more time looking for females—the time and energy constraint hypothesis (Ghiselin, 1974; Reiss, 1989). Second, it is advantageous for females to be bigger than males because their fecundity is directly related to her size—the fecundity advantage hypothesis (Fairbairn, 1990). Third, males begin developing their gonads earlier than females and that has costs in terms of the growth rate that male larvae can sustain—the gonadal constraint hypothesis (Dixon, 2000). Fourth, sexual size dimorphism can be explained in terms of protandry—the developmental constraint hypothesis (Alexander *et al.*, 1979; Fairbairn, 1990). That is, it is a consequence of selection for rapid growth and early sexual maturation in males.

Field observations show that *Adalia bipunctata* has overlapping generations (Hemptinne, 1989). The breeding season is rather long and females mate several times with different males during their live (Majerus, 1994). In such a mating system competition between males for females is unlikely and theory predicts that both sexes should emerge at the same time.

The objective of this paper is to demonstrate that protandry does not occur in *A. bipunctata*.

## MATERIALS AND METHODS

### Ladybird Culture

Two-spot ladybirds, *A. bipunctata*, were reared at  $15 \pm 1^\circ\text{C}$  under a photoperiod of 16 h light and 8 h darkness. Groups of approximately 20 males and 20 females were kept in 5-liter plastic boxes, which also contained a piece of corrugated filter paper to increase the surface area. Every other day the ladybirds were fed an excess of pea aphids, *Acyrtosiphon pisum* (Harris). On that occasion, a section of stem of broad bean, *Vicia faba* L., was put in each of the rearing boxes to provide a source of humidity and to keep the aphids alive as long as possible. Once a week the ladybirds were transferred to clean containers to stimulate egg laying.

Eggs were collected every day and incubated in 175-cm<sup>3</sup> boxes under the same daylength and temperature conditions as experienced by the adults. After hatching the larvae were also fed an excess of pea aphids. To reduce the risk of cannibalism, the number of larvae per box never exceeded 15. Prepupae were transferred to a  $20 \pm 1^\circ\text{C}$  cabinet to reduce the duration of

their developmental time. Containers with pupae were inspected every 6 h and newly emerged adults were immediately isolated in 5-cm-diameter petri dishes. These virgin adults were kept at  $20 \pm 1^\circ\text{C}$  under a photoperiod of 16 h light and 8 h darkness and fed an excess of pea aphids every day. The beetles were sexed by examining the shape of the last abdominal sternite, which is hemispherical in females and notched posteriorly in males (Hodek, 1973). The sex was confirmed by observing whether there were large orange flexure bands between the sternites. These bands are areas of thin cuticle, which are present only in males (Randall *et al.*, 1992).

### Age and Sexual Activity

At 1000 h, a 1-day-old virgin male was placed in a clean petri dish, lined with filter paper, containing a 1-day-old virgin female. Their behavior was observed continuously for the first 30 min and then every hour until 1600 h. The timing of the observation appears suitable, as mating lasts for 3 to 8 h (Majerus, 1994). This was done at  $20 \pm 1^\circ\text{C}$  and repeated 20 times. Similarly 1-day-old males were placed with virgin females 2, 3, 4, 5, 10, 20, and 30 days old. The experiment was then repeated with virgin males 2, 3, 4, 5, 10, 20, and 30 days old. In this way the incidence of mating between virgin males and females of a range of ages was determined. Each ladybird was used only once.

The males either (1) were completely inactive; (2) were active and palpated the surface of the female on each encounter but did not mate; or (3) were active, palpated the female extruded their genitalia, and immediately mated. The third category of behavior was taken as an indication that they were willing to mate. Females showed one of two kinds of behavior when palpated by the male; they either (1) vigorously refused to mate, as described previously by Obata (1988) and Majerus (1994); or (2) were willing to mate, i.e., they stopped walking and raised slightly the tip of their abdomen (Hemptinne *et al.*, 1998).

The proportions of mating attempts or matings, that is, phase 2 or 3, respectively, of female or male behavior, were analyzed by  $\chi^2$  tests. Note that one sex can show willingness to mate, while the other does not. Although sometimes the expected values were small, this test was valid because in each case they were always greater than 6 (Roscoe and Byars, 1971; Zar, 1996). The willingness to mate of males and females was analyzed statistically only in the presence of mates aged 10, 20, or 30 days. As two-spot ladybird beetles that are well fed and between 10 and 30 days old are sexually mature (Hemptinne, 1989; Majerus, 1994), this constituted a control for the influence of the mates.

## RESULTS

### General Trends in Sexual Activity

Mature males reacted very rapidly to virgin females. After palpating the female for a few seconds, the male immediately mounted and attempted to copulate, usually within the first 30 min. The highest incidence of mating occurred when both males and females were aged 5 days or older. No males aged from 1 to 3 days were observed to mate, and only a few females aged from 1 to 2 days mated (Fig. 1). The incidence of mating between beetles aged from 1 to 4 days and older beetles was not symmetrical, as the younger females were more likely to mate with old males than *vice versa*.

### Age-Related Incidence of Mating

*In Males.* The incidence of mating when males were confined with females that were either 10, 20, or 30 days old was similar (Table I). Therefore, the results for each age of males were pooled. This revealed that a few males mated when 4 days old and then the incidence of mating increased with age and reached a maximum of 85% when the males were 20 days old (Fig. 2). The age at which 50% of the males mated, estimated by fitting a curve by eye to Fig. 2, was 6.7 days.

*In Females.* As with males, females behaved similarly when confined with 10- or 20-day-old males. On two occasions, mating frequencies with 30-day-old males were lower than with younger males (Table II). Therefore, the sexual activity of females in the presence of 30-day-old males was not used in this analysis, and the results for each age of female in the presence of 10- and 20-day-old males were pooled. A few 3-day-old females mated. The maximum incidence of mating was 82.5% and occurred when the females were 10 days old (Fig. 2). The predicted age at which 50% of the females mated, estimated by fitting a curve by eye to Fig. 2, was 5.3 days.

### Do Females Mature Sexually Earlier than Males?

The above results indicate that mating began slightly earlier in females than in males (Tables I and II). Comparing the incidence of mating observed when 3-, 4-, and 5 day-old males and females were confined with a mature partner, it is clear that the incidence of mating shown by females was significantly higher than that by males on days 3 and 4 (Table III). That is, two-spot ladybirds show slight protogyny rather than protandry.

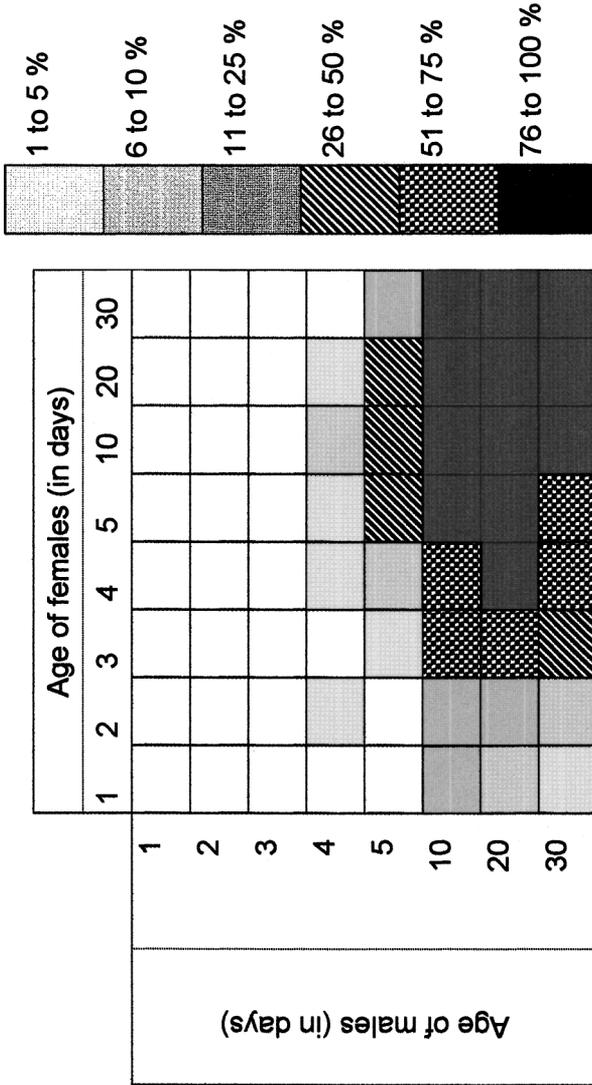


Fig. 1. The proportions of males and females of *Adalia bipunctata* of increasing age that copulated when presented with a mate 1, 2, 3, 4, 5, 10, 20, or 30 days old.

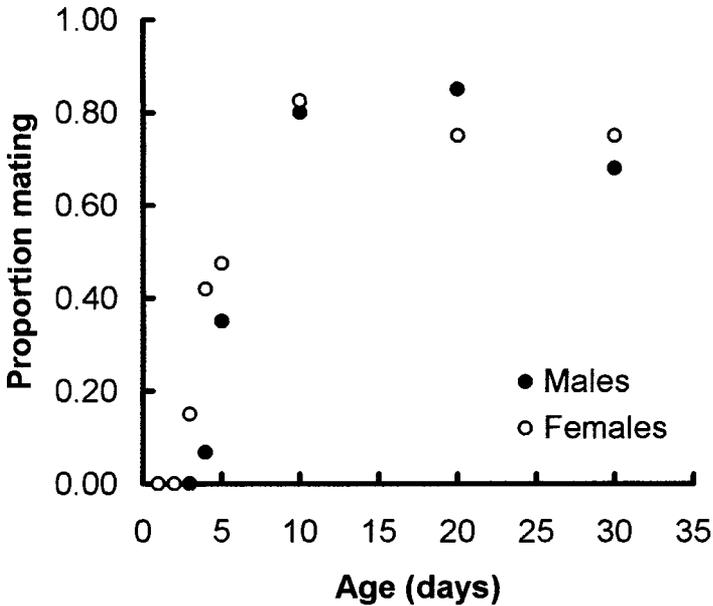
**Table I.** The Numbers of Males of *Adalia bipunctata* of Increasing Age that Attempted to Copulate or Copulated with Mature Females Aged 10, 20, or 30 Days

Male age (days)	Female age (days)						$\chi^2$ test
	10		20		30		
	<i>n</i>	Mating	<i>n</i>	Mating	<i>n</i>	Mating	
1	20	0	20	0	20	0	—
2	20	0	20	0	20	0	—
3	20	0	20	0	20	0	—
4	20	1	20	3	20	0	3.759 (NS)
5	20	9	20	8	20	4	3.077 (NS)
10	20	17	20	14	20	17	1.875 (NS)
20	20	18	20	17	20	16	0.784 (NS)
30	20	12	20	13	20	16	2.003 (NS)

Note. NS, not significant; *n*, number of replicates.

## DISCUSSION

Most of the theory relating to when sexes of insects should emerge and become sexually mature has been developed for butterflies, which generally



**Fig. 2.** The proportions of males and females of *Adalia bipunctata* of increasing age that copulated when presented with a mature mate.

**Table II.** The Numbers of Females of *Adalia bipunctata* of Increasing Age that Were Mated by Mature Males Aged 10, 20, or 30 Days

Female age (days)	Male age (days)						$\chi^2$ test
	10		20		30		
	<i>n</i>	Mating	<i>n</i>	Mating	<i>n</i>	Mating	
1	20	0	20	0	20	0	—
2	20	0	20	0	20	0	—
3	20	4	20	2	20	2	1.153 (NS)
4	20	7	20	10	20	1	10.000**
5	20	12	20	7	20	5	5.417 (NS)
10	20	15	20	18	20	8	12.173**
20	20	14	20	16	20	11	2.928 NS
30	20	15	20	15	20	13	0.656 NS

Note. \*\* $P < 0.01$ . NS, not significant; *n*, number of replicates.

show synchronized emergence and have a restricted breeding season, and the females of which mate only once. When adult mortality is low males should emerge before females, and as the risk of mortality increases the emergence times of the two sexes should become more similar (Wiklund and Fagerström, 1977; Fagerström and Wiklund, 1982; Singer, 1982; Bulmer, 1983, 1994; Iwasa *et al.*, 1983; Parker and Courtney, 1983). If species have overlapping generations, a constant population size, and females that mate several times during their life time, then there can be no advantage in males emerging and/or maturing sexually before females. Males will achieve the same average mating success whenever they emerge. As the two-spot ladybird has overlapping generations (Hemptonne, 1989), then the simplest and most effective strategy for both sexes is to have similar sexual maturation periods.

Data in the literature for 16 species of ladybirds, including *A. bipunctata*, indicate that the sexes take a similar length of time to develop from egg to adult (Dixon, 2000). The results presented here, on the time adults take to

**Table III.** The Proportions of 3-, 4-, and 5-Day-Old Males and Females of *Adalia bipunctata* that Mated with 10-, 20-, and 30-Day-Old Partners

Mating	3-day-old		4-day-old		5-day-old	
	Males	Females	Males	Females	Males	Females
Yes	0	8	4	18	21	24
No	60	52	56	42	39	36
$\chi^2$ test	8.571**		10.909***		0.320 (NS)	

Note. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ . NS, not significant.

reach sexual maturity, indicate that for *A. bipunctata* reared at one temperature there is a slight tendency for the females to mature first (protogyny). That is, males are not completing their larval development and becoming sexually mature before the females (protandry).

The postemergence refractory period shown by the two-spot ladybird may lessen the risk of mating with siblings (Antolin and Strand, 1992) and the incidence of inbreeding depression (Morjan *et al.*, 1999). This is especially so, as ladybirds can fly within hours of emerging from pupae (Hodek and Honek, 1996). However, the ecological significance of the slight protogyny observed in this laboratory study has to be questioned and may indicate only that for females there are advantages in accepting mating and storing sperm in anticipation of their shortly becoming sexually mature.

The question of why male poikilotherms are smaller than females remains open. That adult males of *A. bipunctata* eat considerably less food than females (Hemptinne *et al.*, 1996) seems to give credit to the time and energy constraint hypothesis. However, the gonadal constraint and fecundity advantage need to be tested experimentally, as it is likely that there is not a single explanation of male dwarfism in ladybirds.

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