

Sexual size dimorphism in the two spot ladybird beetle *Adalia bipunctata*: developmental mechanism and its consequences for mating

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Abstract. 1. The literature on ladybirds indicates that males are consistently smaller than females but take the same length of time to complete their development. Rearing *Adalia bipunctata* at 20 and 25 °C confirmed that protandry cannot account for sexual size dimorphism in this species, nor can a difference in egg size.

2. Female larvae consumed more food and had a higher relative growth rate in the fourth instar than did male larvae.

3. When food is limited, small males appear to be more successful at mating than are large males.

4. To account for these results, it is hypothesised that the gonads of male larvae compete more strongly with the soma for resources and that this reduces the growth potential of the soma of male larvae relative to that of female larvae. The greater mating success of small males when food is limited supports the *eat or mate* hypothesis, which predicts that when food is limited small males will spend less time feeding and more time mating than will large males.

Key words. *Adalia bipunctata*, adult size, developmental constraint, eat or mate hypothesis, food consumption, gonad, growth rate, mating behaviour, predatory ladybird, protandry.

Introduction

Sexual size dimorphism is common in animals. Male gigantism is adaptive where males compete for females (e.g. Shine, 1989), however male dwarfism is not adaptive (Vollrath & Parker, 1992). Nevertheless, in most invertebrates males tend to be smaller than females (Wiklund & Karlsson, 1988) and female-biased sexual size dimorphism has been recorded in several groups of invertebrates: aphids (Dixon *et al.*, 1998), butterflies (Wiklund & Karlsson, 1988; Nylin *et al.*, 1993), damselflies (Anholt, 1997), flies (Nunney, 1996), ladybird beetles (Dixon, 2000), midges (Neems *et al.*, 1990), spiders (Vollrath & Parker, 1992), wasps (Coelho, 1997), and waterstriders (Fairbairn, 1990; Blanckenhorn *et al.*, 1995; Preziosi & Fairbairn, 1997).

The simplest explanation for a female-biased sexual size dimorphism is protandry, i.e. males develop faster than

females because it gives them a mating advantage, and this results in the males being smaller. All the studies on ladybirds indicate that males and females take the same length of time to complete their development (Dixon, 2000). If this is a general phenomenon, the developmental biology of males must differ from that of females in some other way. For example, the eggs that give rise to males could be smaller than those that give rise to females, or the eggs could be the same size but the growth rate of males could be lower than that of females.

In addition, in situations where males scramble rather than compete for females, it is thought that selection favours increased activity and mobility in males (Ghiselin, 1974), i.e. small males may be at an advantage in such a situation as they require less food for maintenance than large males and are therefore likely to require less time for foraging for food and have more time for the pursuit of mates than are large males: the *eat or mate* hypothesis (Blanckenhorn *et al.*, 1995).

The objectives of the work reported here were (1) to confirm that the developmental times of males and females of *Adalia bipunctata* (L.) are similar and (2) to identify the

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developmental mechanism that results in male dwarfism. (3) Small and large males of *A. bipunctata*, produced by manipulating the food supply that they received during their development, were then used to test the eat or mate hypothesis.

Materials and methods

Adult ladybirds were collected on the University of East Anglia, U.K., campus (52.7°N, 1.3°E) and fed pea aphids *Acyrtosiphon pisum* Harris. The larvae that hatched from the eggs laid by these ladybirds were used in the experiments. In order to determine the effect of male size on mating success, large and small males were produced. This was achieved by rearing ladybird larvae on an excess of pea aphids individually in 5-cm Petri dishes until they reached the fourth instar, when half were each fed an excess of pea aphids (>45 mg of aphids per day), which resulted in large males, and some were given ≈6 mg of aphids per day, which resulted in small males. All larvae, except those used to produce the small males used in the mating experiment, were fed daily an excess of pea aphids individually in 5-cm Petri dishes throughout their larval development and kept in an incubator at a constant temperature of 20 or 25 °C and a LD 18:6 h photoperiod. In the experiments on the duration of development, observations were made daily at 08.00 and 20.00 hours until the larvae developed into adults, and at each observation it was recorded whether the larvae had moulted or pupated. Each adult was sexed and the length of its elytra and its maximum width were measured using a microscope fitted with a micrometer eyepiece, or it was weighed 1 day after emerging from a pupa.

The effects of temperature on the developmental time of male and female *A. bipunctata* were assessed using a two-way ANOVA. Logistic regression analysis allowed the effect of male size on mounting and mating success to be determined. Other results were analysed using a one-way ANOVA; the sequential Bonferroni procedure was used to control for table-wide Type I error (Rice, 1989).

Duration of development of males and females at two temperatures

Eggs were kept in an incubator at 20 or 25 °C and the hatching larvae were reared individually at these temperatures.

Egg weight

As egg size might account for the small size of males (e.g. Stamps, 1993), eggs were weighed within 12 h of laying and the larvae that hatched from these eggs were reared individually.

Food consumption and relative growth rate

Weights of pea aphids supplied to each fourth-instar larva and weights of the aphids remaining each day were

measured and daily food consumption was estimated as the difference between these weights. Daily food consumption of 1-month-old adults was estimated in the same way.

The relative growth rate of fourth-instar larvae was calculated as: (1) $RGR1 = (WA - W4)/\text{food intake}$, (2) $RGR2 = (\log WA - \log W4)/\text{developmental time}$, where WA is the weight of an adult on day of emergence from pupa, W4 is the weight of a larva on moulting to the fourth instar, food intake is the total amount of food consumed in the fourth instar, and developmental time is the duration of development of the fourth instar. Fourth-instar larvae were used because they are the most voracious of the larvae.

Resource allocation by larvae

Resources are assigned to either somatic or gonadal growth during development. If male larvae invest more in those components of the soma that are not involved in acquiring and processing prey, e.g. fat reserves, but the allocation of resources to gonads is the same as in female larvae, males are likely to be smaller than females. In order to test this, the fat content of freshly emerged adult males and females was determined. Two-day-old adult ladybirds were killed by asphyxiation with carbon dioxide gas and their bodies were pierced with a needle to facilitate fat extraction. The ladybirds were placed individually into labelled glass tubes and oven dried at 38 °C for 30 days then weighed. Fat was extracted by immersing individual ladybirds in closed tubes of petroleum ether, first for 2 days, after which the ether was renewed, then for 4 days. Each beetle was then dried in an oven at 38 °C for 1 week then reweighed to obtain the fat-free dry weight (Mills, 1981; Zhou *et al.*, 1995).

Male size and mating success

In order to determine whether large males have an advantage over small males when seeking mates, a large and a small male, each marked with a dot of a different colour, were kept together in a 5-cm Petri dish and after 5 min a female was placed in the dish. The behaviour of the males was observed for 1 h and their mating behaviour was recorded, i.e. the number of times each male attempted to mount the female or displace the mounted male. To determine whether the amount of food available to the males affected their mating success, the small and large males were each either fed an excess of pea aphids or just two adult pea aphids per day. In this experiment, 49 large and 43 small males were fed an excess of food, while 46 large and 52 small males were fed a limited amount of food. In each of these experiments, 36 females were used, i.e. a total of 72 females, which were all fed an excess of aphids prior to the experiment. The females were at least 1 month old and some individuals were used more than once. As ladybirds are promiscuous (Majerus, 1994), the fact that some of the females had mated before is not thought to have affected the results. The experiments were performed in a laboratory at 23–25 °C.

Results

Duration of development of males and females at two temperatures

The individuals reared at 25 °C developed faster than those reared at 20 °C but the developmental times of the sexes did not differ at the two temperatures (Tables 1 and 2).

Egg weight

There was no difference in the weight of the eggs that developed into males and the weight of the eggs that developed into females ($F=0.03$, $P>0.05$), nevertheless females were heavier than males ($F=26.96$, $P<0.001$; Table 3). Thus differences in egg size did not appear to determine adult sexual size dimorphism.

Food consumption and relative growth rate

During the fourth instar, the food consumption of male larvae was less than that of female larvae ($F=11.90$, $P<0.001$). In addition, the relative growth rate of the female larvae was higher than that of the male larvae ($F=5.73$, $P<0.05$ for relative growth rate calculated with respect to food intake; $F=4.83$, $P<0.05$ for relative growth rate calculated with respect to developmental time; Table 3), i.e. small size in males was associated with a lower food consumption, poorer conversion of food to ladybird biomass, and lower relative growth rate of their larvae compared with those of females.

Resource allocation

There was no significant difference in the amount of fat in recently emerged male and female beetles ($F=2.92$, $P>0.05$ for weight of fat; $F=0.68$, $P>0.05$ for weight of fat/dry weight; Table 3), i.e. there was no evidence that male larvae allocated more resources to their fat reserves than do female larvae.

Male size and mating success

The rearing of larvae on a limited food supply during their fourth instar resulted in the development of smaller males than when fed an excess of prey throughout their

Table 1. Developmental time (days from egg to adult) of *Adalia bipunctata* at two temperatures (mean \pm SE).

	20 °C	<i>n</i>	25 °C	<i>n</i>
Male	19.53 \pm 0.14	30	14.84 \pm 0.15	19
Female	19.93 \pm 0.14	27	14.88 \pm 0.14	21

Table 2. Two-way ANOVA of developmental time (days from egg to adult) at 20 and 25 °C.

Source	d.f.	<i>F</i>	<i>P</i>
Temperature	1	1349.16	<0.0001
Sex	1	2.65	0.107
Temperature \times sex	1	1.78	0.185

larval development ($F=166.86$, $P<0.001$; Table 4). Although mature males consumed only one-third of the amount of food required by females per unit time (Table 3), they nevertheless needed food and its acquisition could affect their mating success.

When supplied with an excess of food, there was no difference in the numbers of successful mountings and matings recorded for the large and small males ($\chi^2=0.93$, $P>0.05$ for mounting; $\chi^2=0.18$, $P>0.05$ for mating). When supplied with a limited amount of food, however, small males were recorded mounting significantly more frequently than were large males ($\chi^2=5.34$, $P<0.05$). Although the small males also showed a higher frequency of mating than the large males when food was limited, the difference was not significant ($\chi^2=2.08$, $P>0.05$; Table 5). The lower incidence of mounting and mating in small males fed excess food compared with limited food is slightly puzzling and needs further study. During mounting and mating, the other male sometimes attempted to displace the mating male. Of the eight such interactions observed, the mating male always won, irrespective of its size.

Discussion

The results for developmental times at two temperatures indicate clearly that protandry cannot account for sexual size dimorphism in *A. bipunctata*. Similar developmental times for males and females have been reported for 17 species of ladybird beetle (Dixon, 2000). Therefore, generally in ladybirds, protandry does not appear to be the main determinant of sexual size dimorphism.

Of the other developmental mechanisms that might account for sexual size dimorphism, egg size would appear to be unimportant as the weights of the eggs that gave rise to male and female *A. bipunctata* were similar; however female larvae consumed more food and had a better conversion of food to ladybird biomass and a higher relative growth rate in the fourth instar than did male larvae.

The low relative growth rate in males might be seen as an adaptation rather than a consequence of a trade-off in the investment in gonads and soma; however as very few (0–7%) ladybird larvae reach maturity in the field because of cannibalism and intraguild predation (Dixon, 2000), it would appear to be more advantageous for male larvae to have the same relative growth rate as female larvae and a shorter developmental time. Thus, it seems unlikely that the lower relative growth rate of male larvae is an adaptation.

Table 3. Weight, food consumption, relative growth rate, and fat content of different stages of *Adalia bipunctata* (mean \pm SE).

	Weight (mg)		Food consumption (mg)†		Relative growth rate		Fat content	
	Egg	Adult	Fourth instar	Adult	(WA – W4)/ food	(log WA – log W4)/ developmental time	Fat weight (mg)	Fat weight/dry weight
Male	0.121 \pm 0.002	10.53 \pm 0.22	66.99 \pm 1.98	11.11 \pm 1.70	0.063 \pm 0.003	0.058 \pm 0.004	0.0605 \pm 0.0065	0.0283 \pm 0.0026
<i>n</i>	18	18	41	18	41	41	18	18
Female	0.120 \pm 0.002	12.05 \pm 0.20	77.13 \pm 2.17	32.32 \pm 1.75	0.075 \pm 0.004	0.070 \pm 0.004	0.0754 \pm 0.0058	0.0311 \pm 0.0023
<i>n</i>	22	22	34	17	34	34	22	22
<i>P</i> ‡	0.86	0.0001	0.0009	0.00001	0.016	0.031	0.096	0.42
Adjusted <i>P</i> §	NS	***	***	***	*	NS	NS	NS

†Food consumption of fourth instars is the total amount of food consumed; food consumption of adults is the daily consumption.

‡Unadjusted *P*.

§Sequential Bonferroni adjustment: NS not significant, **P* < 0.05, ****P* < 0.001.

An optimal energy partitioning model developed for aphids indicates that where there is competition for resources acquired by the soma, the overall growth of the organism will be reduced compared with situations where there is no competition, i.e. all the resources go into further growth of the soma. In the case of unwinged aphids, the competition comes from the developing gonads and in winged aphids also from the development of wing muscles (Kindlmann & Dixon, 1989; Dixon & Kindlmann, 1999). In addition, in aphids the males develop larger fat reserves than the females (Kundu & Dixon, 1997).

The modes of development in aphids and ladybirds differ. The immature stages of insects such as aphids resemble the adults and start developing adult features such as gonads, fat reserves, and wings early in their development. In contrast, the immature stages of insects such as ladybird beetles differ greatly in appearance from the adults and go through a pupal stage during which they switch from a larval to an adult morphology. It used to be thought that gonads, fat body, and wings developed during this stage, however the gonads in other insects that go through a pupal stage, such as Lepidoptera, are now known to start developing in the larvae, with those of the males developing earlier than those of their female sibs (Reed & Beckage, 1997). Similarly, the sexual size dimorphism in *Drosophila* is attributed to some intrinsic feature of male development that causes males to be smaller (Nunney, 1996), and a probable cause is some aspect of gonadal development as the larval testes are much

larger than the larval ovaries (Kerkis, 1931), and spermatogenesis is thought to be a costly process (Partridge, 1993). Thus, as has been suggested for aphids, there could be competition for the resources acquired by the soma and this competition is more severe in male than in female larvae; however in ladybirds this competition does not appear to come from the developing fat body as recently emerged adult males and females had similar absolute fat reserves and the females had larger fat reserves per unit dry weight than the males. The inhibition of gonadal growth by endoparasitoids, parasitic castration, is often associated with gigantism in insects (Reed & Beckage, 1997), therefore it is possible that the relative time of the onset of development of gonads in ladybirds might be a key factor determining the relative size of the sexes. What evidence is there for this?

Unpublished results (B. K. Agarwala and A. F. G. Dixon) indicate that, as in other insects that go through a pupal stage, the gonads of male *A. bipunctata* start developing earlier and are more advanced in their development in terms of their final size in the later larval stages than are those of females. Although similar data need to be collected for other species, the implication is that the gonads of male ladybird beetles start developing earlier than those of females, which may enable the sexes to reach sexual maturity synchronously (Hemptinne *et al.*, 2001). A possible consequence of this is that males are smaller than females. As indicated above, one possible mechanism is that the gonads of males start competing with the soma for resources earlier than in females and that this reduces the growth potential of the soma of males. The cumulative effect of this increases with each instar and as a consequence male larvae have a lower relative growth rate than female larvae. This would be similar to what is thought to happen in aphids, in which it has been suggested that the winged aphids are smaller and take longer to develop than unwinged individuals because their wing apparatus competes with the soma for limited resources (Dixon *et al.*, 1998; Dixon & Kindlmann, 1999). Although there are only data for one species, there is therefore some evidence in support of the hypothesis that sexual

Table 4. Average sizes of the adult males of *Adalia bipunctata* reared on an excess or a limited supply of food during the fourth instar (mean \pm SE).

Excess food	<i>n</i>	Limited food†	<i>n</i>
13.51 \pm 0.17a	39	11.14 \pm 0.12b	46

†See Materials and methods for definition of food availability. Adult size (mm²) = width of body \times length of elytra. Values followed by different letters are significantly different (ANOVA, *P* < 0.001).

Table 5. Total numbers of mounting and successful mating attempts observed for different-sized males of *Adalia bipunctata*.

	Limited food ($n = 72$)		Excess food ($n = 85$)	
	Mounting	Mating	Mounting	Mating
Large male	16	11	19	14
Small male	29	18	14	12
P_{\dagger}	0.02	0.14	0.33	0.67
Adjusted P_{\ddagger}	*	NS	NS	NS

\dagger Unadjusted P .

\ddagger Sequential Bonferroni adjustment: NS, not significant, $*P < 0.05$.

size dimorphism in ladybirds is a consequence of the early development of gonads in males competing for a limited resource.

There is no unequivocal evidence that ladybirds compete for mates and in particular that large males have a mating advantage (Dixon, 2000). In this study, when food was abundant neither small nor large males of *A. bipunctata* appeared to have a mating advantage, in contrast with the situation when food was limited. Under food limitation, small males were observed to mount females more frequently than were large males, as predicted by the eat or mate hypothesis. The implication is that although small size in male ladybirds may be a consequence of a developmental mechanism, it is not a disadvantage but an advantage when mating, i.e. in terms of mating, there is no selection for large size that would counter the effect of the developmental mechanism.

In summary, at least 29 species of ladybird show sexual size dimorphism in which the male is the smaller sex, and in 17 of these species the developmental times of the two sexes are known to be similar (Dixon, 2000). Therefore, the phenomenon would appear to be general in ladybirds and not a consequence of protandry, or in *A. bipunctata*, of differences in birth weight. Other studies, such as that of Wiklund *et al.* (1991), have assumed that birth sizes of males and females are similar. For the first time for an insect, sexual size dimorphism is shown to be associated with a lower growth rate in male larvae that is not a consequence of a smaller birth size. It seems reasonable to hypothesise that this lower growth rate is a consequence of male larvae developing their gonads earlier than female larvae. As males scramble for mates, there appears to be no advantage to being large. Indeed, when food is limited, small males are at an advantage in that they are likely to spend proportionally more time seeking mates and less time feeding than are large males. That is, female-biased sexual size dimorphism in ladybird beetles is likely to be a consequence of a developmental constraint in males reinforced by a mating advantage that operates when food is scarce.

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