Sex-dependent effects of sibling cannibalism on life history traits of the ladybird beetle *Harmonia axyridis* (Coleoptera: Coccinellidae)

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To investigate the sex-dependent effects of sibling cannibalism on variations in life history traits, I analysed body size, weight and instar interval in relation to the occurrence of sibling cannibalism in the ladybird beetle Harmonia axyridis. Sibling cannibalism at the time of hatching significantly affected the body size and weight of adults. There was a 2.32% and 1.05% increase in the body size of males and females, respectively, and a 3.55% increase and a 2.30% decrease in their respective body weights. Sibling cannibalism also significantly shortened the total and larval instar intervals, by 4.24% in males and by 1.22% in females, mainly due to shortening of the first instar. These results suggest that the effects of sibling cannibalism on life history traits are sex-differentiated and are greater in males than in females. A simulation of aphid density indicated that shortening the instar interval affected larval survival; the aphid density when the larvae completed development was 39.71% and 10.52% larger for cannibalistic males and females, respectively, than for non-cannibals. These results suggest that sibling cannibalism promotes more rapid development and larger adult size, although the effect was more pronounced in males than in females. Faster development may be adaptive for resource tracking, and the large adult size may increase fecundity in females and mating success in males through female mate choice, both resulting in an increase in the fitness of cannibals.   © 2002 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2002, **76**, 349–360.

ADDITIONAL KEYWORDS: body size – body weight – development rate – fitness – instar interval – sex.

INTRODUCTION

Traditionally, cannibalism had been regarded as abnormal behaviour or an artefact that has little ecological significance in wild populations (e.g. Colinvaux, 1973). However, recent evidence from field and laboratory studies suggests that cannibalism is an important component of the biology of a taxonomically broad range of species, from lower eukaryotes to higher primates (e.g. Fox, 1975; Polis, 1981; Elgar & Crespi, 1992). Cannibalism confers many nutritional and competitive advantages to the cannibals, suggesting that it may be common in many species, but cannibalism is usually less common than we might expect (Dawkins, 1976), and in many species it is observed only in certain stages of development. The cannibalis-

tic trait may evolve if its evolutionary cost is less than its benefit. The costs of cannibalism are thought to be (i) increased risk of injury to the cannibal (Dawkins, 1976); (ii) diminished inclusive fitness (e.g. Hamilton, 1964a, 1964b; Fox, 1975); and (iii) potential disease transmission via cannibalism (Elgar & Crespi, 1992; Hurst & Majerus, 1993; Boots, 1998; Pfennig *et al.*, 1998; Chapman *et al.*, 1999b). Of these three factors, the risk of injury to cannibals may not always be important because cannibalism is typically size-dependent; smaller, defenceless individuals are usually cannibalized (e.g. Semlitsch & West, 1988; Buskirk, 1989; Leonardsson, 1991; Elgar & Crespi, 1992). To evaluate cannibalism through kin-relatedness, kin recognition must be considered. Many empirical studies have shown that cannibals preferentially avoid eating kin (e.g. Pfennig *et al.*, 1993; Fellows, 1998), although there are many exceptions

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(e.g. Walls & Blaustein, 1995; Boots, 2000). The discrepancy in the results of these empirical studies suggests that both direct and indirect mechanisms of kin discrimination may be involved in cannibalism during several developmental stages in many animals. Furthermore, some authors have suggested that the existence of kin recognition does not necessarily imply that natural selection has favoured organisms to discriminate kin *per se* (e.g. Grafen, 1990). In contrast, infections by pathogens as a result of cannibalism have recently been given much attention as one of the most important costs in the evolution of cannibalism (e.g. Boots, 1998; Pfennig *et al*., 1998). A number of studies have stressed the large benefit of cannibalism (e.g. Fox, 1975; Polis, 1981; Baur, 1990; Crespi, 1992). Furthermore, several studies have investigated the consequences of cannibalism among non-siblings on variation in life history characteristics, including survivorship, size, growth rate and time constraints (e.g. Church & Sherratt, 1996; Lounibos *et al*., 1996; Chapman *et al*., 1999a, 1999b; Johansson & Rowe, 1999).

In the Coccinellidae, egg cannibalism, wherein newly hatched or young larvae eat conspecific eggs, occurs primarily in wild populations of many species (e.g. Stevens, 1992; Hodek & Honek, 1996; Dixon, 2000). Hamilton (1964a, 1964b) classified cannibalism into two categories: cannibalism of kin (sibling cannibalism) and cannibalism of non-kin (non-sibling cannibalism). Sibling cannibalism in beetles is defined as cannibalism among siblings in an egg batch upon hatching (Osawa, 1989; Stevens, 1992). Sibling cannibalism is observed in many taxa in which the feeding conditions for newly hatched offspring are thought to be severe, e.g. gastropods (Baur & Baur, 1986; Baur, 1990, 1992), amphibians (Duellman & Trueb, 1986) and marine prosobranchs (Fretter & Graham, 1962; Fioroni, 1966, 1988). The ecological circumstances of sibling cannibalism in a wild population of the aphidophagous ladybird beetle *Harmonia axyridis* are well documented. Cannibals eat all infertile eggs and fertile eggs with delayed hatching in an egg batch, only at hatching (Kawai, 1978). The proximate factors for sibling cannibalism are thought to be (i) the existence of infertile or unhatched eggs (Kawai, 1978; Osawa, 1992a); and (ii) asynchronous hatching among eggs in an egg batch (Kawai, 1978). When the cannibal and the victim are full or half siblings (e.g. the victim is a fertile egg), by kin selection the cannibalistic trait is beneficial to the cannibal and the victim at a low density of alternate prey, while it is not always beneficial at high prey density (Osawa, 1992a). The mechanisms of producing infertile or unhatched eggs, or asynchronous hatching in an egg batch are not fully understood. The existence of male-killing microorganisms has recently been discovered as one important

factor affecting the production of fertilized, but unhatched, eggs (e.g. Hurst & Majerus, 1993; Hurst *et al*., 1993). Male-killing microorganisms have been reported in many insect taxa (Hurst & Majerus, 1993). Male-killers, vertically transmitted through egg cytoplasm, but not sperm (i.e. only from mother to daughters), are also observed in many species of ladybird beetles, including *H. axyridis* (e.g. Matsuka *et al*., 1975; Gotoh & Niijima, 1986; Hurst & Majerus, 1993; Majerus *et al*., 1998). In *H. axyridis*, about half of the male eggs in an egg batch died due to the infection by the male-killer (e.g. Majerus *et al*., 1998), suggesting that sibling cannibalism in female hatchlings is more intense than that in males because all the male eggs are not hatched and cannibalized. When the victim is an infertile or an unhatched egg, the cannibalistic trait is usually adaptive for male and female cannibals, and sibling cannibalism will be favoured. The horizontal disease transmission of the male-killers is thought to result from non-sibling egg cannibalism by larvae of the same species and intraspecific predation of eggs (Majerus, 1994). Twenty percent of eggs were killed by non-sibling cannibalism in a field population of *H. axyridis* (Osawa, 1993), suggesting that non-sibling cannibalism can be one of the important factors in the horizontal transmission of the disease (Majerus, 1994). In *H. axyridis*, the male-killers were inherited by more than 99% of the progeny of infected females (Majerus *et al*., 1998), but the infected proportion varies largely among populations (Majerus *et al*., 1998). Male-killers are sensitive to antibiotics (Matsuka *et al*., 1975; Gotoh & Niijima, 1986; Majerus *et al*., 1998). Furthermore, a reversion to normal sex ratios has been observed in females that initially produced female-biased sex ratios in *H. axyridis* (Hu, 1979; Niijima & Nakajima, 1981; Gotoh, 1982; Hurst & Majerus, 1993). These results imply that male-killers can be eliminated from a population. Therefore, sibling cannibalism (i.e. infected female larvae cannibalizing infected and unhatched male eggs) may play a certain role in maintaining male-killers in ladybird populations. When females are infected with male-killers, the fitness of these infected females, as well as the males that mate with them, is decreased by 50% when the sex ratio is equal. Therefore, mating between an uninfected male and an infected female is thought to be the second route of horizontal transmission of male-killers. In the evolution of male-killers, the cost of disease transmission through mating may exist only in males that mate with infected females. Whether sibling cannibalism has any consequences on variation in the life history traits of cannibal and non-cannibal males and females remains unknown when the occurrence of sibling cannibalism may be female-biased and sibling cannibalism involves disease transmission only in females.

Body size, especially in females, is an important fitness component that evolves through natural selection in many animals (e.g. Roff, 1981, 1986). Moreover, in *H. axyridis*, larger males have an advantage over smaller males in mating (Osawa & Nishida, 1992; Ueno *et al.*, 1998), suggesting that body size is directly involved in determining the mating success of males. Furthermore, instar interval may be one of the important factors determining the fitness of predatory ladybird beetles; aphidophagous coccinellids tend to lay eggs before the prey density peaks (Hemptinne *et al.*, 1992; Osawa, 2000), but a severe food shortage often occurs during the later larval stages (Osawa, 1992b). Therefore, shortening the developmental interval is extremely important in allowing aphidophagous coccinellid larvae to escape a food shortage in later developmental stages. Interestingly, only extremely low food supplies influence the final larval size of the adult in predatory coccinellids, while the growth rate is very sensitive to food consumption (Hodek & Honek, 1996). Therefore, instar interval and final adult size in a predatory coccinellid may be related (Ueno, 1994). Thus, attaining a certain size in adulthood, along with controlling larval growth rate in relation to food availability, is one of the important strategies that guarantees a certain level of inclusive fitness in adulthood. It has been demonstrated that sibling cannibalism guarantees safe development in first instar larvae of a coccinellid (Kawai, 1978; Osawa, 1992a), but the role of sibling cannibalism in determining instar interval of larvae and final body size in a predatory coccinellid is less well documented. Life history traits must vary between a cannibal and a non-cannibal if sibling cannibalism promotes not only safe but also immediate development, resulting in the size variations of the adults.

This study examined the sex-dependent effects of sibling cannibalism on body size and weight in adults and larvae of the ladybird beetle *H. axyridis*.

MATERIAL AND METHODS

LABORATORY EXPERIMENTS

Husbandry of ladybird females for egg stocks
This experiment investigated the effect of sibling cannibalism on variation in life history traits of male and female *Harmonia axyridis*. The traits investigated were body size, body weight and instar intervals. Controls were run without sibling cannibalism, and all experiments were performed between April and May 2000. Sixteen adult females of the spring generation (the first generation after hibernation) were caught at the Botanical Garden, Kyoto University, Japan (35–02′ N, 135–47′ W) from 17 to 19 April. All females were thought to have mated because mating is often observed during the closing stage of dormancy in many coccinellids (Hodek & Honek, 1996). The beetles were individually reared with a surplus of aphids (*Aphis spiraecola* Patch, Hemiptera: Aphididae) and a shoot of *Spiraea thunbergii* Sieb. (Rosaceae) at 25°C, 16L/8D, in a plastic Petri dish (9 cm in diameter, 1.5 cm in height) with a piece of wet filter paper (7 cm in diameter). The cap of the each Petri dish was marked with the code for each female. Shoots of *S. thunbergii* with aphids were collected daily near the university campus. Shoots were changed daily and examined for signs of oviposition. The numbers of eggs in each batch were counted and the date and mother code were written on the cap of each Petri dish. In total, the 16 females produced 396 eggs from 27 egg batches.

Sibling cannibalism experiment
With the tip of a fine paintbrush, approximately half of the eggs in each batch were detached, counted, and kept in a Petri dish, labelled with the mother code, number of eggs and date of birth. These eggs were refrigerated at 5°C to delay the time of hatching and were 'to-be-cannibalised'; there were 121 such eggs from the 27 batches. After removing these eggs, the remaining eggs ('hatching' eggs; $N = 275$ from the 27 batches) were detached from the batches using a paintbrush and individually reared in plastic Petri dishes at 25°C, 16L/8D, and *c*. 60–70% relative humidity Each hatching egg was provided with a 'to-be-cannibalised' egg with the same mother code [one egg for a cannibal; zero (control) for a non-cannibal] from the stock of cooled eggs laid between 18 and 23 April. Each hatching egg was placed on a moist piece of filter paper in a Petri dish, along with a 'to-be-cannibalised' sibling egg, to promote safe hatching and the occurrence of sibling cannibalism before larval dispersal. The supplied eggs were oviposited 1 or 2 days before the cannibal eggs, and all the cannibals ate the provided eggs within 3 days. Cannibalistic larvae in *H. axyridis* eat infertile sibling eggs and fertile ones with delayed hatching. Of the eggs killed by sibling cannibalism (34.98%), 24.33% were infertile eggs and 10.65% were fertile eggs with delayed hatching; the cannibals consume sibling eggs regardless of their fertility (Osawa, 1992a). Furthermore, sibling cannibalism occurred in >90% of egg batches in the Botanical Garden of Kyoto, Japan (Osawa, 1989), implying that the sex ratio of the adult population may be extremely female-biased if all the male eggs were killed by male killers. However, no extremely female-biased sex ratio was observed in the adult population (Osawa, unpublished). These results suggest other factors in addition to male killers may be involved in the occurrence of sibling cannibalism. Therefore, their fertility and potential infection of the stocked sibling eggs by male killers were not checked. The eggs were reared indi-

vidually in Petri dishes on piece of wet filter paper and held laboratory conditions (25°C, 16L/8D, and *c*. 60–70% relative humidity) with a shoot of *S. thunbergii* and a surplus of aphids without distinguishing adult and apterous larvae. Every 12 h a fresh shoot of *S. thunbergii* with excess aphids and a new filter paper were provided, and the larvae were all checked to see whether they had moulted. Okamoto (1978) showed that the availability of food to larvae during development affected life history traits (e.g. body size, body weight and instar interval) in the predatory ladybird beetle *Menochilus secmaculatus*; limited food availability caused the larvae to consume less food in total, resulting in smaller, lighter adult beetles with longer instar intervals. This suggests that the effect of sibling cannibalism in *H. axyridis* is more pronounced when less food is available. Furthermore, in laboratory experiments using 48 families of *H. axyridis*, Grill *et al*. (1997) showed that plasticity in development time, and adult size and weight may evolve given appropriate selection pressure. Heritability was low for size and intermediate for development time in beetles that were reared under conditions of high food quality (using aphids for food), whereas heritability was high for size and development time in beetles that were reared under conditions of low food quality (using an artificial diet for food). The authors suggested that relatively low quality food exaggerates genetic differences in quality (e.g. size, weight and developmental rate) among the families (Grill *et al*., 1997). Therefore, I used surplus food to avoid (i) the possibility of all *H. axyridis* larvae developing at a low rate due to food shortage and therefore potentially obscuring the effect of sibling cannibalism on body size and development rate; and (ii) the acceleration of genetic differences among families of beetles. Thus, I confirmed the effect of sibling cannibalism on the variation in life history traits in *H. axyridis* when food conditions for the developing larvae are good, although the effects of sibling cannibalism may have been underestimated in this experiment. Throughout larval development, living aphids were usually found in the Petri dishes. Therefore, the larvae were had sufficient food during the experiments.

In all experiments, the young shoots of *S. thunbergii* collected in the field frequently contained the eggs, larvae, pupae and adults of many aphidophagous predators in addition to *A. spiraecola*. To remove the effect of other predators, I carefully removed all of them from the shoots before providing them to the larvae. The numbers of apterae and alatae of *A. spiraecola* per young *S. thunbergii* shoot were 415 ± 34.34 and 0.80 ± 0.29 (mean $\pm$ SE), respectively, and the numbers of leaves per shoot and the shoot length (mm) were 21.70 ± 1.28, and 78.40 ± 2.92, respectively (based on 10 random samples). After the larvae

pupated and emerged, new adults were sexed and their body size (total body length to the nearest 0.01 mm) and fresh body weight (mg) were measured approximately 12 h after emergence. Thirty-one eggs (cannibal $N = 16$, non-cannibal $N = 15$) did not hatch and 10 cannibals and 19 non-cannibals died during the experiments. I eliminated these data from the subsequent analysis. In total, 215 adults from 16 females (non-cannibal: males $N = 56$, females $N = 64$; cannibal: males $N = 50$, females $N = 45$) were used for the analysis. Of the 16 adult females, one female produced only female progeny: seven female cannibals and two female non-cannibals (Fisher's exact test for number of progeny, $\chi^2 = 5.885$, $P < 0.05$; 16 eggs randomly chosen from 52 eggs in 2 batches were used for the experiment), implying that male-killing microorganisms exist in this population.

Developmental rate of the larvae

The developmental rate was calculated as $\log_e$[fresh adult weight (mg)]/(instar interval), assuming an exponential growth curve (Ueno, 1994).

SIMULATION OF APHID DENSITY

A simulation model of aphid density was used to evaluate the effects of sibling cannibalism on variation in the larval instar interval between cannibals and non-cannibals. The simulation was run with Mathematica software (Wolfram, 1999).

Aphid life cycles are generally well synchronized with bud burst and leaf fall of plants (Dixon, 1985). Generally, aphid density in a habitat (e.g. a whole tree) reaches a peak, and then decreases rapidly primarily due to intraspecific mechanisms, e.g. producing winged morphs, and partly due to foraging by aphidophagous insects in the declining phase (e.g. Dixon, 1971, 1985). *H. axyridis* oviposits shortly before the peak aphid density (Osawa, 2000), although *H. axyridis* is unable to locate aphids at very low densities because of its limited searching ability. I modified the normal distribution curve $[(1/\sqrt{(2\pi)}) \times \exp(-x^2/2)]$ in the calculation of aphid density because the normal distribution curve approximates the population dynamics of many species of aphids in wild populations (see Osawa, 2000). Aphid density was calculated using the following equation:

$$f(x) = (a/\sqrt{(2\pi)}) \times \exp[-(-4 + x/3)^2/2] \tag{1}$$

where the parameters a ($a > 0$), $a/\sqrt{(2\pi)}$, and x represent the constant values for the aphid population, the peak aphid density and the age of the population (days), respectively (see Fig. 1a). From Eqn 1, $df(x)/dx$ and $d^2f(x)/dx^2$ were calculated to understand the characteristics of the dynamics of the simulation model (see Fig. 1b and c, respectively). The virtual aphid
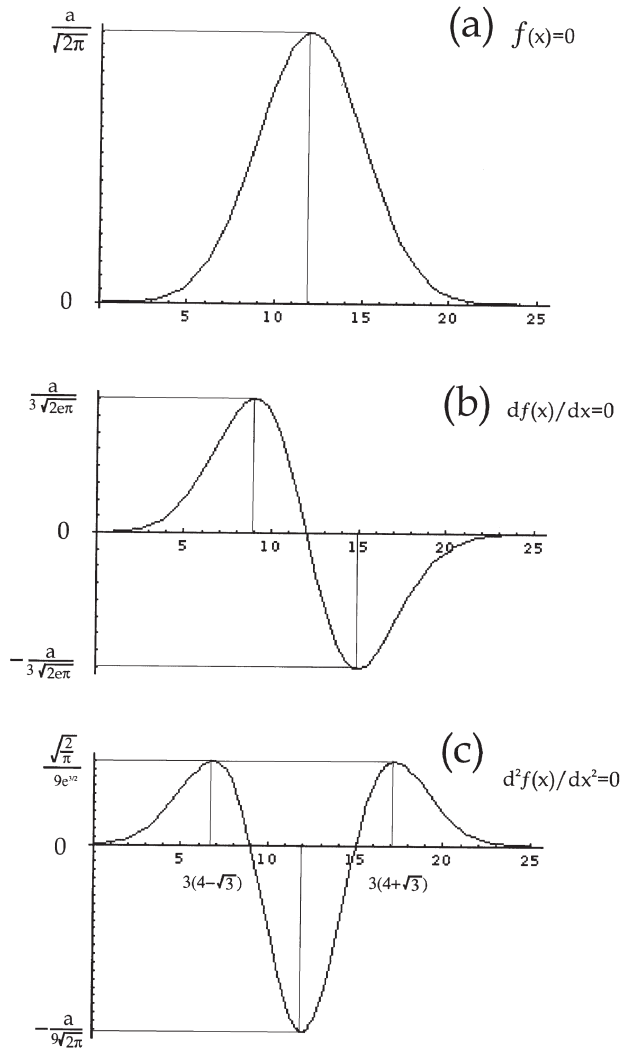
Figure 1. The population dynamics model of aphid. (a) Eqn 1, $d(x) = (a/\sqrt{(2\pi)}) \times \exp[-(-4 + x/3)^2/2]$; (b) Eqn 2, $df(x)/dx = \{a(4 - x/3) \times \exp[-(-4 + x/3)^2/2]\}/3[\sqrt{(2\pi)}]$; and (c) Eqn 3, $d^2f(x)/dx^2 = -\{(a \times \exp[-(-4 + x/3)^2/2])/9\sqrt{(2\pi)}\} + \{(a(4 - x/3)^2 \times \exp[-(-4 + x/3)^2/2])/9\sqrt{(2\pi)}\}$.

population peaked on the 12th day and decreased rapidly thereafter (Fig. 1a). The ranges of x and $f(x)$ in the population were $0 < x < 25$, and $a\sqrt{(2\pi)} \times \exp(-169/18) < f(x) < a\sqrt{(2\pi)}$, respectively (Fig. 1a).

In this virtual aphid population, *H. axyridis* hatches when the increase rate of the aphid population (= $f(x)/dx$) is at its maximum (the 9th day; Fig. 1a and b), when a few alatae may exist in the population. Newly hatched coccinellid larvae cannot easily capture aphids; they can catch aphids primarily in the first instar (apterous morph; Dixon, 1959; Brown, 1972; Kawai, 1978). These results suggest that *H. axyridis* larvae hatch during optimal aphid conditions for the larvae, when there are more apterae and fewer alatae

in the virtual aphid population. This is fairly consistent with field observations of *H. axyridis*, which lays eggs shortly before the aphid density peaks (Osawa, 2000). In *H. axyridis*, severe food shortage often occurs in the forth instar larvae in wild populations, and *H. axyridis* populations often become extinct due to starvation (Osawa, 1992a, 1993; Hironori & Katsuhiro, 1997). To evaluate the differences in instar interval between cannibals and non-cannibals, the virtual aphid populations of both cannibals and non-cannibals were not allowed to be annihilated before the larvae of *H. axyridis* finished their development (Fig. 2).

STATISTICAL ANALYSIS

Two-way ANOVA was used to detect effects of sex and sibling cannibalism on total and larval instar interval, body size, body weight and developmental rate. All ANOVAs are for an unbalanced sample size and are based on type III model testing. The preliminary analysis of the relationship between total instar interval and body size, and between total instar interval and body weight determined that there were maximal extremes in each relationship. Therefore, polynomial curves (degree 2) were used to analyse the relationships. SAS Discovery Software (SAS Institute, 1995) was used for the above-mentioned statistical analyses. For all other comparisons, SuperANOVA software was used to run one-way ANOVAs with Scheffe's range test (Abacus Concepts, 1989).

RESULTS

EFFECTS OF SEX AND CANNIBALISM ON THE LENGTH OF OF EACH DEVELOPMENTAL STAGE

Figure 3 shows the differences in instar interval during each developmental stage. In first and third instar larvae, cannibalism significantly affected the instar intervals by shortening the first instar and lengthening the third instar (two-way ANOVA: $F_{1,211} = 55.459$, $P < 0.0001$; $F = 10.220$, $P < 0.01$, respectively). However, sex and the interaction between sex and cannibalism did not significantly affect the instar interval (two-way ANOVA, first instar: $F_{1,211} = 0.324$, $P = 0.570$; $F_{1,211} = 2.358$, $P = 0.126$, respectively; third instar: $F_{1,211} = 2.427$, $P = 0.121$; $F_{1,211} = 0.00003$, $P = 0.986$, respectively). In prepupa and pupa, the interaction between sex and cannibalism significantly affected the instar interval (two-way ANOVA, $F_{1,211} = 4.545$, $P < 0.05$; $F_{1,211} = 6.430$, $P < 0.05$, respectively). However, the single effects of sex and cannibalism on the instar interval were not significant (two-way ANOVA; prepupa: $F_{1,211} = 3.387$, $P = 0.067$; $F_{1,211} = 0.042$, $P = 0.837$, respectively; pupa: $F_{1,211} = 0.861$, $P = 0.355$; $F_{1,211} = 0.789$, $P = 0.376$, respectively). In the second
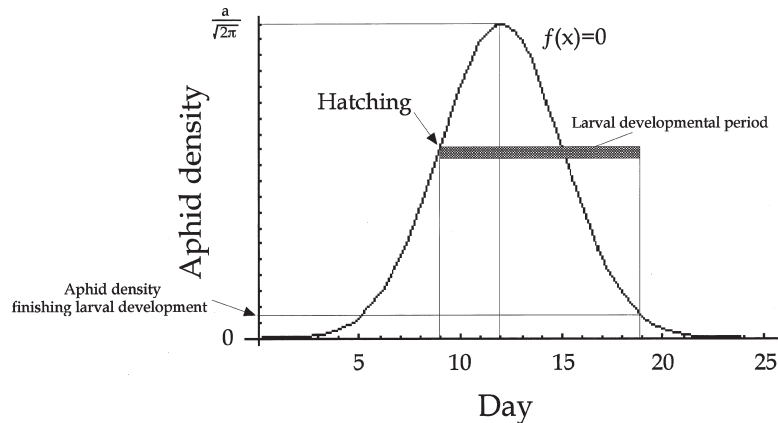
Figure 2. The schematic model for population dynamics of the aphid and instar interval of *H. axyridis*.

and fourth instar larvae, sex, cannibalism, and their interaction did not affect the instar interval (two-way ANOVA, second instar: $F_{1,211} = 0.032$, $P = 0.858$; $F_{1,211} = 0.008$, $P = 0.928$; $F_{1,211} = 0.210$, $P = 0.647$, respectively; fourth instar: $F_{1,211} = 1.088$, $P = 0.298$; $F_{1,211} = 0.048$, $P = 0.827$; $F_{1,211} = 0.933$, $P = 0.335$, respectively).

SEX-DEPENDENT EFFECTS OF SIBLING CANNIBALISM

Body size and weight of adults

Sex significantly affected body size; females were significantly larger than males (two-way ANOVA: $F_{1,211} = 186.470$, $P < 0.0001$) (Fig. 4a). Furthermore, the effect of cannibalism on the body size of adults was significant (in males: cannibal, 6.968 ± 0.048 (mean $\pm$ SE) mm; non-cannibal, 6.810 ± 0.036; in females: cannibal, 7.518 ± 0.056, non-cannibal, 7.440 ± 0.035; two-way ANOVA, $F_{1,211} = 7.447$, $P < 0.01$); male and female cannibals were 2.32% and 1.05% larger than non-cannibals, respectively (Fig. 4a). The difference was significant in males (Scheffe's range test, $P < 0.01$), but not in females (Scheffe's range test, $P = 0.217$). The interaction between sex and cannibalism did not affect adult body size (two-way ANOVA, $F_{1,211} = 0.855$, $P = 0.356$; Fig. 4a).

Females were significantly heavier than males (two-way ANOVA, $F_{1,211} = 169.782$, $P < 0.0001$; Fig. 4b). The interaction between sex and cannibalism significantly affected adult body weight, with a 3.55% increase in males and a 2.30% decrease in females (in males: cannibal, 33.320 ± 0.427 (mean $\pm$ SE) mg, and non-cannibal, 32.179 ± 0.420; in females: cannibal, 38.044 ± 0.553, and non-cannibal, 38.938 ± 0.375; two-way ANOVA, $F_{1,211} = 5.329$, $P < 0.05$; Fig. 4b. However, the single effect of cannibalism was not significant (two-way ANOVA, $F_{1,211} = 0.079$, $P = 0.778$; Fig. 2b).

Larval and total instar interval and developmental rate

The occurrence of cannibalism significantly affected larval and total instar interval (two-way ANOVA, $F_{1,211} = 7.708$, $P < 0.01$; $F_{1,211} = 5.942$ $P < 0.05$, respectively; Fig. 5a). The difference in sex was significant for the larval instar interval, with 4.24% and 1.22% decreases in males and females, respectively (two-way ANOVA, $F_{1,211} = 4.058$, $P < 0.05$; Fig. 5a). In males, these differences were significant for larval and total instar intervals (Scheffe's range test, $P < 0.01$, and $P < 0.05$, respectively). In females, however, they were not significantly different (Scheffe's range test, $P = 0.4036$, and $P = 0.279$, respectively). The interaction between cannibalism and sex was not significant for larval or total instar interval (two-way ANOVA, $F_{1,211} = 2.325$, $P = 0.129$; $F_{1,211} = 0.925$ $P = 0.337$, respectively; Fig. 5a).

The difference in sex significantly affected larval and total developmental rates (two-way ANOVA, $F_{1,211} = 90.368$, $P < 0.0001$; $F_{1,211} = 155.888$, $P < 0.0001$, respectively; Fig. 5b). Furthermore, the occurrence of cannibalism had a significant effect on the larval development rate (two-way ANOVA, $F_{1,211} = 4.932$, $P < 0.05$); cannibals had accelerated growth by 6.827% in males and 0.870% in females (see Fig. 5b). However, cannibalism did not affect the total development rate (two-way ANOVA, $F_{1,211} = 2.291$, $P = 0.132$; Fig. 5b). The interaction between cannibalism and sex was also significant for the larval and total developmental rate (two-way ANOVA, $F_{1,211} = 8.961$, $P < 0.01$; $F_{1,211} = 7.717$, $P < 0.01$, respectively; Fig. 5b).

Relationships between body size, weight, and instar interval

In males, there was no significant relationship between total instar interval and body weight, or
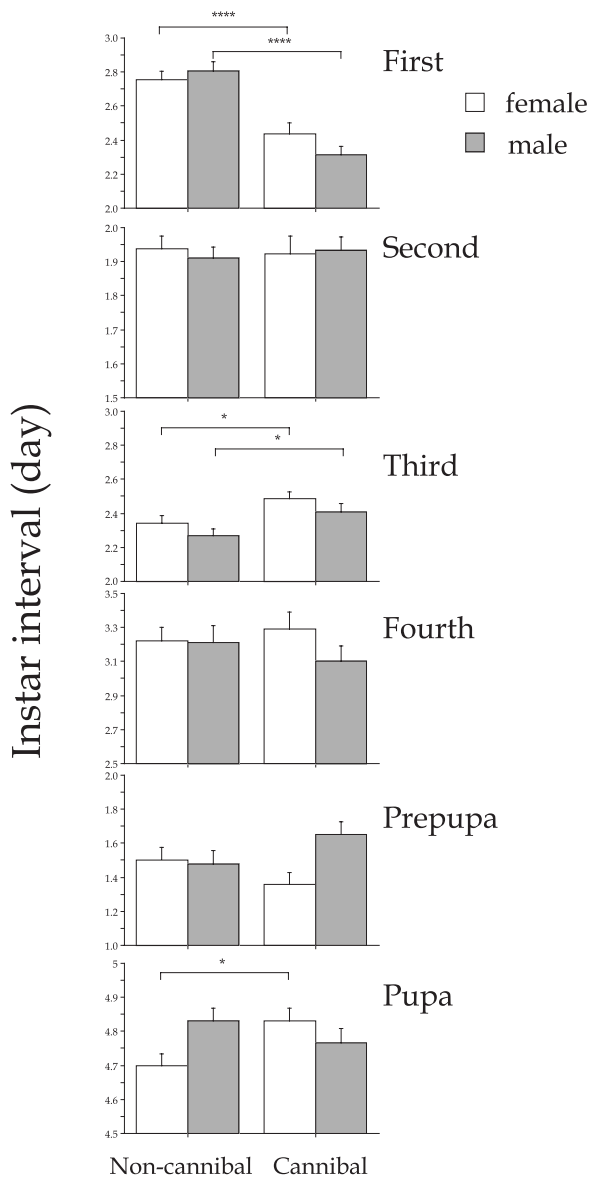
Figure 3. The differences in instar interval, at each developmental stage, between cannibals and non-cannibals. Vertical lines indicate SE. *$P = 0.05$ and ****$P = 0.0001$, comparing cannibals and non-cannibals in males and females, using Scheffe's range test.

Figure 4. The differences in body size (a) and body weight (b) between cannibals and non-cannibals. Vertical lines indicate SE. **$P = 0.01$, comparing cannibals and non-cannibals in males and females, using Scheffe's range test.

between total instar interval and body size ($R^2 = 0.033$, $F = 1.756$, $P = 0.178$; $R^2 = 0.029$, $F = 1.549$, $P = 0.217$, respectively; Fig. 6). However, in females, there was a weak, but significant, relationship between total instar interval and body weight ($R^2 = 0.056$, $F = 3.152$, $P < 0.05$; Fig. 6). In females, the relationship between total instar interval and body size was weak and only marginally significant ($R^2 = 0.053$, $F = 2.941$, $P = 0.057$; Fig. 6).
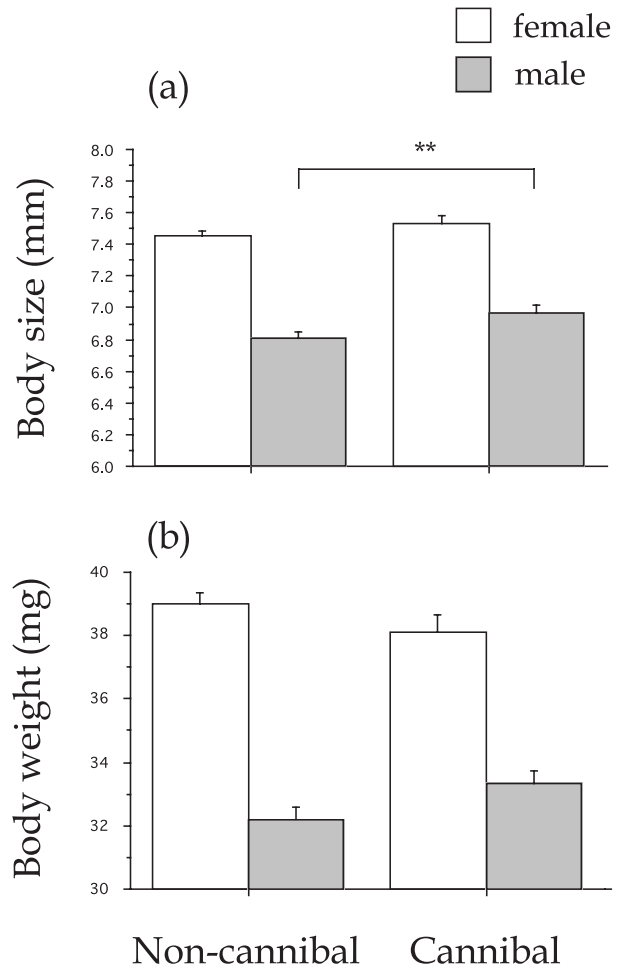
EVALUATION OF INSTAR INTERVAL SHORTENING BY SIBLING CANNIBALISM IN RELATION TO PREY DYNAMICS

The total instar interval between cannibals and non-cannibals was shortened by 0.432 days in males and 0.125 days in females (Table 1). However, there was a large difference in aphid densities between cannibals and non-cannibals when the ladybird larvae completed their development. For male cannibals, the aphid density was 39.71% larger than for non-cannibal males. In contrast, the aphid density for female cannibals was 10.52% larger than for non-cannibals (Table 1).
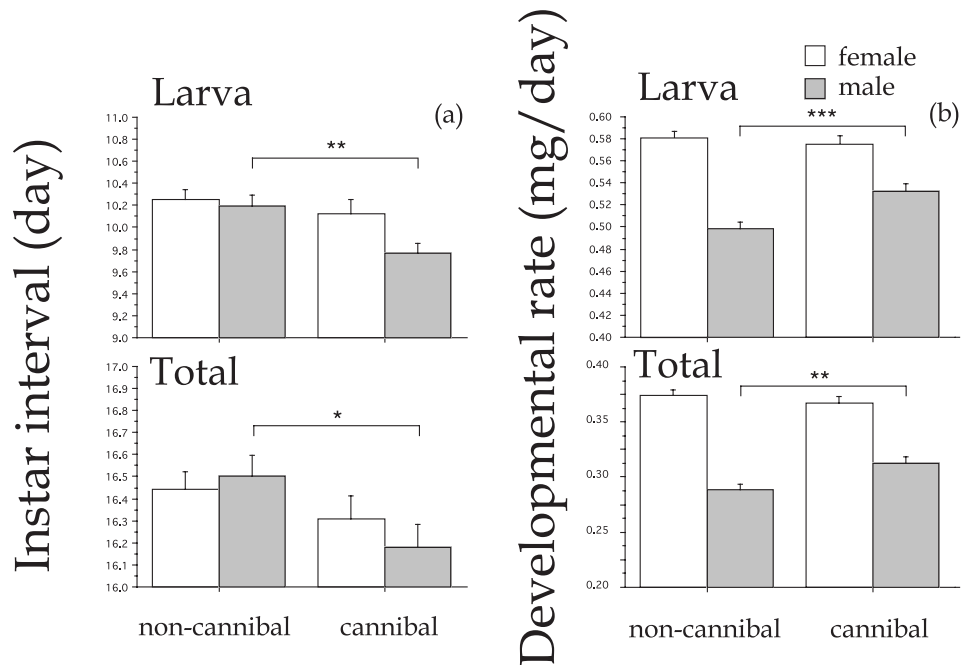
Figure 5. The differences between cannibals and non-cannibals in instar intervals of larvae and total (a), and in developmental rate of larvae and total (b). Vertical lines indicate SE. *$P = 0.05$, **$P = 0.01$ and ***$P = 0.001$, comparing cannibals and non-cannibals in males and females, using Scheffe's range test.

DISCUSSION

Variation in body size and body weight of males and females due to sibling cannibalism

This study demonstrated that sibling cannibalism significantly increased the body sizes of males and females, by 2.32% and 1.05%, respectively. In general, female fecundity and longevity, and hence lifetime reproductive success, is correlated with body size (e.g. Partridge, 1988). Furthermore, it has been suggested that sex- and colour morph-related differences in cold tolerance during hibernation may be involved in the variation of body size in *H axyridis*; large melanistic females were favoured during cold winters (Osawa, 2001). Females are usually more cannibalistic than males in many animal species (Polis, 1981). Church & Sherratt, 1996) suggested that the greater incidence of non-sibling cannibalism in females of many species is related to their higher energy requirements, particularly for egg maturation and oviposition. Furthermore, there is a positive relationship between the body size of females and the number of offspring in many insect species (e.g. Thornhill & Alcock, 1983; Birkhead *et al.*, 1998). This study found that the effect of sibling cannibalism on body size was significant in male and female *H. axyridis*, indicating that sibling cannibalism, at least in female *H. axyridis*, may directly increase the number of offspring of cannibalistic

females. This suggests that natural selection favours larger body sizes in female *H. axyridis*, although larger size and faster development are not compatible traits, especially in females (Figs 3 and 6).

This study also found that sibling cannibalism significantly increased the body weight in males, but decreased it in females. In a laboratory experiment, Ueno (1994) showed that the heritability of body size in females (0.563) was significantly larger than in males (0.324), while the difference in body weight was not significant between sexes (0.321 in males and 0.383 in females). This result suggests that: (i) the role of body size in determining fitness in males is larger than in females, but this is not the case for body weight, because in many animals, traits closely associated with fitness are generally less heritable than traits more loosely connected with fitness (Mousseau & Roff, 1987); and (ii) the role of genetic factors in determining body size in females is larger than in males. Furthermore, body size and body weight in females were weakly correlated, which is not the case in males. Aphidophagous coccinellids adopt the strategy that females decrease egg numbers and maintain their size if food supply is limited (Hodek & Honek, 1996). Moreover, both the numbers of eggs laid per day and the size of individual clusters of eggs in the coccinellids are greatly affected by the availability of food (Dixon, 2000). These results suggest that early
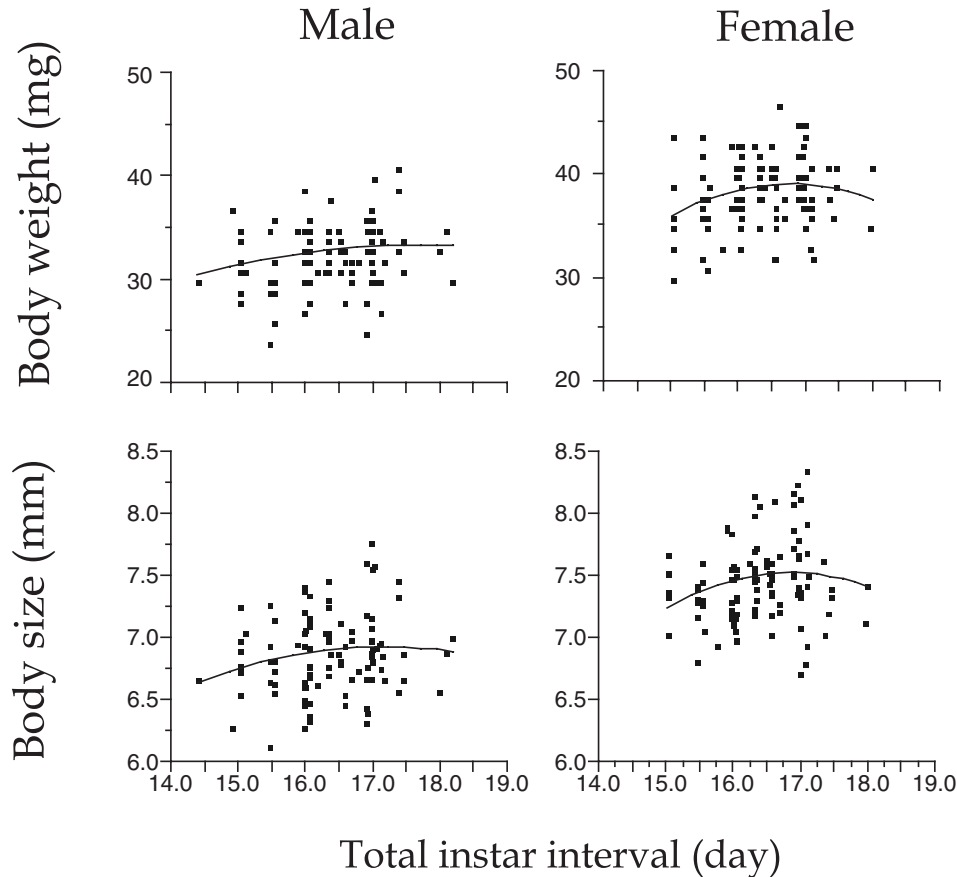
Figure 6. The relationship between total instar interval and body weight (above), and that between total instar interval and body size (below) in males and females. The polynomial (degree 2) curves were fitted for the analysis.

Table 1. The estimated aphid density at larval completion of cannibals and non-cannibals in *H. axyridis* in males and females

	Larval instar interval (mean in days)	Equation 1 x-value	Aphid density at larval completion	Density ratio[1]
Male				
Cannibal	9.763	18.763	$0.0788b^2$	1.3971
Non-cannibal	10.195	19.195	$0.0564b^2$	1
Female				
Cannibal	10.119	19.119	$0.0599b^2$	1.1052
Non-cannibal	10.244	19.224	$0.0542b^2$	1

[1]Aphid density at the development completion of cannibals was compared with that of non-cannibals (non-cannibal = 1) in males and females. [2]$b = a/\sqrt{2\pi}$ in Eqn 1.

reproduction by females permits flexibility in body weight.

This study found that sibling cannibalism significantly increased body size and body weight of male *H. axyridis*. Sexual selection towards larger body sizes is frequent in males and females in many taxa (e.g. Andersson, 1994). In wild populations of *H. axyridis*, larger males have an advantage over smaller males in mating, which is primarily explainable by female-mate choice (Osawa & Nishida, 1992; Ueno *et al.*,

1998). These results suggest that larger males, that may have cannibalized sibling eggs, have more advantages in mating than smaller males that may not have cannibalized sibling eggs; thus larger males with cannibalistic traits are favoured during mating.

Traditionally, attention has only been paid to female mate choice in sexual selection in studies of the evolution of sexual dimorphism. This is mainly due to the low encounter and acceptance probability in mating from the male point of view. However, the role of male mate choice may not be negligible in non-sexually dimorphic species like *H. axyridis*. In addition to male–male competition and female mate choice, a number of physiological and behavioural conditions in males and females may be involved in mating success (Osawa & Nishida, 1992), although female mate choice certainly plays an important role in the mating success of males. Recently, Randerson *et al*. (2000) modelled the evolution of a male choice gene that allows discrimination between infected and uninfected females by male-killing microorganisms. These authors showed that the stable maintenance of both female variation and male choice is likely as long as males make mistakes when discriminating between females, which may furnish a solution to the 'paradox of the lek'. It is known that through long and short distance movements, male and female adult *H. axyridis* concentrate on the quantity and quality of favourable food habitats and mate there (Osawa, 2000). This suggests that a kind of lek, which balances the sex ratio, is formed during the mating of *H. axyridis* in favourable habitats. Interestingly, *H. axyridis* males tended to avoid mating with larger females during the spring in a wild population (Osawa & Nishida, 1992), which may contradict the notion that larger females have more offspring in many insect species (e.g. Thornhill & Alcock, 1983; Birkhead *et al*., 1998). However, this study showed that sibling cannibalism significantly increased the body sizes of female cannibals. Furthermore, the progeny of females ($N = 9$) strongly suspected of being infected by male-killers were significantly larger and heavier than the offspring of uninfected females (one-way ANOVA: in body size, $F_{1,107} = 20.604$, $P < 0.0001$; in body weight, $F_{1,107} = 4.012$, $P < 0.05$, respectively). Moreover, the instar interval of the infection-suspected progeny was significantly longer than that of the uninfected ones (one-way ANOVA in total instar interval, $F_{1,107} = 8.306$, $P < 0.01$). These results imply that the infected females are larger and heavier than uninfected ones because the male-killers decelerated larval development. However, not all larger females may be infected, and the males that mate with large and uninfected females may increase their fitness if lifetime reproductive success in females is correlated with body size (e.g. Mousseau & Roff, 1987; Partridge, 1988); this

results in variation in male mate preference. Thus, males avoid larger females, which have a higher probability of infection by male-killers, and females favour larger males, which have cannibalistic tendencies. Therefore, evaluating the size of a potential mate may cause the variation in body size and mating preferences among *H. axyridis* populations.

SHORTENING THE INSTAR INTERVAL BY SIBLING CANNIBALISM

This study showed that cannibalism shortened the larval and total instar intervals of cannibals, mainly because of shortening of the first instar interval. The total instar interval for male and female cannibals was only 0.432 and 0.125 days shorter, respectively, than for non-cannibals. However, Table 1 shows that this small difference was important for the completion of larval development, because of the food shortage caused by the decrease in aphid density later in the season. High mortality in first and fourth instar larvae was observed in wild populations of *H. axyridis* (Osawa, 1992b; Hironori & Katsuhiro, 1997). This phenomenon may occur because females lay eggs well before aphid populations peak (Hemptinne *et al*., 1992; Osawa, 2000), and aphid consumption by larvae increases drastically in the later stages of development (Okamoto, 1978). Moreover, a high aphid density increases the number of alatae, which can migrate to a secondary host (Robert, 1987). This may cause food shortage for coccinellid larvae during later developmental stages, even if fourth instar larvae have the greatest ability to capture prey among the four larval stages (Dixon, 1959).

All instars in this study were reared with an excess of food; surplus aphids were provided in the Petri dishes. The effect of sibling cannibalism in the first instar may be larger in a natural population, because earlier instar larvae are less adept at capturing prey (Dixon, 1959; Brown, 1972; Kawai, 1978). In a natural population of *H. axyridis*, Osawa (1992a) demonstrated that a first instar cannibal that ate one egg increased its survival rate at a low aphid density by five times compared with an individual at a high aphid density. Furthermore, Kawai (1978) showed that first instar larvae preferred their own eggs to aphids, and that all first instar larvae moulted without feeding on other food when three eggs were provided. These results suggest that (i) sibling eggs in *H. axyridis* may be nutritionally more suitable than aphids for newly hatched larvae; and (ii) sibling eggs may provide additional nutrients during larval development, just like trophic eggs in social insects (Hölldobler & Wilson, 1990; Crespi, 1992). Thus, cannibals in wild populations may gain a greater advantage in survival and development from sibling cannibalism than shown

in this experiment, which demonstrated the higher fitness of cannibals vs. non-cannibals.

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