

## Kin recognition and avoidance of kin cannibalism by the larvae of co-occurring ladybirds: a laboratory study

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**Abstract.** The role of relatedness and diet in kin recognition was investigated in the aphidophagous ladybirds *Propylea dissecta* and *Coccinella transversalis*. Third instar larvae of both species exhibited kin recognition, as they were reluctant to consume related first instar larvae. They spent significantly more time prior to cannibalism when interacting with genetically related victims; this indicates the presence of a kin recognition system. Kin recognition, however, was not evident in fourth instar larvae as they ate both related and unrelated victims. This result reveals that the need of fourth instar larvae to attain a critical weight for pupation might subdue kin recognition. The diet of cannibals did not affect cannibalism, number of encounters or time that elapsed prior to cannibalism. This indicates that kin recognition is not dependent on exogenous cues derived from the diets of the cannibal and victim.

### INTRODUCTION

Kin recognition, the differential treatment of conspecifics that vary in genetic relatedness (Pfennig et al., 1999) is known in many animal taxa (Fletcher & Michener, 1987; Pfennig & Sherman, 1995; Faraji et al., 2000; Bilde & Lubin, 2001). It is seemingly maintained by natural selection, as it can enhance direct (genes contributed to next generation by an individual via reproduction) or indirect (genes contributed to next generation by an individual indirectly by helping non-descendent kin) components of a discriminator's inclusive fitness (Brown, 1987). Little is known about kin recognition in cannibalistic species, but it is predicted that these species should avoid eating their offspring and siblings (Pfennig, 1997).

Cannibalism can be an important survival strategy and has been documented in ten insect orders (Elgar & Crespi, 1992) and probably remains undescribed in many others. It could increase fitness because it provides nourishment and decreases competition for resources among survivors. There are many factors, such as hunger, food availability, food quality, size disparity, degree of relatedness and population density that can affect cannibalism rates in insects, especially in predaceous ladybirds (Coleoptera: Coccinellidae) (Michaud, 2003). Conspecific eggs represent a higher quality nutritional resource for cannibals than conspecific larvae (Michaud, 2003) and are less risky to consume, as they are undefended. Furthermore, sibling larvae have a higher inclusive fitness value than sibling eggs, having completed a greater proportion of their development. Collectively, this means that we should expect to see greater avoidance of sibling larval cannibalism than of sibling egg cannibalism.

Little is known about kin recognition in predaceous ladybirds (Agarwala & Dixon, 1993; Joseph et al., 1999).

Adult females reluctantly consume their own eggs, possibly due to the recognition of chemical odours, whereas males readily eat the eggs they sired (Agarwala & Dixon, 1993). However, the cues responsible for kin recognition among larvae are poorly understood. Ladybird larvae normally indulge in non-sibling egg cannibalism rather than sibling cannibalism (Mills, 1982; Osawa, 1992). Third instar larvae of *Harmonia axyridis* (Pallas) avoided eating relatives and preferentially consumed unrelated conspecifics (Joseph et al., 1999). In contrast, no kin recognition was observed in two Nearctic ladybirds (Michaud, 2003). Avoidance of sibling cannibalism could be beneficial under most circumstances, yet the ability to recognize and avoid cannibalizing relatives varies both within and between species (Pfennig, 1997). Within species, this may be due to differences in the voracity and prey-handling abilities of different predatory stages. It also seems plausible that certain maternal cues might enable kin recognition among larvae and that these cues may be genetically or environmentally influenced. For instance, the chemicals derived from the maternal diet prior to oviposition might be involved in kin recognition among offspring. To better understand the mechanism of kin-recognition, we addressed the following questions. Does the expression of kin recognition vary with larval stage? What are the possible cues? Can the diet of a victim affect its susceptibility to cannibalism?

The experiments were designed using two co-occurring ladybirds, *Coccinella transversalis* Fabricius and *Propylea dissecta* (Mulsant). These Oriental ladybirds are aphidophagous but also prey on a variety of other taxa (Omkar & James, 2004; Omkar & Pervez, 2004; Pervez & Omkar, 2004).

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## MATERIAL AND METHODS

### Stock colonies

Adults of *C. transversalis* and *P. dissecta* were collected from local fields of *Raphanus sativus* (radish), where they were preying on aphids, *Lipaphis erysimi* and used to establish a laboratory colony. The stock cultures were maintained by keeping pairs of adults in Petri dishes (9.0 × 2.0 cm) and feeding them on *L. erysimi* ad libitum on the above host at 27 ± 1°C, 65 ± 5% R.H. and 12L: 12D. Eggs were removed daily and the larvae were reared separately on the above food in glass beakers (9.0 × 11.0 cm) until they emerged as adults.

### Experimental design

Endogenous cues were manipulated by presenting sib and non-sib victims to 12 h starved third and fourth instar larvae (cannibals) reared on *L. erysimi*. The food provided to the cannibals and victims was the same (*L. erysimi*). Incidence of cannibalism was observed by placing one 12 h starved third or fourth instar larva with one first instar (sib or non-sib) larva in a Petri dish (1.5 × 9.0 cm). The observations lasted for a period of 30 min or until cannibalism occurred. Replicates in which no contact occurred between larvae were excluded from the analysis. We recorded the number of encounters between the larvae and the time to cannibalism in each replicate. The percentage of encounters resulting in cannibalism (= number of larvae cannibalized × 100 / number of encounters between larvae) was then calculated for each treatment. The experiment was replicated twenty times with both *C. transversalis* and *P. dissecta*.

The effect of food (prey) was studied by splitting clutches of *C. transversalis* and *P. dissecta* eggs into two. The neonates hatching from one half of each cluster were provided with an optimal diet (*L. erysimi*) and those of the other half with a relatively sub-optimal diet [i.e. *Myzus persicae* (Sulzer) on *Solanum nigrum* (Omkar & James, 2004; Pervez & Omkar, 2004)]. These larvae were reared to third or fourth instar and used as potential cannibals. Candidate victims were first instar larvae obtained from the same mother as the potential cannibal and fed *Myzus persicae* for 24 h. This generated two treatments: cannibals presented with sibling victims raised on the same diet, and cannibals presented with sibling victims raised on a different diet. Twelve hour starved cannibals and victims were confined together for 30 min under the same conditions as described above and there were twenty replicates of each treatment.

Percentage cannibalism was compared between treatments using a  $\chi^2$  Goodness-of-fit test (MINITAB, 2000). The data on numbers of encounters and time to cannibalism were subjected to one-way ANOVA on MINITAB (2000). The effect of species, cannibal stage and kin-treatment (kin v/s non-kin) on kin recognition was analyzed using a three-way ANOVA on statistical software (SAS, 2002) with ladybird species (two levels), cannibal life stage (two levels) and kin-treatment (two levels) as independent variables, and number of encounters and time to cannibalism as dependent variables. The effect of species, cannibal stage and diet (same v/s different) on kin recognition was also analyzed using a three-way ANOVA (SAS, 2002) with ladybird species (two levels), cannibal life stage (two levels) and diet treatment (two levels) as independent variables, and number of encounters and time to cannibalism as dependent variables. Prior to Three-way ANOVA, the data on number of encounters and time to cannibalism were confirmed for the assumptions of normality of distribution and equality of variance using Levene's Test (MINITAB, 2000) and found to be normally distributed.

## RESULTS

### Cannibalism by third instar larvae

The incidence of cannibalism by third instar larvae of both species was higher when they were presented with non-sib first instar larvae as potential victims than when presented with sibs (*P. dissecta*:  $\chi^2 = 50.51$ ;  $P < 0.0001$ ; d.f. = 1; *C. transversalis*:  $\chi^2 = 35.17$ ;  $P < 0.0001$ ; d.f. = 1; Fig. 1). However, cannibalism was not affected by their diet (*P. dissecta*:  $\chi^2 = 0.51$ ;  $P > 0.1$ ; d.f. = 1; *C. transversalis*:  $\chi^2 = 0.50$ ;  $P > 0.1$ ; d.f. = 1; Fig. 2). Cannibalism occurred sooner between non-sibs of *P. dissecta* than between sibs ( $F = 98.40$ ;  $P < 0.001$ ; d.f. = 1, 17) and required significantly fewer encounters between larvae ( $F = 44.47$ ;  $P < 0.001$ ; d.f. = 1, 17). Similarly, the number of encounters between *C. transversalis* larvae ( $F = 11.79$ ;  $P < 0.01$ ; d.f. = 1, 9; one-way ANOVA; Fig. 1) and time to cannibalism ( $F = 13.59$ ;  $P < 0.01$ ; d.f. = 1, 17; one-way ANOVA) were greater when larvae were related.

The number of encounters between larvae did not vary as a function of diet for either species (*P. dissecta*:  $F = 1.29$ ;  $P > 0.1$ ; d.f. = 1, 16; *C. transversalis*:  $F = 4.05$ ;  $P > 0.1$ ; d.f. = 1, 17; Fig. 2) but the mean time to cannibalism was significantly affected when larvae were fed different diets (*P. dissecta*:  $F = 8.63$ ;  $P < 0.01$ ; d.f. = 1, 16; *C. transversalis*:  $F = 4.96$ ;  $P < 0.05$ ; d.f. = 1, 17).

### Cannibalism by fourth instar larvae

Cannibalism by fourth instar larvae of *C. transversalis* was more marked when they were confined with non-sib compared with sib first instars ( $\chi^2 = 16.21$ ;  $P < 0.0001$ ; d.f. = 1; Fig. 3) but not in the case of fourth instar larvae of *P. dissecta* ( $\chi^2 = 1.80$ ;  $P > 0.1$ ; d.f. = 1). Cannibalism occurred at similar rates in both species whether cannibals and victims were fed on the same or different diets (*C. transversalis*:  $\chi^2 = 0.87$ ;  $P > 0.1$ ; d.f. = 1; *P. dissecta*:  $\chi^2 = 1.14$ ;  $P > 0.1$ ; d.f. = 1; Fig. 4).

Relatedness did not affect the number of encounters between *P. dissecta* larvae ( $F = 0.09$ ;  $P > 0.1$ ; d.f. = 1, 36; Fig. 3) or the mean time to cannibalism ( $F = 0.79$ ;  $P > 0.1$ ; d.f. = 1, 36). However, significantly more encounters occurred between related than unrelated *C. transversalis* larvae ( $F = 27.15$ ;  $P < 0.001$ ; d.f. = 1, 35; Fig. 3), but the mean-time to cannibalism did not vary ( $F = 2.90$ ;  $P > 0.05$ ; d.f. = 1, 35).

Rearing *C. transversalis* larvae on different diets significantly increased the number of encounters between larvae ( $F = 6.25$ ;  $P < 0.01$ ; d.f. = 1, 31; Fig. 4) and reduced mean-time to cannibalism ( $F = 4.88$ ;  $P < 0.05$ ; d.f. = 1, 31) relative to pairs of larvae fed on the same diet. However, no such differences were evident for *P. dissecta* (number of encounters:  $F = 0.21$ ;  $P > 0.1$ ; d.f. = 1, 31; time to cannibalism:  $F = 0.43$ ;  $P > 0.1$ ; d.f. = 1, 31).

### Kin treatment

The three-way ANOVA revealed a significant main effect of "larval stage" ( $F = 184.64$ ;  $P < 0.0001$ ; d.f. = 1) and "kin-treatment" ( $F = 104.22$ ;  $P < 0.0001$ ; d.f. = 1) on the number of encounters. The main effect of "ladybird species" was not significant ( $F = 0.19$ ;  $P > 0.1$ ; d.f. = 1).

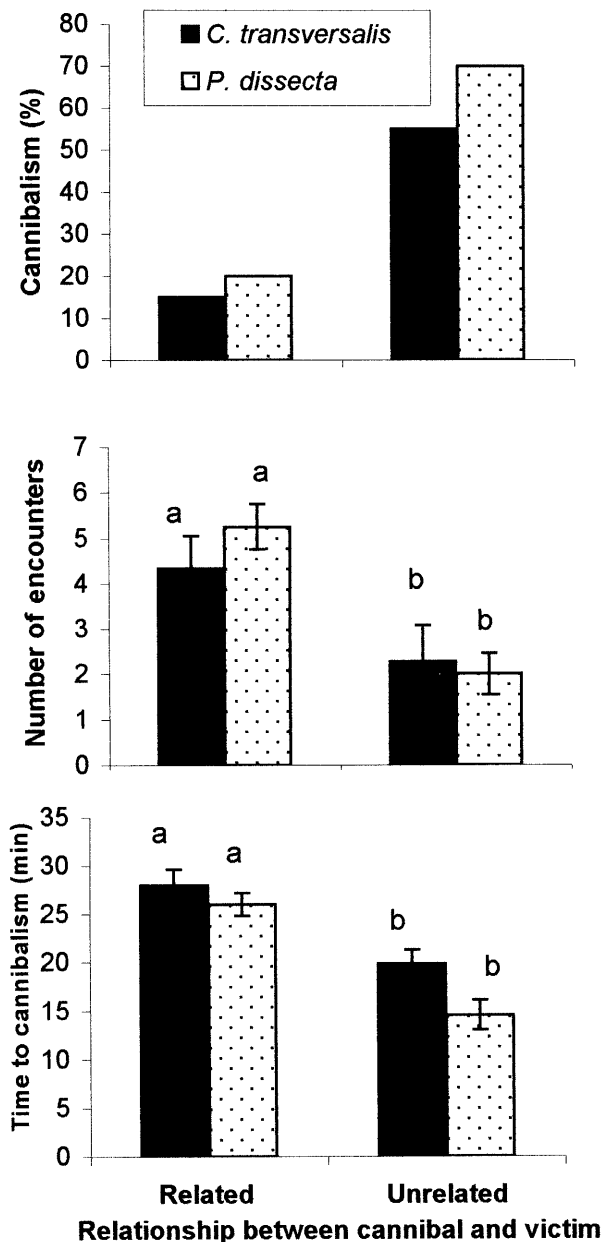


Fig. 1

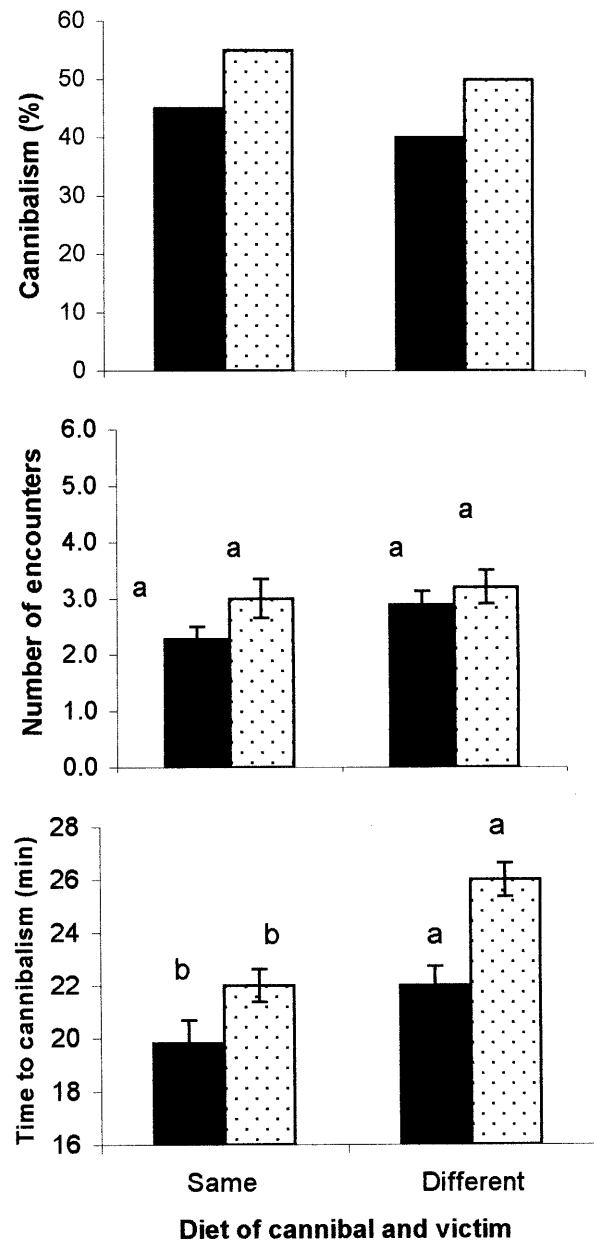


Fig. 2

Figs 1–2. 1 – Percentage cannibalism, mean ( $\pm$  SEM) number of encounters and mean ( $\pm$  SEM) time to cannibalism by third instar larvae of *Coccinella transversalis* and *Propylea dissecta* when confined with related and unrelated first instar conspecifics. Bars bearing the same letter were not significantly different within a species. 2 – Percentage cannibalism, mean ( $\pm$  SEM) number of encounters and mean ( $\pm$  SEM) time to cannibalism by third instar larvae of *Coccinella transversalis* and *Propylea dissecta* when confined with first instar conspecifics fed on the same or different diets. Bars bearing the same letter were not significantly different within a species.

Significant interactions were found between “larval stage” and “kin-treatment” ( $F = 58.20$ ;  $P < 0.0001$ ; d.f. = 1), and “ladybird species” and “larval stage” ( $F = 6.77$ ;  $P < 0.01$ ; d.f. = 1), while that between “ladybird species” and “kin-treatment” was not significant ( $F = 0.15$ ;  $P > 0.1$ ; d.f. = 1). In terms of the number of encounters the interaction between “ladybird species”, “stage” and “kin-treatment” was significant ( $F = 13.05$ ;  $P < 0.001$ ; d.f. = 1).

Significant main effects of “ladybird species” ( $F = 47.80$ ;  $P < 0.0001$ ; d.f. = 1), “larval stage” ( $F = 932.97$ ;  $P < 0.0001$ ; d.f. = 1) and “kin-treatment” ( $F = 75.00$ ;  $P < 0.0001$ ; d.f. = 1) were found on time to cannibalism. The interactions between “larval stage” and “kin-treatment” ( $F = 106.26$ ;  $P < 0.0001$ ; d.f. = 1) and “ladybird species” and “kin-treatment” ( $F = 4.29$ ;  $P < 0.05$ ; d.f. = 1) were significant. However, interaction between “ladybird species” and “larval stage” ( $F = 0.01$ ;  $P > 0.1$ ; d.f. = 1) was not

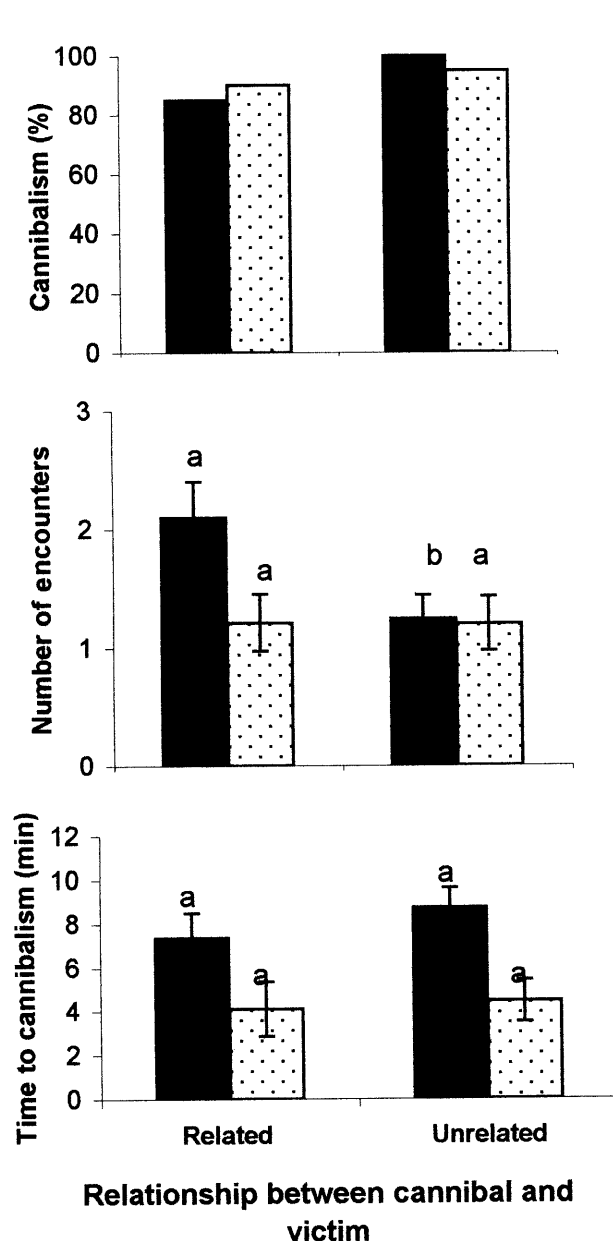


Fig. 3

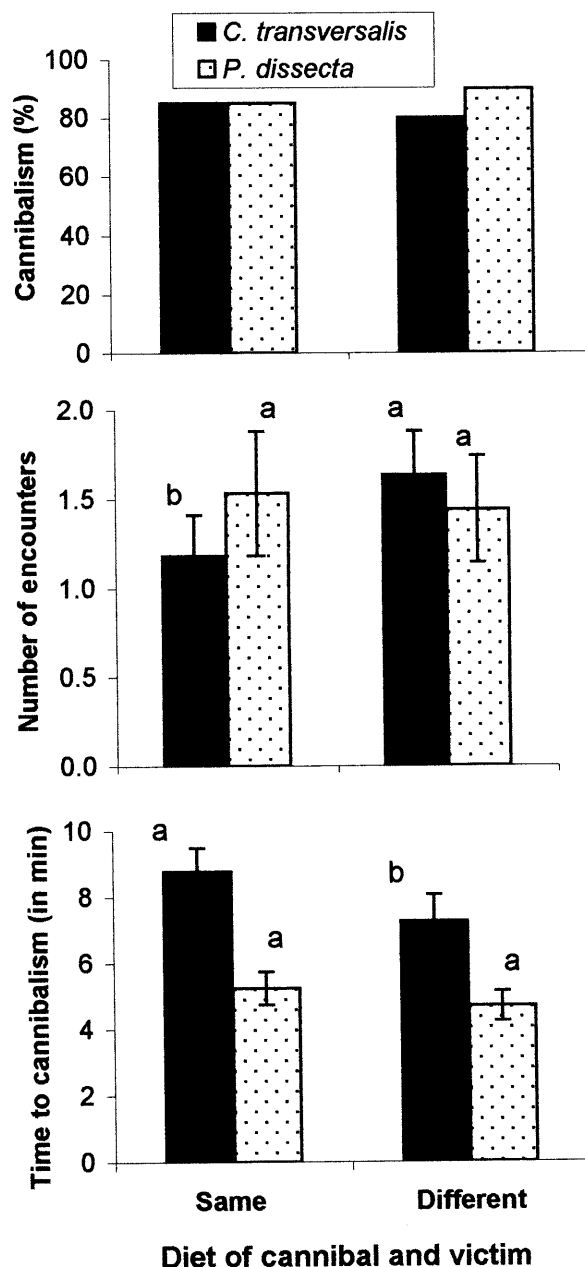


Fig. 4

Figs 3–4. 3 – Percentage cannibalism, mean ( $\pm$  SEM) number of encounters and mean ( $\pm$  SEM) time to cannibalism by fourth instar larvae of *Coccinella transversalis* and *Propylea dissecta* when confined with related and unrelated first instars conspecifics. Bars bearing the same letter were not significantly different within a species. 4 – Percentage cannibalism, mean ( $\pm$  SEM) number of encounters and mean ( $\pm$  SEM) time to cannibalism by fourth instar larvae of *Coccinella transversalis* and *Propylea dissecta* when confined with first instars conspecifics fed on the same or different diets. Bars bearing the same letter were not significantly different within a species.

significant. The interaction between “ladybird species”, “stage” and “kin-treatment” was significant ( $F = 4.21$ ;  $P < 0.05$ ; d.f. = 1).

#### Diet treatment

The three-way ANOVA revealed a significant main effect of “larval stage” ( $F = 246.68$ ;  $P < 0.0001$ ; d.f. = 1) on the number of encounters. The main effects of “ladybird species” ( $F = 0.08$ ;  $P > 0.1$ ; d.f. = 1) and “diet” ( $F = 0.00$ ;  $P > 0.1$ ; d.f. = 1) were not significant. There were

significant interactions between “larval stage” and “diet” ( $F = 7.77$ ;  $P < 0.01$ ; d.f. = 1), and “ladybird species” and “larval stage” ( $F = 11.68$ ;  $P < 0.01$ ; d.f. = 1), however the interaction between “ladybird species” and “diet” was not significant ( $F = 0.12$ ;  $P > 0.1$ ; d.f. = 1). The interaction between “ladybird species”, “stage” and “diet” was also not significant ( $F = 3.10$ ;  $P > 0.05$ ; d.f. = 1).

Significant main effects of “ladybird species” ( $F = 29.45$ ;  $P < 0.0001$ ; d.f. = 1) and “larval stage” ( $F =$

246.68;  $P < 0.0001$ ; d.f. = 1) were found on time to cannibalism. However, the main effect of “diet” was not significant ( $F = 0.25$ ;  $P > 0.1$ ; d.f. = 1). The interactions between “ladybird species” and “diet” ( $F = 7.78$ ;  $P < 0.01$ ; d.f. = 1), “larval stage” and “diet” ( $F = 21.15$ ;  $P < 0.0001$ ; d.f. = 1) and “ladybird species” and “larval stage” ( $F = 90.67$ ;  $P < 0.0001$ ; d.f. = 1) were significant. However, the interaction between “ladybird species”, “larval stage” and “diet” was not significant ( $F = 2.51$ ;  $P > 0.1$ ; d.f. = 1).

## DISCUSSION

The results revealed that third instar larvae of both *P. dissecta* and *C. transversalis* were more likely to eat non-sib than sib conspecific larvae in no choice situations, which indicates a reluctance to cannibalize sibs perhaps suggesting kin recognition. A similar kin recognition mechanism is reported in *H. axyridis* (Joseph et al., 1999). In the present study, there was a significant main effect of “kin-treatment” on the number of encounters and time to cannibalism. This suggests that the larvae responded differently towards sib and non-sib victims in no choice situations, which resulted in an increase in the number of encounters and an increase in the time to cannibalism. The main effect of “ladybird species” on the number of encounters in the kin-treatment analysis was not significant, which reveals a similar behavioural response in terms of cannibalism and kin recognition by the two ladybird species. The neonates of these two species responded similarly by eating greater number of conspecific eggs when provided with both conspecific and heterospecific eggs (Omkar et al., 2004).

Larval stage had a significant main effect on number of encounters and time to cannibalism in the kin treatment, which reveals that avoidance of kin cannibalism was larval stage dependent with third instars of both species being more reluctant to consume their sibs. They committed fewer acts of cannibalism on siblings than on unrelated larvae revealing the existence of a kin recognition mechanism. However, the effect of kin recognition was less marked in fourth instar larvae, possibly because of increased food demand prior to pupation. Fourth instar larvae possibly place a higher priority on food acquisition than avoiding sibling cannibalism. The differential responses of third and fourth instar larvae towards their sibs also might be due to the greater size disparity between cannibal and victim. The degree of starvation after 12 h of starvation could have been greater for fourth than for third instar larvae, which could also account for the observed differences. Difference in the incidence of kin cannibalism by third and fourth instar larvae could also be due to the enormous difference in their voracity.

The insignificant main effect of “diet” on the number of encounters and time to cannibalism suggests that diet of cannibal and victim did not affect the cannibalistic tendency of the ladybird larvae. Thus, instead of diet generating an exogenous cue, certain other factors might be involved, which facilitate distinct kin recognition by third instar larvae. These cues might be intraspecific chemical

odours, which have direct or indirect genetic foundations (Roberts et al., 2003). Chemically mediated kin recognition is reported in spiders (Miller, 1989; Evans, 1999). Cannibalism did not occur more or less frequently when the larvae were fed the same diet compared to different diets, suggesting that diet has no role as an exogenous cue for detecting kinship. Nevertheless, some paper wasps use environmentally derived cues for kin recognition (Pfennig, 2002). Consistency of kin recognition cues across clutches would be increased if genetically based markers were maternally derived rather than expressed in the offspring (Moore et al., 1997). There is also a possibility that larvae could learn the chemical recognition factor upon eclosion and contact with each other, and that it is independent of maternally derived cues.

Little is published on cannibalism in *C. transversalis* and *P. dissecta* (Agarwala & Yasuda, 2001; Omkar et al., 2004). The present study suggests a parallel evolution between these two coexisting ladybirds with respect to larval responses towards sibs and non-sibs. There exists kin recognition in these ladybirds; however, its intensity varies depending on instar. It can be concluded that: (i) third instar larvae of *C. transversalis* and *P. dissecta* are able to recognize their kin, (ii) the tendency to avoid eating kin may diminish in the last larval instar when the demand for food is high, and (iii) diet appears to have no direct effect on kin-recognition, however it might have an indirect effect.

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