

# Cannibalizing *Harmonia axyridis* (Coleoptera: Coccinellidae) larvae use endogenous cues to avoid eating relatives

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## Abstract

Cannibalism is widespread among many different organisms, and can have both negative and positive fitness consequences. Avoiding eating relatives can minimize negative fitness consequences of cannibalism. Such avoidance requires kin discrimination, but evidence for this ability among cannibals is limited with little data that address the cues used in such discrimination. We examined whether larvae of the ladybird beetle *Harmonia axyridis* avoid eating their relatives. We further manipulated environmental factors to create individuals that had similar or dissimilar rearing environments to begin to test for endogenous versus exogenous recognition cues. In our experiments, third-instar larvae were much less likely to cannibalize if they were interacting with a relative. Larvae that did cannibalize kin required more encounters, and significantly delayed cannibalism, compared to larvae cannibalizing unrelated individuals. Acquired cues were less important. Even though the different rearing environment resulted in significantly different phenotypic effects, similarity or dissimilarity of rearing environment had no effect on cannibalism, and there was no interaction between environments and relatedness in cannibalism. We suggest that *H. axyridis* has a well-developed kin discrimination system, and that kin recognition in this ladybird beetle is based on endogenous rather than exogenous cues. We also argue that these cues reflect either direct or indirect genetic effects on larval phenotypes.

## Introduction

Cannibalism has been observed in species ranging from *Tribolium* beetles to humans and can have wide ranging effects both for individuals and for populations (Polis, 1981; Elgar & Crespi, 1992). Most humans find cannibalism socially unacceptable and this contributes, perhaps, to the initial assumption that cannibalism is a maladaptive behaviour (Dawkins, 1976; Arnqvist & Henriksson, 1997). However, preying on conspecifics can have evolutionary benefits such as added nourishment and decreased competition for resources (Polis, 1981; Elgar & Crespi, 1992; Pfennig, 1997). There are also potential costs to cannibalism: individuals risk injury or increase their chance of acquiring parasites when they

cannibalize (Polis, 1981; Elgar & Crespi, 1992; Pfennig, 1997). Also, cannibalism may carry costs if individuals live in populations where encounters with relatives occur more often than expected by chance alone; for example, populations subdivided into structured demes or populations where relatives live in social groups. In such structured populations there may be inclusive fitness costs if cannibalism is indiscriminate (Pfennig, 1997).

Avoiding eating a relative may be beneficial under most circumstances, yet the ability to recognize and avoid cannibalizing relatives varies both within and between species (Pfennig, 1997). For example, in spadefoot toads (*Scaphiopus bombifrons*), cannibal morphs avoid associating with kin and preferentially eat unrelated individuals when they do cannibalize (Pfennig *et al.*, 1993). In tiger salamanders (*Ambystoma tigrinum nebulosum*), both cannibal and noncannibal morphs cannibalize, but the cannibal morphs have better developed kin discrimina-

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tion abilities (Pfennig *et al.*, 1994). In insects, egg cannibalism is common but is not always associated with kin discrimination. Larvae of the imported leaf beetle (*Plagioderma versicolora*) eat eggs, but there is no evidence that these beetles avoid eating relatives (Breden & Wade, 1989). Instead, the benefits of cannibalism appear to outweigh any disadvantages of cannibals eating siblings (Breden & Wade, 1989). In contrast, flour beetles (*Tribolium confusium*) appear to gain a fitness advantage when they avoid eating siblings. Evidence for this comes from studies that demonstrate that populations quickly evolve towards lower levels of cannibalism if cannibals are fed related rather than unrelated eggs (Wade, 1980). In the ladybird beetle *Adalia bipunctata*, the life history stages and sexes differ in kin discrimination. Adult females and second instars avoid eating their own or sibling eggs, but adult males do not avoid eating eggs they fathered (Agarwala & Dixon, 1993). Thus, kin discrimination abilities can exist, but are not universal among cannibalistic species.

One of the factors important in the evolution of kin discrimination is the presence of a reliable cue that allows individuals to discriminate kin from nonkin (Grafen, 1990; Sherman *et al.*, 1997). Such cues can be derived from internal sources (endogenous – genetically influenced or passed from mother to offspring) or external sources (exogenous – acquired or environmental) (see Sherman *et al.*, 1997). Responses to either form of cue can only evolve if these cues are consistent from generation to generation, have low error rates, and allow the greatest dissimilarity among individuals (Sherman *et al.*, 1997). Thus, the type of cue that is likely to be used will depend on both the presence of sufficient genetic variation and the population ecology of the species.

Although we currently have no information on the type of cues used by cannibals to discriminate kin from nonkin, manipulating both exogenous and endogenous factors should facilitate the detection of kin discrimination within the context of cannibalism. Both exogenous and endogenous kin recognition cues have been shown to exist under contexts other than cannibalism, and the type of cue appears to depend on the ecology of the species studied. In social insects, where nestmate recognition may be important for kin selection and the environment (nest or hive) is predictable because of social living, recognition cues have been shown to be exogenous. Cues are acquired from nest material by paper wasps (Gamboa *et al.*, 1986) and comb wax by honey bees (Breed *et al.*, 1995). Genetic factors influencing variation among these cues may still be important in both taxa (Gamboa *et al.*, 1986; Breed *et al.*, 1995). However, the increased variability of the exogenous cue and the predictability of the association between kin and environment may facilitate the use of exogenous cues in these species. In contrast, larval tunicates (*Botryllus schlosseri*) live in potentially much more unpredictable associations, and are able to settle nonrandomly

with respect to relatedness based on an endogenous cue, a single histocompatibility locus (Grosberg & Quinn, 1986).

To document kin discrimination by cannibals, and whether the cue used is exogenous or endogenous, we studied intraspecific predation by the ladybird beetle *H. axyridis*. Both larvae and adults of this ladybird are highly cannibalistic (Osawa, 1989; Hodek & Honek, 1996; Wagner *et al.*, 1999; Snyder *et al.*, in preparation). In this study we allowed larvae the opportunity to cannibalize other larvae based on either genetic relatedness (endogenous cues) or similarity of food environment (exogenous cues – same or different food). Our experimental design allowed us maximum control over the similarity or dissimilarity of environments because potential cannibals and victims were from different clutches, so that individuals never shared a common environment. Similarity was thus due to the factors we provided (food) or relatedness (including common but not shared maternal effects).

## Methods

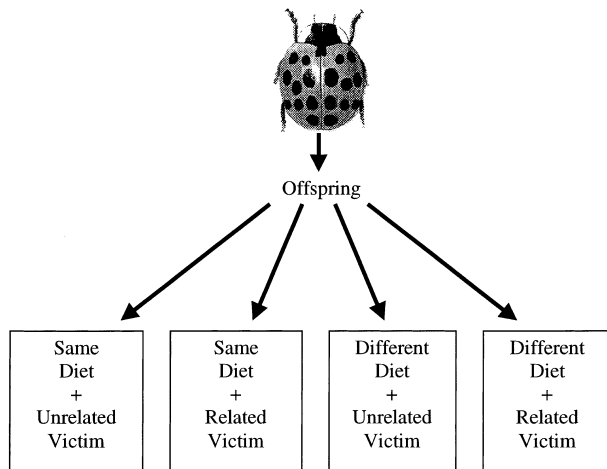
### Experimental design and husbandry

We simultaneously manipulated both the rearing environment and the relatedness during potential cannibalism events by larvae of the ladybird, *H. axyridis*. In addition to facilitating the detection of specific types of cues used, manipulating both cue types in a completely crossed design allows us to examine whether there was an interaction between cue types (Fig. 1).

Larvae for our experiments represented offspring from field-caught adults. Female *H. axyridis* were collected at the University of Kentucky Research Farm (Lexington, KY, USA) during April and May 1998. These females were reared individually in 100-mm Petri dishes with moist cotton wicks and fed a diet of *ad libitum* *Trichoplusia ni* larvae. The *T. ni* were collected as fifth instar larvae and frozen prior to our experiments. These larvae were then thawed and chopped into small pieces prior to being provided as food. All Petri dishes were checked daily for eggs. As soon as females oviposited, they were moved to another Petri dish with *ad libitum* *T. ni* larvae and water. Within 24 h of hatching, larvae were separated and placed singly in Petri dishes with water wicks and *ad libitum* *T. ni* larvae. Cannibalism prior to separation and isolation was not observed.

### Manipulation of exogenous cues

We manipulated the food resources available to larvae because this is a relevant factor that is likely to vary in nature. *Harmonia axyridis* forage on ephemeral resources, and the availability of a particular food may vary from day to day (Hodek & Honek, 1996). This ladybird is also extremely polyphagous, foraging primarily on aphids and



**Fig. 1** Experimental design. Clutches from individual females were split into both different (mix of *T. ni* + *A. nerii*) or same (only *T. ni*) food treatments and different cannibalism trials (related, unrelated). Relatives were derived from later clutches from the same female. Clutches were from field-caught females who may have been multiply mated (Osawa, 1994) and therefore may have been mixed full- and half-sib.

any other invertebrate as soft or softer than it is (Hodek & Honek, 1996). In Kentucky, the larvae of *H. axyridis* have been observed feeding on the eggs and larvae of lepidoptera and the eggs and larvae of other ladybird species (Cottrell & Yeargan, 1998a,b; Cottrell, in preparation).

Female *H. axyridis* lay eggs on a variety of host plants, and females lay multiple clutches over several days. Furthermore, multiple females lay eggs near the same host. Thus, larvae in nature encounter other larvae of various stages that may have foraged on the same or different prey.

To manipulate exogenous cues, we split clutches and provided half with one food treatment and half with a different food treatment. Other differences (e.g. specific environmental effects associated with Petri dishes or water wicks) represent random effects across individuals with a mean effect of zero. As a result, the only source of consistent and predictable exogenous cues in our experiments was food type.

All larvae from the first clutch laid by a female were designated to be potential cannibals. Twenty-four hours after hatching, these larvae were separated into two food treatments having known phenotypic effects. Half of the larvae were provided *ad libitum* *T. ni* (a high-quality diet), while the other half were provided *ad libitum* *Aphis nerii* combined with *ad libitum* *T. ni* (a low-quality diet). Larvae in both food treatments were observed eating the designated food and all individuals were reared to the third-instar on that food. In contrast, all potential victims were taken from second and later clutches, and reared to first-instar with *T. ni* as the only food provided. Thus,

potential cannibals and potential victims were raised on the same or different food types from each other, and differed in instar.

### Manipulation of endogenous cues

We chose to examine individuals of different instars and relatedness to mimic the variation in endogenous conditions found in nature. Larvae forage widely within a host plant. Because females lay several clutches over several days, in nature individuals are likely to encounter other larvae from different clutches that vary in size and level of relatedness. Furthermore, females can mate multiply (Osawa, 1994), and within clutches individuals may vary from full to half sibs.

In order to manipulate the endogenous cues (relatedness), larvae from the two food treatments were further divided, with half of the larvae in each treatment offered a first-instar sibling as a potential victim and the other half offered an unrelated first-instar conspecific as a potential victim. Because the related and unrelated third-instar individuals could share the same or different rearing environments, our design also allows us to examine interactions between these two.

Larvae from the first clutch laid by a female were reared under the above food regimes and designated potential cannibals. Subsequent clutches from that same female were used as potential victims, and offered to either related or unrelated third-instars. All clutches from a given female were laid in different Petri dishes so that potential cannibals and potential victims never encountered each other prior to a trial.

### Cannibalism trials

We carried out cannibalism trials by placing one third and one first-instar larva into a neutral arena (35 × 10-mm Petri dish). All trials were continuously observed for 30 min or until cannibalism occurred. Only trials in which the two larvae encountered one another and interacted (touched) were included in the analysis. Encounters were scored as cannibalism if the first-instar larva was killed and eaten. Cannibalism of third-instars by first-instars never occurred.

### Statistical methods

Statistical tests were performed using *SYSTAT*. All data were checked for assumptions of normality, and either transformed or significance determined by randomization if the distributions were non-normal. Significant associations between categorical variables were examined using the log-linear models in *SYSTAT* (see Wilkinson *et al.*, 1996, for a comparison to other log-linear models), which allows us to examine interaction effects as well as main effects. *ANOVA*, using *GLM* in *SYSTAT*, was

used for other tests. All tests of significance were two-tailed except where otherwise noted when we had *a-priori* expectations regarding the direction of the hypothesis tested.

Before all trials, sizes of all larvae were determined from pronotum widths. These sizes were measured using computerized image analysis and NIH Image software (Grill *et al.*, 1997).

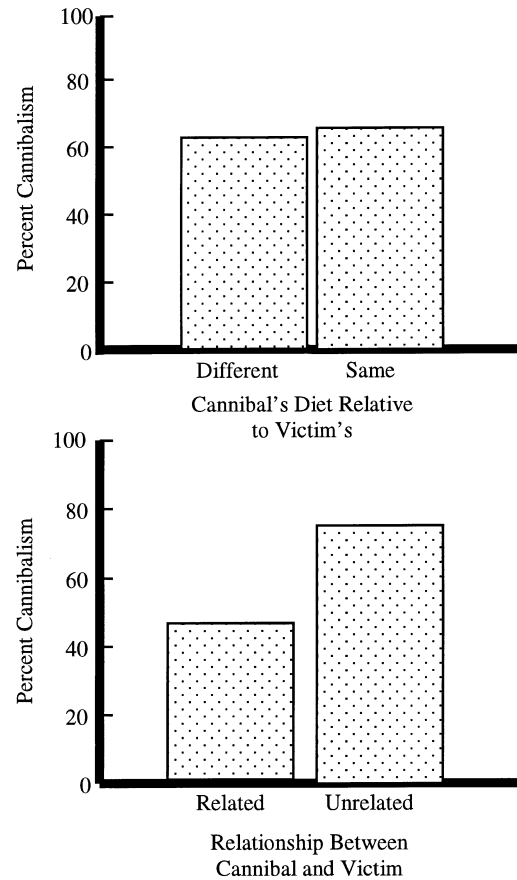
## Results

We ran a total of 101 trials, of which 83 resulted in contact between individuals, and 52 of our 83 trials resulted in cannibalism. In most of the trials where individuals never interacted, the larger individual appeared to be preparing to moult.

Our diet treatment had significant effects on the phenotypes of individuals. Individuals reared on *T. ni* grew to a larger size at third-instar (mean width of pronotum = 1.28 mm  $\pm$  0.02,  $n$  = 34) than did individuals reared on a mixed diet of *T. ni* plus *Aphis nerii* ( $\bar{x}$  = 1.22 mm  $\pm$  0.02,  $n$  = 47;  $F$  = 5.545, d.f. = 1,79,  $P$  = 0.021). Although the different diets affected the size of the individuals, there was no effect of relative size difference between the cannibal and its victim on rate of cannibalism ( $F$  = 0.013, d.f. = 1,76,  $P$  = 0.909). Absolute size also did not influence rate of cannibalism. Larger individuals were no less likely to cannibalize than were smaller individuals ( $F$  = 1.739, d.f. = 1,78,  $P$  = 0.191).

Relatedness was the sole factor that affected cannibalism with unrelated individuals being cannibalized nearly twice as often as related individuals (Fig. 2a;  $\ln(\text{MLE})$  = -24.736,  $\chi^2$  = 8.94, d.f. = 1,  $P$  = 0.0028). The rate of cannibalism did not depend on the diet of the cannibal being the same or different than the diet of the victim (Fig. 2b; loglinear analysis  $\ln(\text{MLE})$  = -20.414,  $\chi^2$  = 0.30, d.f. = 1,  $P$  = 0.583). Likewise, the preferential cannibalism of unrelated over related victims was not influenced by developing on the same or different diets (test for diet-relatedness interaction;  $\ln(\text{MLE})$  = -20.994,  $\chi^2$  = 1.46, d.f. = 1,  $P$  = 0.2266). In a hierarchical loglinear analysis, only removal of the effects of relatedness on cannibalism was significant ( $\ln(\text{MLE})$  = -25.467,  $\chi^2$  = 10.41, d.f. = 2,  $P$  = 0.0055).

Cannibalism occurred more quickly when a cannibal encountered an unrelated rather than a related individual. Third-instar larvae required significantly more encounters with relatives than with unrelated individuals before engaging in cannibalism (Fig. 3a; one-tailed ANOVA;  $F$  = 3.214, d.f. = 1,50,  $P$  = 0.0395; significance determined by 10 000 randomizations). It also took significantly longer for larvae to begin to cannibalize relatives (time from first contact until cannibalism commenced) compared with the time it took for nonrelatives to become victims (Fig. 3b; one-tailed ANOVA;

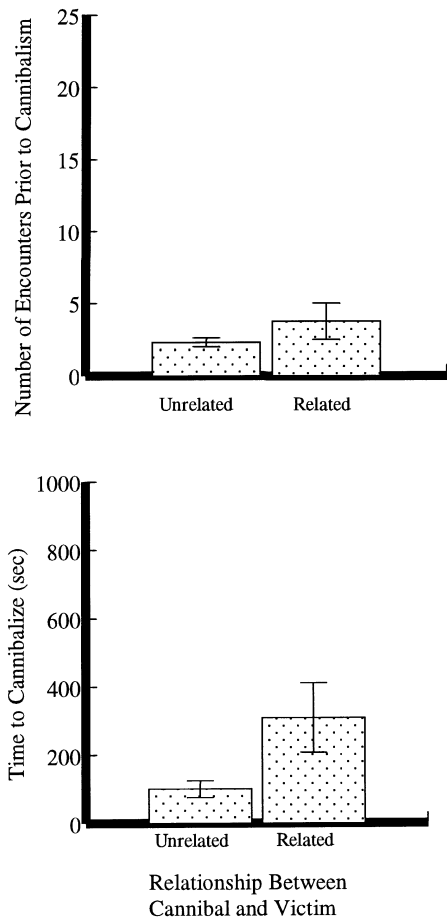


**Fig. 2** The effects of food or relatedness on cannibalism. In contrast, rearing on the same or different food type had no effect on cannibalism (a) even though there was a significant effect of food on growth. There is a significant difference in the probability of a relative being cannibalized compared with the cannibalism of unrelated individuals (b). Percentages based on 83 trials where there was an opportunity for cannibalism.

$F$  = 7.070, d.f. = 1,50,  $P$  = 0.0045; significance determined by 10 000 randomizations).

## Discussion

Our study shows that third-instar larvae are less likely to cannibalize relatives than nonrelatives. Related victims required more encounters prior to cannibalism than did nonrelatives and nonrelatives were cannibalized more quickly. Furthermore, experimentally manipulating diet so that the cannibal and victim were reared on the same or different foods yielded no difference in the likelihood of cannibalism. Within the size classes that we presented as potential prey, relative difference in size between the cannibal and victim did not influence rate of cannibalism. Absolute size of the predator also did not influence tendency to cannibalize, as larger individuals were no



**Fig. 3** The differences in encounter rate (a) and latency to cannibalize after first contact (b) as a function of interacting with related or unrelated larvae. There were significantly more encounters of relatives prior to cannibalism, and cannibalism was significantly delayed when interacting with a relative compared with interactions with unrelated larvae.

more likely to eat conspecifics than were smaller individuals.

In *H. axyridis*, propensity to cannibalize varies among families and is heritable (Wagner *et al.*, 1999). Given a higher than average likelihood of encountering relatives, cannibalistic families could be at a disadvantage compared with noncannibalistic families if individuals are indiscriminate in whom they eat. There are fitness benefits to cannibalism – increased development rate and survivorship under reduced quantity (Wagner *et al.*, 1999) or quality (Snyder *et al.*, in preparation) of food – but the fitness benefits gained under poor quality or quantity of food are small compared with the inclusive fitness costs of eating relatives. Thus, the presence of kin discrimination abilities is likely to be adaptive for *H. axyridis* larvae.

Kin discrimination by *H. axyridis* appears to be based on endogenous rather than exogenous cues. Cannibals and

victims were derived from different clutches, even when related, in order to prevent kin recognition based on familiarity. Individuals were also isolated from their clutchmates and reared individually. Thus the major source of predictable environmental cues was the food they were provided. Cannibal diet was manipulated so that it was identical or different from the first-instar victim. Any cues that were acquired from the food had no significant effect on the cannibal's ability to detect kinship.

Indirect evidence suggests that the endogenous cue in *H. axyridis* (probably cuticular odour) is based on genetic differences among individuals. Relative size, to the extent that related individuals are more similar in size than unrelated individuals, could be an endogenous cue. However, because size of larvae is very dependent upon diet (Preziosi *et al.*, in press; Snyder *et al.*, in preparation), relative size would be a nongenetic endogenous cue. In our study size played no role in victim selection. Another environmentally based endogenous cue is the egg environment (i.e. a maternally provided cue). Environmental effects provided by the mother would be predictable within, but not necessarily across, clutches. Predictability across clutches would be increased if the maternal effect were genetically influenced, e.g. through an indirect genetic effect (i.e. variation reflecting genes expressed in the different mother rather than in the offspring; Moore *et al.*, 1997). Although we did not manipulate maternal diet, and therefore cannot rule out an environmental maternal effect, in our study the cue was reliable across clutches laid several days apart. We therefore suggest that either direct genetic effects (genes expressed in the offspring) or indirect genetic effects (genes expressed in the different mothers that affect the offspring phenotype) play a central role in kin recognition cues in *H. axyridis*.

A remaining question is why endogenous rather than exogenous cues should evolve. It seems unlikely that the use of endogenous cues is required for the expression of kin recognition in the context of cannibalism. Rather, we suggest that predictability of environments is more important in determining whether individuals will evolve kin recognition based on endogenous or exogenous cues. This may be true even when the proximate basis is similar for both endogenous and exogenous cues (e.g. cuticular odours). Colonizing species or species that disperse widely, such as these ladybirds or tunicates, may need to evolve endogenous cues. In addition, extreme polyphagy such as that practised by *H. axyridis*, can result in foraging individuals existing in a series of microenvironments that are spatiotemporally unpredictable making exogenous cues also unpredictable.

The presence of a well-developed kin recognition system in *H. axyridis*, and the biology of this species allows us to address additional aspects of kin discrimination. Grafen (1990) has argued that all recognition cues are ultimately genetically based, and we will be able to examine this question in *H. axyridis*. We have already

shown that there is genetic variation underlying the propensity of larvae to cannibalize (Wagner *et al.*, 1999). The ease of rearing *H. axyridis* will allow us to measure genetic variation underlying kin recognition. We can also manipulate maternal effects to separate genetically based and maternally provided aspects of the cue. Finally, we can determine if the ability to recognize kin changes across life history stages.

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