Incidence of Egg-cannibalism By Stage Specific Kins of Two Predaceous Ladybirds (Coleoptera: Coccinellidae)

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Abstract: Egg cannibalism by stage-specific kin of two predaceous ladybirds, *Coccinella septempunctata* L. and *Menochilus sexmaculatus* (Fabricius) was studied by providing eggs from the same mother in the absence of natural aphid prey. The pattern of egg cannibalism was similar in both species, however, *C. septempunctata* consumes more eggs than *M. sexmaculatus*. It was observed that during the first hour of exposure, the first and second instars of both the species of coccinellids were most willing to cannibalize kin eggs than the third and fourth instar larvae. Adult males though attracted towards the eggs they sired showed less willingness compared to larval instars. Adult females were least willing to cannibalize their own eggs. After 24 hours of exposure to eggs, the magnitude of egg cannibalism was shifted to the later instars and the adults, as they cannibalized more eggs than the early instars. This shift in the cannibal drive from early instars to the later instar larvae with time indicates that later instars and adults indulged in egg cannibalism as a last resort to suppress their hunger in the absence of natural aphid food.

Keywords: *Coccinella septempunctata Menochilus sexmaculatus* • Aphids Egg cannibalism Biocontrol

Introduction

Cannibalism is a wide-spread and frequent behaviour, which is well-documented in insects (Elgar and Crespi, 1992). It is more operational in prey scarcity (Pervez et al., 2006), occurs commonly in ladybirds (Coleoptera: Coccinellidae) (Omkar and Pervez, 2004, 2016) and provides nutritional and competitive advantages to them (Osawa, 2002). Its nutritional benefits vary from slight changes in developmental rate to major modifications in life history traits (Richardson et al., 2010). However, a recent paper states no nutritional benefit of egg cannibalism on the development and reproductive success of a ladybird, *Coleomegilla maculata* (deGeer) in presence of high quality food (Abdelwahab et al., 2017). Martini et al. (2009) discussed that elimination of competitors and energy gain are sufficient reasons for the evolution of cannibalism, even in the presence of natural prey. However, cannibalism also carries heavy costs in terms of loss of inclusive fitness (Joseph et al., 1999), injury to cannibals (Dawkin, 1976) and disease transmission (Rudolf and Antonovics, 2007). Sibling cannibalism, however less frequent than non-sibling ones (Mills 1982) is gaining much attention because of its adaptive and evolutionary significance (Osawa, 1989, 1992, 2002, 2003; Pervez et al., 2005). It provides critical energy and nutrients that expedite development and increase the survival (Roy et al 2007). Ladybirds consuming sibling eggs have larger body size and weight with faster development rate (Osawa, 2002; Michaud and Grant 2004; Omkar et al. 2007). Sibling cannibalism occurs by only those larvae coming from same egg cluster (Fox, 1975; Osawa, 1989). Such larvae gain instant nourishment feeding on delayed hatched eggs (Osawa, 1993) and the nurse/ trophic eggs (Perry and Roitberg, 2005a). Kin-recognition and avoidance of kin cannibalism is evident at the larval level in ladybird species, *Propylea dissecta* (Mulsant) and *Coccinella transversalis* (Fabricius) (Pervez et al. 2005). However, little is known about the egg cannibalism by the genetically related cannibals.

Coccinella septempunctata L. and *Menochilus sexmaculatus* (Fabr.) are two most common predaceous cosmopolitan ladybirds found abundantly in the Indian subcontinent with a vast prey range (Omkar and Pervez, 2004; Pervez and Rajesh 2017). Both the ladybird species can resort to cannibalism, however, meager information is available on their cannibalistic behaviour (Hodek et al., 2012). Hence, it would be interesting to know how by the genetically related instars and adults will respond when only eggs are provided as diet. Dixon and Kindlmann (2012) gave "*meet and eat*" hypothesis to explain cannibalism to be a possible function of encounters between the cannibal and victim. Hence, the present investigation was carried out to testify the above-proposed hypothesis on different predatory stages of the two ladybird species.

Materials and Methods

Stock Culture: Adults of *C. septempunctata* and *M. sexmaculatus* were collected from agricultural fields near the suburbs of Kashipur, Uttarakhand and brought to the laboratory. The adults were sexed and paired in plastic Petri dishes (9.0 cm diameter × 2.0 cm height) and reared in Environmental Test Chamber (14L: 10D) at 25 ± 1°C and 65 ± 5% RH. They were fed on *ad libitum* supply of aphids, *Aphis gossypii* (Glover) infested on the leaves of *Lagenaria vulgaris*. After mating, the females laid eggs in clusters. These F_1 eggs were collected and reared in glass beakers in the group of five larvae per beaker till they develop into adults (aphid and host as above). The F_1 adults were further sexed and paired to obtain F_2 eggs. The F_2 eggs, thus obtained were collected and isolated for the experiment. The F_2 eggs obtained from the same mother were also reared on the above prey to obtain different larval instars to be used in the experiments.

Experimental design: Ten eggs (10-hour-old) of *C. septempunctata* were placed in a single straight line in a plastic Petri dish (9.0 cm diameter × 2.0 cm height). Thereafter, a 6-hour starved conspecific first instar, raised from the eggs of the same mother but from different egg-cluster was released in the Petri dish and allowed to consume the eggs. The initial behaviour of the cannibal towards the eggs in terms of (i) number of eggs contacted, (ii) number of eggs uneaten after being contacted, and (iii) number of egg cannibalized for one hour was observed. Thereafter, the Petri dish was kept in the Environmental Test Chamber (ETC) under constant temperature of 25 ± 1°C and 65 ± 5% R.H. After 24 hours from the start of the experiment, the Petri dish from the ETC was taken out. The first instar was removed from Petri dish and the number of remaining eggs were counted to quantify the number of eggs consumed by the first instar larva. The experiment was replicated for ten times. The experiment was repeated using the related second, third, fourth instar, adult male (that sired those eggs) and adult female (that laid those eggs). Similarly, we repeated the experiment using related eggs and predatory stages of *M. sexmaculatus.*

The data on egg cannibalism were tested for normality using Kolmogorov – Smirnoff Test and homogeneity of variance using Bartlett's Test using statistical software, SAS 9.0. The number of eggs contacted and number of eggs cannibalized by the predatory stages of two ladybird species, within 1 hour of exposure, were subjected to two sample t-test using the statistical software, SAS 9.0 and means compared. Willingness for egg cannibalism by the life stages of predaceous ladybirds during the first hour was calculated by following formula (Perry and Roitberg, 2005b).

$$\text{Willingness for egg cannibalism} = \frac{\text{numbeer of eggs cannabalized}}{\text{number of eggs contacted}}$$

Willingness for egg cannibalism was subjected to Two-way ANOVA using 'stage' and 'species' as independent variables and 'willingness' as dependent variable using SAS 9.0. The data on egg cannibalism by a specific predatory stage of two species of ladybirds were subjected to two sample t-test using SAS 9.0 and means compared. The data were further subjected to Two-way ANOVA using (i) 'species' and (ii) 'stage' as dependent variables and 'egg cannibalism' as an independent variable using SAS 9.0.

Results

The data pertaining to the number of eggs contacted and cannibalized for the first hour of the experiment is presented in Table-1. It was revealed that the number of contacts increased with the increase in the instar stage and adult females of both species had significantly maximum number of contacts ($t = 4.49$; $P<0.01$; d.f. = 1). Despite of many contacts, these females were least willing to consume their own eggs during the first hour of the experiment (Table -1; Figure-1). Two Way ANOVA revealed a significant main effect of 'stage' ($F = 11.86$; $P<0.001$; d. f. = 5) and 'species' ($F = 7.53$; $P<0.001$; d. f. = 1) on willingness for egg-cannibalism. Interaction between 'species' and 'stage', however, was not statistically significant ($F = 3.22$; $P<0.01$; d. f. = 5, 119). The data recorded after 24 hours of experiment revealed that first ($t = 0.13$; $P = 0.72$; d. f. = 1) and second ($t = 1.07$; $P = 0.31$; d. f. = 1) instars of both ladybird species cannibalized related eggs, however the consumption did not vary significantly (Figure- 2). The third ($t = 13.63$; $P < 0.001$; d. f. = 1) and fourth ($t = 14.82$; $P < 0.001$; d. f. = 1) instars of *C. septempunctata* consumed significantly greater number of eggs than those of *M. sexmaculatus*. Adult males of *C. septempunctata* cannibalized significantly ($t = 16.03$; $P < 0.001$; d. f. = 1) greater number of those eggs which were sired by them than those of *M. sexmaculatus*. Similarly, adult female *C. septempunctata* cannibalized significantly ($t = 16.94$; $P < 0.001$; d. f. = 1) greater number of her own eggs as compared with female *M. sexmaculatus* as a cannibal. Two Way ANOVA revealed a significant main effect of 'stage' on sibling egg-cannibalism ($F = 34.32$; $P<0.001$; d. f. = 5). The main effect of 'species' on sibling egg-cannibalism was also statistically significant ($F = 33.67$; $P<0.001$; d. f. = 1). Interaction between 'species' and 'stage' was also significant ($F = 3.22$; $P<0.01$; d. f. = 5, 119).

Discussion

Life stages of *C. septempunctata* were better cannibals in terms of number of related eggs consumed than *M. sexmaculatus*. The first instars were most willing to cannibalize eggs during the first hour of observation, as they didn't even forage for natural prey after having the first encounter with eggs. Conspecific egg as a first meal was considered as nutritious to the neonates and first instars (Omkar et al., 2006). Mostly, first and second instar larvae contacted and cannibalized the first encountered kin egg, thereby proving the "*meet and eat*" hypothesis (Dixon and Kindlmann, 2012), however, only at early instar level. Female ladybirds lay trophic (apparently infertile) eggs in the cluster, especially to mitigate the risk of offspring starvation (Perry and Roitberg, 2005a). This might explain the behaviour of the early instars to cannibalize the eggs of kins.

Egg cannibalism by neonates of *Hippodamia convergens* Guerin-Meneville resulted in faster development and heavier adults (Bayoumy and Michaud, 2015). Similarly, neonates of *P. dissecta* and *C. transversalis* were more willing to consume conspecific eggs rather than heterospecific eggs (Omkar et al., 2004). Adults showed reluctance to cannibalize sibling eggs during the first few encounters, especially the adult females during the first hour of exposure. It appears that they might have recognized their own eggs and hence refrained themselves from consuming them. Female tends to recognize her own eggs due to the presence of surface alkanes on them (Omkar et al., 2004; Pattanayak et al., 2014, 2016). However, after a few hours, the surface odours might have faded and the hunger level increased that could have triggered the increased consumption of eggs. Bayoumy et al.,

(2016) found egg cannibalism to be beneficial to both parents in *Coccinella undecimpunctata* L., in terms of increased fitness level, their reproductive performance, and better offspring.

Table 1 Total number of eggs contacted and number of eggs cannibalised by the life stages of two ladybirds, *viz. C. septempunctata* and *M. sexmaculatus.*

	First Instar	Second Instar	Third Instar	Fourth Instar	Adult Male	Adult Female
(Eggs contacted)						
C. septempunctata	1.10 ± 0.10 a	1.50 ± 0.17 a	3.00 ± 0.37 a	4.20 ± 0.65 a	4.70 ± 0.56 a	8.10 ± 0.74 a
M. sexmaculatus	0.60 ± 0.16 a	1.00 ± 0.15 b	1.70 ± 0.21 b	3.20 ± 0.49 b	4.40 ± 0.67 a	5.90 ± 0.38 b
t-value	t = 1.86; p=0.096	t = 2.29; p<0.05	t = 4.99; p<0.001	t = 1.50; p=0.016	t = 0.39; p=0.70	t = 4.49; p<0.01
(Eggs cannibalized)						
C. septempunctata	0.90 ± 0.10 a	1.10 ± 0.10 a	1.60 ± 0.16 a	2.10 ± 0.18 a	1.50 ± 0.17 a	0.20 ± 0.13a
M. sexmaculatus	0.50 ± 0.17 b	0.80 ± 0.20 a	0.50 ± 0.17 b	0.60 ± 0.22 b	1.10 ± 0.18 a	0.10 ± 0.10 a
t-value	t = 2.45; p<0.05	t = 1.15; p=0.279	t = 3.97; p<0.05	t = 5.58; p<0.0001	t = 1.50; p=0.16	t = 0.56; p=0.591

Data are Mean ± S.E. Different letters mean significance within the distribution.

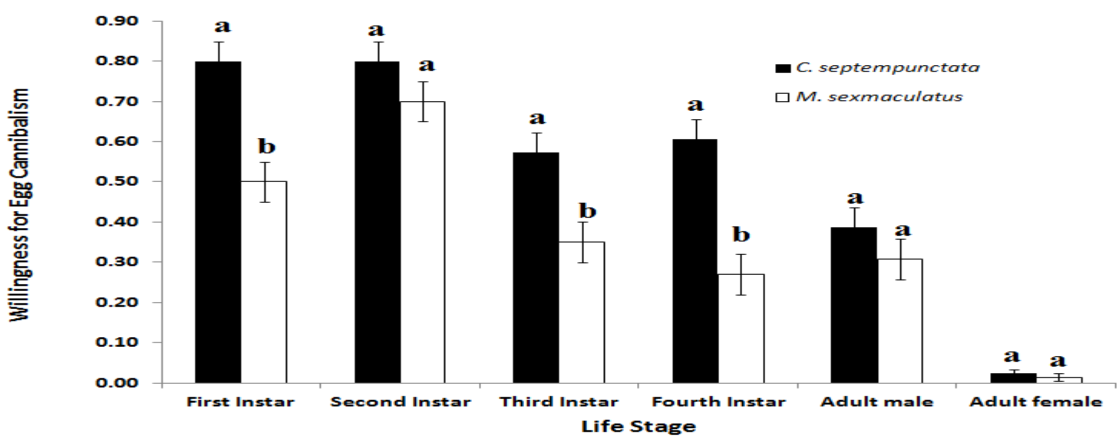

Figure-1: Willingness for cannibalism by the life stages of *C. septempunctata* and *M. sexmaculatus*. Different letters mean significance within the distribution.

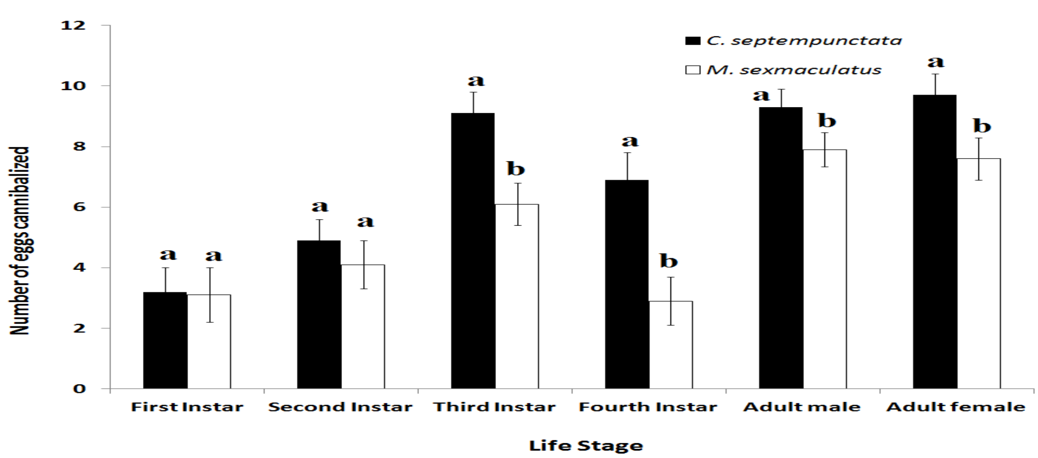

Figure-2: Cannibalism of the related eggs by life stages of *C. septempunctata* and *M. sexmaculatus* . Different letters mean significance within the distribution.

The third and fourth instar larvae were less willing to cannibalize sibling eggs as compared to those of early instar larvae. In the later course of experiment, the later instars cannibalized more related eggs than the early ones, which could be attributed to their hunger level and intense developmental pressure regarding moulting and pupation. Two-way ANOVA revealed a significant main effect of 'species' on sibling egg cannibalism, which indicate that egg cannibalism, however, exhibited similar pattern differed in magnitude. That is, *C. septempunctata* consumed more eggs than *M. sexmaculatus*, which could be attributed to the bigger body size of the former leading to greater food requirement than *M. sexmaculatus*. Pervez et al. (2006) reported the stronger cannibalistic behavior of ladybird, *Propylea dissecta* (Mulsant) than *Coccinella transversalis* (Fabr.). Agarwala et al. (1998) found that adults of *M. sexmaculatus* consumed conspecific eggs more readily than heterospecific ones. On the basis of present experiment, it may be concluded that: (i) *C. septempunctata* more readily cannibalize eggs than *M. sexmaculatus*, (ii) early instar larvae are most willing to cannibalize eggs, (iii) adults, particularly females, show initial reluctance in consuming their own eggs probably due to kin recognition, and (iv) the adults can readily cannibalize the same eggs as the time passes and hunger level increases.

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