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Impact of egg-quality and its laying pattern on the fate of egg-cannibalism and intraguild predation in ladybirds

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Abstract

We investigated the impact of egg-quality and its laying pattern on the incidence of cannibalism and intraguild predation in ladybirds. We manipulated quality by using two species of ladybirds' eggs and the pattern of egg-laying by arranging the eggs singly or in cluster and using second, third and fourth instar larva of two ladybird species, *Coccinella septempunctata* L. and *Menochilus sexmaculatus* (Fabricius), as predators. We found that second instars of both ladybird species indulged in egg-cannibalism, whereas third and fourth instars were potential intraguild predators and consumed heterospecific eggs. On the other hand, linearly arranged and clustered eggs of *M. sexmaculatus* were least protected by higher instars of *C. septempunctata*, while clustered eggs of *C. septempunctata* got a refuge from *M. sexmaculatus* higher stage larvae due to aposematism in eggs. It is concluded that *C. septempunctata* was an intraguild predator of eggs of *M. sexmaculatus* in starved conditions, and the egg-quality and pattern of egg-laying can affect the fate of egg-cannibalism and intraguild predation.

Keywords: *Coccinella septempunctata*, *Menochilus sexmaculatus*, cannibalism, intraguild predation, pattern

Introduction

Ladybirds' ability to suppress prey can be reduced by complex predator-predator interactions, such as, cannibalism (eating the individuals of same species) and intraguild predation (eating the individuals of different predatory species), causing difficulties to predict under what circumstances the diversity of these predators will aid or impede pest management (Rosenheim and Harmon 2006; Grez *et al.* 2012) [21, 10]. Intraguild predation of eggs occurs most commonly in ladybirds in absence of natural food. It does, however, have substantial advantages, such as the eradication of a prospective predator or rival and, concurrently, the eating of a protein-rich diet (Lucas 2012) [18]. Eggs are in the most vulnerable stage as they are immobile hence an easily available meal for intruders. Both conspecific and heterospecific larvae are better potential predators of unhatched eggs than the adults (Hodek and Evans 2012) [13]. To protect their offspring, ladybirds induce strategies of protection *via* chemicals and arranging eggs into clusters.

Egg chorion chemicals emphasize the presence of toxic repellents while the shield of chemicals inside and outside the egg surface protects from adversaries (King and Meinwald 1996; Hemptinne *et al.* 2000) [17, 11]. The protective substances in or on coccinellid eggs and the predators' capacity to deal with them both affect how predation turns out (Weber and Lundgren, 2009) [24]. Ladybirds oviposit eggs either in clusters or in batches. For a number of reasons, including the availability of trophic eggs, sibling cannibalism, and the "social feeding theory," female lady beetles are hypothesized to improve the survival of their larvae by depositing eggs in clusters (Agarwala and Dixon 1993; Hemptinne *et al.* 2000) [1, 11].

Coccinella septempunctata Linnaeus or C7 is a widespread ladybird found in the Palearctic, Nearctic, and Oriental areas. Its nature is euryphagous. Due to heterogeneous voltinism and diapause, a percentage of C7 populations can continue to feed on aphids far into the late summer and fall. Particularly in arid regions, life history plasticity may be essential to the survival of the species. If ingested by avian predators, *C. septempunctata* is unpleasant and harmful (Dolenska *et al.* 2009) [7]. This shows that the species is aposematic in nature, along with its unusual color and patterning. Two endogenously produced alkaloids, N-oxide

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coccinelline and its free base precoccinelline, provide chemical defense (Daloze *et al.* 1994) [5]. *Menochilus sexmaculatus* (Fabricius) is the most misidentified ladybird species due to polymorphism, *i.e.* it is found in different colour patterns and forms, and since it is so common in North Indian agricultural fields, researchers can use it as a model to study how ladybirds interact with aphids raised on various crops. Fifty-seven species of aphids are known prey of *M. sexmaculatus*. It is more capable of reproducing in a hostile environment (Agarwala and Yasuda 2000) [2]. We studied the consumption of conspecific (Cannibalism) and heterospecific eggs (Intraguild predation) along with its pattern when the eggs are arranged in cluster or non-cluster (singly laid) by *C. septempunctata* and *M. sexmaculatus*, which are the two most commonly occurring ladybird species of North India.

Materials and Methods

Stock culture

adults of *C. septempunctata* and *M. sexmaculatus* were collected from the agricultural fields preying on aphid, *Aphis gossypii* (Glover) infesting Cucurbitacea climber, *Coccinia grandis* (L.) near the city of Kashipur, India. They were brought to the laboratory and reared on the above aphid and host plant by keeping pairs in Petri dishes (9.0 cm diameter x 2.0 cm height) in an Environmental Test Chamber (Remi, Remi Instruments) under controlled suitable conditions ($27 \pm 1^\circ\text{C}$; $65 \pm 5\%$ RH; 14L:10D). The pairs were allowed to mate and the eggs laid were isolated and reared on above prey in separate Petri dishes (size as above) to attain second, third and fourth larval instars to be used as predators in the experiment.

Experimental design

Ten *M. sexmaculatus* eggs (10 hours old) were kept in a single straight line and ten *C. septempunctata* eggs (10 hours old) were arranged in a second straight line in a plastic Petri dish (size as above). A 5-hour starved second-instar larva of *C. septempunctata* was released in this Petri dish for two hours to forage and consume eggs. It was removed from the Petri dish after 2.0 hours, and the number of unconsumed eggs in each arrangement was tallied to determine the number of eggs consumed. The experiment was replicated ten times ($n=10$). The experiment was repeated using third and fourth instars as predators. The experiment was repeated using all four *M. sexmaculatus* larval instars as predators.

Ten eggs (10 hours old) of *M. sexmaculatus* were arranged in a single straight line and ten eggs (10 hours old) of *C. septempunctata* were arranged in clusters in a plastic Petri dish. A 5-hour starved second-instar larva of *C. septempunctata* was released in the same Petri dish for two hours, as above. After two hours, the second instar was removed from the Petri dish and the number of unconsumed eggs was counted to find out the number of eggs consumed. Ten replicates of the experiment were made ($n=10$). The

experiment was repeated using third and fourth instar larvae as predators. The experiment was repeated using all four larval instars of *M. sexmaculatus* separately as predators. The data on number of eggs consumed in each treatment was subjected to the Wilcoxon Signed rank test using a statistical software SAS (Version 9.0) on our personal computer. The data on 'egg consumption' as a dependent variable was then subjected to Three-way ANOVA with the following independent variables: (i) predator species, (ii) predator stage, and (iii) prey species using SAS (Version 9.0).

Results

The second ($Z = 2.32$; $P = 0.01$) and third ($Z = 2.73$; $P = 0.01$) instar larvae of *C. septempunctata* significantly ingested more conspecific eggs than the heterospecific eggs, as compared to fourth instar larva ($Z = 0.00$; $P = 1$). However, consumption of conspecific eggs by second ($Z = 0.23$; $P = 0.408$) and third ($Z = 1.3$; $P = 0.09$) instar larvae of *M. sexmaculatus* was not found to be statistically significant. Fourth instar of *M. sexmaculatus* consumed greater number of heterospecific eggs. However, this difference was not found to be statistically significant ($Z = -0.55$; $P = 0.28$). According to Three-way ANOVA, "prey species" significantly affected CANN/IGP ($F = 11.15$; $P = 0.0012$; d. f. = 1). The "ladybird species" had no bearing on the results ($F = 0.001$; $P = 0.9542$; d. f. = 1). The "larval stage" has a highly significant effect on CANN/IGP ($F = 14.34$; $P = 0.0001$; d. f. = 2). "Prey species" and "ladybird species" interacted statistically significantly ($F = 5.30$; $P = 0.0232$; d. f. = 1). "Prey species" and "larval stage" were found to interact significantly ($F = 5.05$, $P = 0.0080$; d. f. = 2). The interaction between "ladybird species" and "larval stage" also had a significant effect ($F = 7.20$; $P = 0.0012$; d. f. = 2). However, the interaction between "ladybird species," "larval stage," and "prey species" had no discernible effect ($F = 0.56$; $P = 0.5741$; d. f. = 2).

The second instar larva ($Z = 0.88$; $P = 0.288$) of *C. septempunctata* insignificantly devoured single-arrangement eggs marginally more than the third ($Z = -1.45$; $P = 0.07$) and fourth ($Z = -0.07$; $P = 0.47$) instars, which consumed marginally more clustered eggs insignificantly. On the other hand, second ($Z = 0.799$; $P = 0.211$), third ($Z = 1.34$; $P = 0.08$), and fourth ($Z = 0.23$; $P = 0.47$) instars of *M. sexmaculatus* insignificantly consumed single-arrangement eggs. The influence of "ladybird species" on pattern (single/cluster) was found to be significantly different ($F = 10.36$; $p < 0.0001$; d. f. = 2,119) at the level of "larval stage." However, the main effects of "pattern" (single/cluster) ($F = 2.57$; $P = 0.112$; d. f. = 2,119) and "Prey species" were not found to be statistically significant ($F = 1.23$; $P = 0.2707$; d. f. = 1). Additionally negligible ($F = 3.34$; $P = 0.0705$; d. f. = 1) was the influence of "ladybird species". The pattern of eggs significantly affected by the "larval stage" ($F = 19.49$; $P = 0.0001$; d. f. = 2).

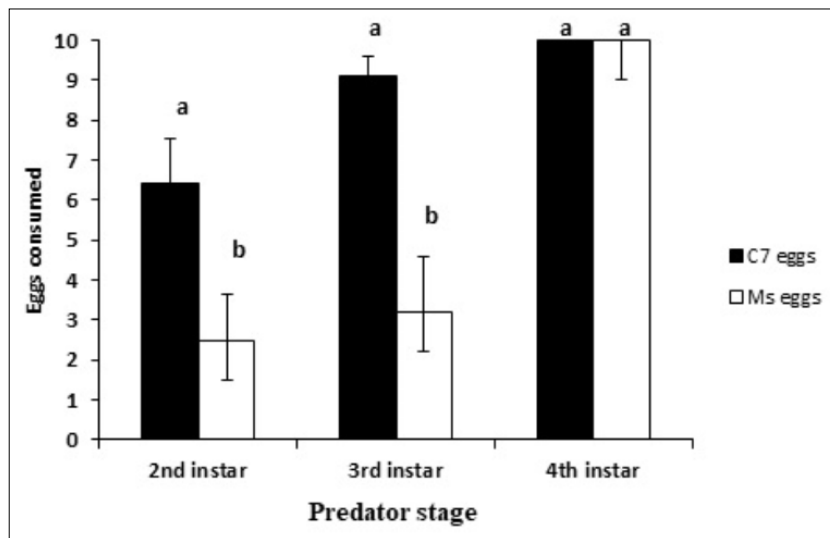


Fig 1(a): Number of C7/ Ms eggs consumed when *C. septempunctata* larva was foraging as predator.

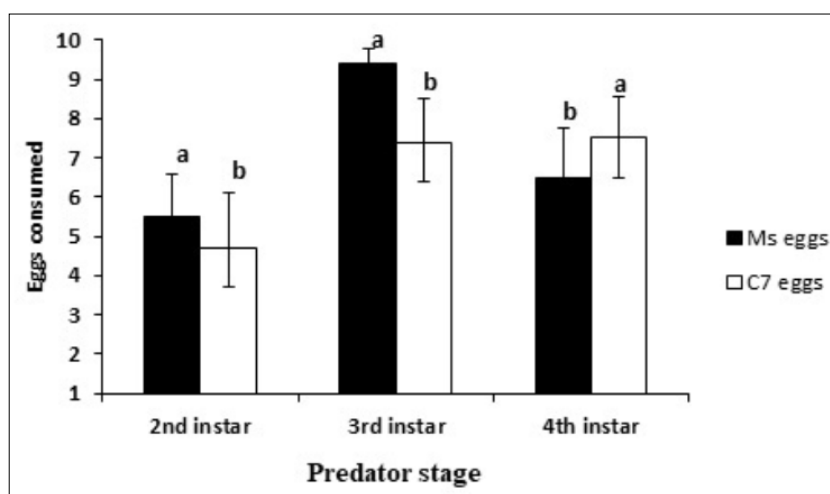


Fig 1(b): Number of C7/ Ms eggs consumed when *M. sexmaculatus* larva was foraging as predator.

Insignificant interaction existed between “prey species” and “larval stage” ($F = 1.35$; $P = 0.2476$; d. f. = 1). “Prey species” and “larval stage” did not interact significantly ($F = 0.84$, $P = 0.4357$; d. f. = 2), while “larval stage” and “ladybird species”

did not interact significantly ($F = 0.50$, $P = 0.6087$; the. f. = 2). Additionally, it was shown that the interaction between “prey species,” “larval stage,” and “ladybird species” had no significant effect ($F = 0.51$; $P = 0.6004$; d. f. = 2).

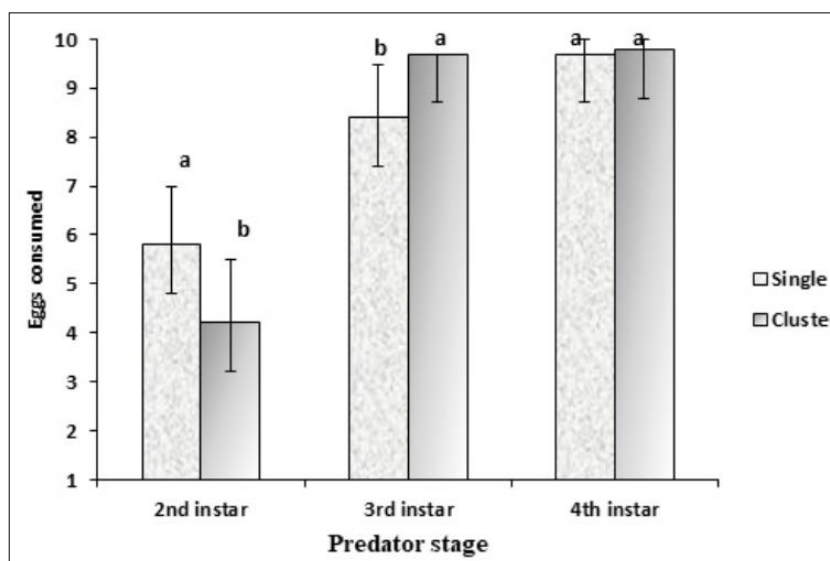


Fig 2(a): Number of eggs consumed by the larvae of *C. septempunctata* when arranged singly/ cluster.

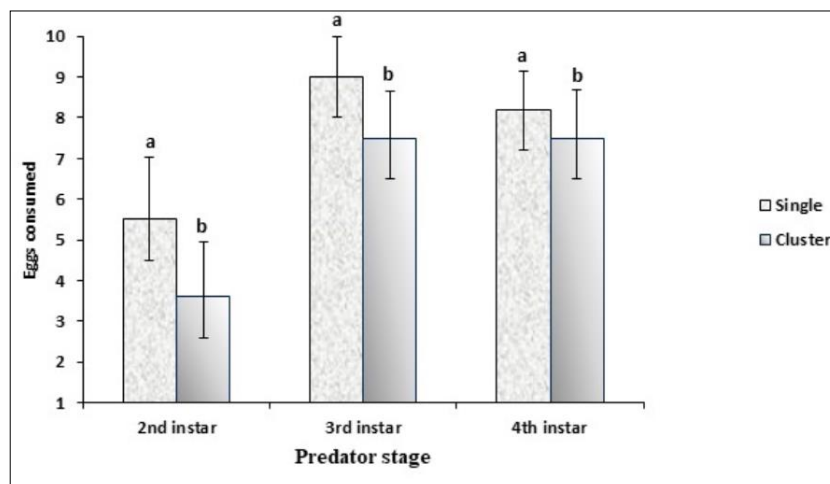


Fig 2(b): Number of eggs consumed by the larvae of *M. sexmaculatus* when arranged singly/ cluster.

Discussion

The results revealed that second and third instars of *C. septempunctata* and *M. sexmaculatus* were predisposed to consume conspecific eggs. However, the fourth instar, which was massive and food-starved, preyed on both types of egg diet equally. Usually, the young instars or newly hatched larvae prefer feeding on conspecific eggs, as their first meal due to the easy availability and nutritive quality of the eggs (Omkar *et al.* 2006) [19]. Conspecific eggs' surface chemistry appears to function as a feeding stimulant, which may be less effective or missing in heterospecific eggs because ladybird larvae don't appear to use visual cues to recognize prey and have underdeveloped eyes (Dixon 2000) [6]. Conspecific eggs are preferred because of the hydrocarbons on their chorion surface. Saturated hydrocarbons on the surface of eggs do not oxidize quickly and remain to act as signals, allowing conspecifics to feast on them and discouraging heterospecifics (Hemptonne *et al.* 2000) [11]. In addition to playing a key role in predator attraction and repulsion, alkanes/alkaloids also have relative costs and benefits for predator survival (Hemptonne *et al.* 2001) [12]. Larvae may simply store and utilize interspecific conspecific alkaloids for future defense (Kajita *et al.* 2010) [15]. Our findings are in agreement with those of Gagne *et al.* (2002) [9] on the neonates of ladybird, *Coleomegilla maculata* (De Geer). The primary factor influencing cannibalism and its level of satiation is food deprivation (Dixon 2000) [6]. If the natural food is readily available, the incidences of cannibalism decline suggesting that cannibalism among larvae is inversely correlated with food supply (Khan and Yoldas 2018) [16]. Starved fourth instar larvae disregarded the toxicity of non-conspecific eggs due to their size and voracity. Compared to earlier stages, fourth instar foraged all conspecific and non-conspecific eggs. Due to their greater body size and higher nutritional needs, fourth instar larvae ingested more eggs than younger ones (Cottrell 2005; Sato *et al.* 2011; Jafari 2013) [4, 23, 14]. When ladybird larvae are in their fourth stage, they must devote more energy transitioning from the pupal to adult stage. We agree with the finding of Kajita *et al.* (2010) [15], which suggest that larger individuals of *C. septempunctata* would be able to withstand higher concentrations of non-conspecific alkaloids.

In the second set of experiment, second instar of *C. septempunctata* ate eggs that were lined up in a row. All of the clustered eggs were consumed by third stage instars, while fourth stage instars exhibited no preference for either configuration. The second stage instar of *M. sexmaculatus* ate

a lot of eggs laid in a single arrangement. Similar to this, the average intake of third and fourth stage instars of *M. sexmaculatus* seemed to favour single-hatched eggs. The larval instars of *C. septempunctata* provided the least protection for linearly oviposited eggs of *M. sexmaculatus*. The defense of egg clustering in *M. sexmaculatus* eggs was breached by third and fourth instar larvae of *C. septempunctata*. *M. sexmaculatus* lays eggs in batches rather in clusters. Predators who like solitary laying eggs target single eggs more frequently (Agarwala and Dixon 1993) [1]. Less likely than eggs in clusters to be consumed by newly emerging larvae are solitary eggs. Santi and Maini (2007) [22] identified solitary eggs in *Adalia bipunctata* and *Harmonia axyridis* as "trophic eggs." However, trophic eggs are thought to be a method of feeding newborn larvae in social insects (Perry and Roitberg 2006) [20]. They therefore constitute an additional female reproductive effort for the immediate benefit of their neonates (Ferrer *et al.* 2011) [8]. Eggs are laid in groups by *C. septempunctata*. They have aposematism due to their vivid yellow colouring. The hue of the egg and the elytra precisely reflects the level of chemical defence that is crucial for that stage of development (Winters *et al.* 2014) [25]. By placing clusters of eggs in numerous layers, the parasitism level is reduced (Bessera and Parra 2005) [3]. Hence, we conclude *C. septempunctata* could act as an intraguild predator in absence of essential prey and the egg-quality and pattern of egg-laying in the form of egg-clustering can affect the fate of egg-cannibalism and intraguild predation.

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