Article

Intraguild predation and cannibalism between two co-occurring ladybird species (*Coccinella septempunctata* and *Menochilus sexmaculatus*): A fight for supremacy

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Abstract: *Coccinella septempunctata* L. (*C7*) and *Menochilus sexmaculatus* (Fabricius) (*Ms*) are aphidophagous ladybirds (Coleoptera: Coccinellidae), which dominates the ladybird fauna of Oriental region. Since these species co-occur and are highly predaceous on various aphids, it was hypothesized that in the scarcity or absence of aphids, i.e., extraguild prey, one ladybird species will start attacking and killing the other one, which will endanger their co-occurrence. Thus, we aim to determine, which ladybirds’ larvae will subdue the other; their attack rates (rate at which a superior individual attacks the inferior one), escape rates (rate at which an inferior individual escapes from the attack of superior one) and predation rates (rate at which a superior individual eats the inferior one) were investigated, as they indulged in cannibalism and intraguild predation. Larvae of *C7* were slightly bigger and heavier than those of *Ms*, thereby they gained a slight competitive advantage. This enabled *C7* a better attacker and an escapist when confronted with larvae of *Ms* in a predatory guild, which resulted in lesser larval mortality due to intraguild predation by *Ms* larvae. However, *C7* successfully preyed upon the larvae of *Ms*. The highest rate of cannibalism and intraguild predation was executed by *C7* which made it a potent cannibal and an intraguild predator. Despite being outplayed by *C7*, the inferior *Ms* larvae didn’t suffer much loss during the intraguild combat due to their armoured morphological features in the form of spines and rough texture. This is the reason why *Ms* still exists as the second most commonly occurring ladybird in the field.

Keywords: Coccinellidae; *Coccinella septempunctata*; *Menochilus sexmaculatus*; cannibalism; intraguild predation

1. Introduction

The majority of ladybirds (Coccinellidae: Coleoptera: Insecta) are predaceous and considered as potential biological control agents of aphids, scale-insects, mealybugs, whiteflies, and tiny phytophagous insects, which inflict serious crop damage[1]. Ladybirds have seasonal synchrony with these phytophagous pests and their early arrival to at the pest-sites is beneficial for the crops due to their optimal foraging[2]. However, the interactions between a few ladybirds both in the presence and absence of the prey species could result in a decline in pest suppression. The most notable interaction between heterospecific ladybirds is intraguild predation (IGP), where a dominant predatory species attacks the recessive one and eats it. IGP involves at least three species: an intraguild predator (the dominant predator), an intraguild prey (the weak predator), and the extraguild prey (prey species that both predators share). The intraguild predator and intraguild prey have different rates of resource exploitation that are lower in the former than in the latter[3]. The main reasons for larval mortality of aphidophagous ladybirds are cannibalism, and intraguild predation when aphids are scarce[4,5]. However, high risk of intraguild predation may even prolong development, which may be compensated during early larval stage by high foraging activities and accelerated development in predatory...
mites[6]. However, these mites may adjust their predatory behaviour when at risk or vulnerability[7] and have a preference for a particular intraguild prey[8]. Body size modulates intraguild predation because large species are more likely to become intraguild predators[9]. For instance, a ladybird Coccinella septempunctata brucki Mulsant is commonly preyed upon by a dominant invader ladybird species, Harmonia axyridis (Pallas), whereas the reverse trophic interaction is rare[10]. Similarly, first-instar larvae of Adalia bipunctata L. are eaten by bigger instars of H. axyridis, but vice-versa never occurred[11]. Harmonia axyridis possesses intrinsic advantage in the guild over other predators due to its bigger size and voracity[12] and often coexists with them in its native habitats[13].

Seven-spot ladybird, Coccinella septempunctata is a cosmopolitan species, native to Palearctic region, and globally dominates numerous indigenous predatory species[14,15]. However, a few dominant and bigger species, like H. axyridis and Coleomegilla maculata DeGeer, usually out-compete C. septempunctata in intraguild interactions[16]. Its dietary history of non-availability of natural food during instar level tends to eat the same food during adulthood[17]. Similarly, six-spotted zigzag ladybird, Menochilus sexmaculatus (Fabricius) is a co-occurring generalist aphidophagous ladybird species, which is a dominant species in the Oriental region[18]. During prey scarcity, it easily attacks and dominates its smaller co-occurring ladybird species, like Oenopia conglobata (Linnaeus)[19] and Propylea dissecta (Mulsant)[20]. Both C. septempunctata and M. sexmaculatus are dominant species, however, little is known about their intraguild interactions. Their aggression and co-existence raise questions on their mutual behaviour. Morphological traits, like waxy coverings[21] and spiky structures[22] guard ladybird larvae from intraguild predation. Larvae of C. septempunctata lack waxy covering and spikes[20], while those of M. sexmaculatus possess spines. However, these structures affect the incidences of predation and cannibalism. We aim to find out which ladybirds’ larvae will act as intraguild predator and subdue the other. Thus, we investigated their attack rates (rate at which a superior coccinellid attacks the inferior one), escape rates (rate at which an inferior coccinellid escapes from the attack of superior one) and predation rates (rate at which a superior coccinellid eats the inferior one), as they interact with each other in the absence of prey. Hence, the competitive ability between the above two coccinellid species were investigated in the light of the dynamics of cannibalism and IGP between them to better understand their coexistence in the absence of aphid.

2. Materials and methods

2.1. Stock maintenance

Adults of C. septempunctata (C7) and M. sexmaculatus (Ms) were collected from fields of common wireweed, Sida acuta Burm preying upon aphid, Aphis gossypii (Glover) in the college campus of Kashipur, India (29.2104° N, 78.9619° E) and brought to the laboratory to maintain stock culture. Adult male and female of conspecific ladybirds were paired in Petri dishes (9.0 cm diameter × 2.0 cm height) to were allowed to mate, and were fed daily on ad libitum A. gossypii on pieces of S. acuta leaves under constant conditions (27 ± 1 ℃; 65 ± 5% R.H; 12:12 LD) in an Environmental Test Chamber (Remi, Remi Instruments). The female laid eggs, which were collected daily and incubated separately in Petri dishes (size as above). The hatched instars were reared separately in Petri dishes (size and prey, as above) to obtain desired larval stages (L1, L2, L3 and L4) to be used in the experiments.

2.1.1. Body length, weight, and walking activity of ladybird’s larva

Initially, we measured the body lengths, weights and walking activities of all the four instars of C. septempunctata and M. sexmaculatus in the absence of intraguild predator and extraguild prey. This was done before launching them in the IGP experiment (experiment (ii)) just to identify the reasons behind their success
or failure in the guild. For this purpose, we measured the lengths, weights, and walking activities (in terms of the distance covered in one minute) of all four instars of *C. septempunctata* and *M. sexmaculatus*. The larva was placed on a point on graph paper, held firmly but gently, and allowed to extend normally. The body length was calculated by measuring the distance between the tip of the head and the abdomen in ten replicates (\(n=10\)). The larva was then weighed using a SARTORIUS-H51 electronic scale (\(n=10\)) at 0.1 mg precision. A 12-h starving larva was placed on a 100-cm glass rod and allowed to move to measure walking activity. The distance the larva covered in a minute was noted (\(n=10\)). Larval body measurements (length, weight, and walking activity) were subjected to one-way ANOVA and the means were compared using Tukey’s Test using a statistical software called SAS 9.0[23].

2.1.2. Attack, escape and predation rates of the ladybird’s larva

A larval instar and a 6-hour starved older instar (one instar older) were paired in a Petri dish without any food (size as above), thereby making three combinations, viz., \(L_1\) with \(L_2\), \(L_2\) with \(L_3\), and \(L_3\) with \(L_4\). This results in four different intraspecific and interspecific predation combinations (cannibal–victim), viz., (a) \(C_7–C_7\), (b) \(Ms–Ms\), (c) \(C_7–Ms\), and (d) \(Ms–C_7\), each comprising of three subsets (i.e., \(L_1\) with \(L_2\), \(L_2\) with \(L_3\), and \(L_3\) with \(L_4\)). The behaviour of the two larvae in the Petri dish was carefully recorded using a trinocular assembly (Lyzer) connected to a personal computer (Dell). The behavioural interactions were categorized into (i) contact (when a superior larva touches the inferior one), (ii) attack (when a superior larva attacks the inferior one), (iii) escape (when an inferior larva escapes from the attack of superior one), and (iv) predation (when the superior larva preys upon the inferior one). The number of behavioural events, viz., contact, attack, escape, and predation events between larvae were continuously observed and recorded for one hour. The experiment was repeated using the same staged cannibals and victims in all the above combinations. The attack rate (number of inferior larvae attacked × 100/number of inferior larvae contacted), escape rate (number of inferior larvae escaped × 100/number of inferior larvae attacked), and predation rate (number of individuals eaten × 100/total number of replicates) were calculated using above data. The escape was counted as 1 when a predator attacked a prey and the latter escaped from the clutches of the predator after it was contacted and attacked. After undergoing an arcsine square root transformation, the data on attack, escape, and predation rates were subjected to one-way ANOVA followed by a Fisher’s Protected Least Significant Differences post hoc test by SAS 9.0[23].

3. Results

3.1. Body length, weight, and walking activity of ladybird’s larva

The body lengths and body weights of four instars of *C. septempunctata* were significantly greater than those of *M. sexmaculatus* (Table 1). The walking activities of the first and second instars of *C. septempunctata* were significantly greater than those of *M. sexmaculatus*, while these activities of the third and fourth instars were not significantly different.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Larval stage</th>
<th><em>C. septempunctata</em></th>
<th><em>M. sexmaculatus</em></th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length (in mm)</td>
<td>(L_1)</td>
<td>3.03 ± 0.08a</td>
<td>2.02 ± 0.08b</td>
<td>784.69**</td>
</tr>
<tr>
<td></td>
<td>(L_2)</td>
<td>5.1 ± 0.1a</td>
<td>3.3 ± 0.21b</td>
<td>63.44**</td>
</tr>
<tr>
<td></td>
<td>(L_3)</td>
<td>8.3 ± 0.42a</td>
<td>5.5 ± 0.17b</td>
<td>37.9**</td>
</tr>
<tr>
<td></td>
<td>(L_4)</td>
<td>8.5 ± 0.34a</td>
<td>6.0 ± 0.26b</td>
<td>34.1**</td>
</tr>
</tbody>
</table>
### Table 1. (Continued).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Larval stage</th>
<th>C. septempunctata</th>
<th>M. sexmaculatus</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (in mg)</td>
<td>L₁</td>
<td>1.66 ± 0.07a</td>
<td>1.15 ± 0.06b</td>
<td>34.99**</td>
</tr>
<tr>
<td></td>
<td>L₂</td>
<td>2.43 ± 0.08a</td>
<td>1.50 ± 0.07b</td>
<td>73.37**</td>
</tr>
<tr>
<td></td>
<td>L₃</td>
<td>11.5 ± 1.14a</td>
<td>4.4 ± 0.36b</td>
<td>34.9**</td>
</tr>
<tr>
<td></td>
<td>L₄</td>
<td>23.3 ± 1.64a</td>
<td>9.1 ± 0.58b</td>
<td>66.75**</td>
</tr>
<tr>
<td>Speed of walking activity (cm/min)</td>
<td>L₁</td>
<td>46.5 ± 2.93a</td>
<td>22.1 ± 3.9b</td>
<td>25.07**</td>
</tr>
<tr>
<td></td>
<td>L₂</td>
<td>70.0 ± 2.73a</td>
<td>44.1 ± 9.7b</td>
<td>6.61*</td>
</tr>
<tr>
<td></td>
<td>L₃</td>
<td>67.3 ± 15.34</td>
<td>77.5 ± 10.35a</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>L₄</td>
<td>81.9 ± 9.4a</td>
<td>96.8 ± 9.96a</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Significant at: *P < 0.05; **P < 0.0001.
Tukey’s test: range = 2.97; d. f. = 1, 19.
Different letters within a row indicate that the data is significant.

#### 3.2. Attack, escape and predation rates of the ladybird’s larva

Both C7 and Ms significantly attacked their inferior stages, as cannibals (Table 2; Figure 1). C7 also significantly attacked its heterospecifics, while those of Ms were not significant (Table 2; Figure 1). The older larvae of both species have a similar assault strategy. The larva assaulted from the back by gripping an inferior larva’s anal section (Figure 1(a)) until the latter totally surrendered and then sucked all the bodily fluid from the neck area (Figure 1(b)). The larva’s tough exterior was disintegrated (Figure 1(c)). Heterospecifics at the same stage and fourth instar were observed to engage in this form of attack and predation. Older fourth instars attacked the third instar conspecifics from the neck region (Figure 1(d)). The attack rate of C7 was 70.1% towards conspecifics, while it was 69.0% towards heterospecifics. The attack rate by Ms larvae was 68% and 70.3% towards conspecific and heterospecific larvae, respectively. C7 attacked all the younger instars of M. sexmaculatus. The maximum individual attack rate of C. septempunctata was 94.9% between the same third-stage instar Ms larvae followed by 88.9% of second instar Ms on same stage C7 (Figures 2–5). The details of the attack rates of conspecifics and heterospecifics of the two ladybird species have been presented in Figure 3. The overall escape rate for C7 larvae towards cannibals was 87.0 %, and differences in the individual escape rates were statistically significant (Table 2) but were lesser than that of heterospecific (91.3%) larvae. The overall escape rate of C7 towards Ms was also significant (Table 2). Ms escaped much more from cannibals (91.9%) than intraguild predators (72.9%). However, its escape rates were not significant. The details of the escape rates of conspecifics and heterospecifics of the two ladybird species have been presented in Figure 4. The overall predation rates for C7 and Ms larvae towards their cannibals and intraguild predators were significantly different (Table 2). Cannibalism was 65% and 35% by C7 and Ms instars, respectively. The details of the cannibalism and intraguild predation have been presented in Figure 5.
Table 2. Attack rate, escape rate, and predation rates in four larval stage combinations of *C. septempunctata (C7)* and *M. sexta* (*MS*).

<table>
<thead>
<tr>
<th>Independent factor</th>
<th>Attacker</th>
<th>Statistical values</th>
<th>Prey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval stage combination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attack rate</td>
<td>Escape rate</td>
<td>Predation rate</td>
<td></td>
</tr>
<tr>
<td><em>C7–C7</em></td>
<td>F = 6.61; <em>P</em> &lt; 0.0001; d.f. = 6, 105</td>
<td>F = 4.57; <em>P</em> = 0.0004; d.f. = 6, 105</td>
<td>F = 7.35; <em>P</em> &lt; 0.0001; d.f. = 6, 105</td>
</tr>
<tr>
<td><em>MS–MS</em></td>
<td>F = 3.22; <em>P</em> = 0.006; d.f. = 6, 112</td>
<td>F = 0.96; <em>P</em> = 0.4553; d.f. = 6, 112</td>
<td>F = 2.96; <em>P</em> = 0.0104; d.f. = 6, 112</td>
</tr>
<tr>
<td><em>C7–MS</em></td>
<td>F = 12.84; <em>P</em> &lt; 0.0001; d.f. = 6, 93</td>
<td>F = 4.09; <em>P</em> = 0.002; d.f. = 6, 72</td>
<td>F = 4.83; <em>P</em> &lt; 0.0001; d.f. = 6, 93</td>
</tr>
<tr>
<td><em>MS–C7</em></td>
<td>F = 1.179; <em>P</em> = 0.114; d.f. = 6, 72</td>
<td>F = 2.70; <em>P</em> = 0.019; d.f. = 6, 72</td>
<td>F = 11.19; <em>P</em> = 0.0001; d.f. = 6, 72</td>
</tr>
</tbody>
</table>

![Figure 1](image1)

**Figure 1.** Photographs showing intraguild predation by *C7* larva (a) contacting, (b) attacking, and (c) killed and consumed a *Ms* larva (d) *C7* larva attacking a conspecific and (e) *Ms* preying upon a conspecific.
Figure 2. Percent attack, escape and predation by the larvae of two ladybird species, *C. septempunctata* and *M. sexmaculatus*. Note: Different letters denote that data are significant at $P < 0.01$ (Fisher’s PLSD). Pooled data of all instars.

Figure 3. The percent individuals attacked by the conspecific and heterospecific larvae of *C. septempunctata* and *M. sexmaculatus*. Note: Texture filled bars/attacked by the same instar; black bars/attacked by older instar.
Figure 4. The percent individuals escaped by the conspecific and heterospecific larvae of *C. septempunctata* and *M. sexmaculatus*. Note: Texture filled bars/escaped by the same instar; black bars/escaped by older instar.

Figure 5. The percent individuals of *C. septempunctata* and *M. sexmaculatus* cannibalized and preyed upon. Note: Texture filled bars/predation by the same instar; black bars/predation by older instar.

4. Discussion

The results revealed that in the absence of food, *C7* became a potent intraguild predator of *M. sexmaculatus*. In terms of body size and body weight, all four instars of *C7* were larger and heavier than *Ms*. However, the third and fourth instars of *Ms* were more active while walking than those of *C7*. Cannibalism occurred in all four instars of both ladybird species; however, IGP was more prevalent. The heterospecific larvae were attacked more frequently than conspecifics in both species, as in the case between *H. axyridis* and *C7*.[22] The vulnerability of a species to IGP largely depends on its size, with bigger species acting as an IG-predator, whereas its third and fourth instars act as ferocious attackers and predators[24,25], which in the present study was *C7*. This agrees with Khan and Yoldas[26] that adults and older larvae (third and fourth) frequently...
consume lower immature stages. Attacks occurred more frequently in the setups comprising of younger third instars and the older fourth instars of C7–C7 and C7–Ms combinations. This forces the dominant larva to consume the weaker ones (both instar and species).

The frequency of escapes by C7 larvae in the above combinations depends on the severity of the attacks. When a larva was captured, escape was accomplished by “falling downwards”. As coccinellid larvae have evolved and acquired morphological, chemical, and behavioural defences for fending off conspecific or heterospecific aggressors, it is a form of behavioural strategy to escape from being eaten or cannibalized.[25]

Since C7 larvae are not extremely spiny and do not have a waxy covering like Scymnus species, the primary method of defence against intraguild predation is to drop down.[21] To avoid being devoured by H. axyridis, C7 first instars drop quickly from host plants to evade predators.[27] Since Ms weighs lesser than heavier species,[28] it was able to successfully escape from conspecifics at the same stages of the second, third, younger, and older stages of heterospecific combination. Additionally, the third and fourth larval instars of Ms walk faster and more actively than those of C7. Furthermore, females of Ms protect their offspring by laying a greater number of trophic eggs, which is a survival strategy showing maternal effects against the risk against intraguild predators.[29] Similarly, C7 may also show non-disruptive behaviour on the fate of biocontrol and the co-existence of other species by exhibiting coincidental intraguild predation, where it supports natural enemies in biocontrol of aphid by consuming more non-parasitized aphids and declining its attacks on co-occurring ladybirds.[30]

Due to its larger size and heavier weight, C7 larvae preyed on Ms, revealing the predatory character of this species. According to Cabral et al.[31], the fourth instars were the most voracious stages, demonstrating that C7 larvae attack younger as well as same stage instars of heterospecifics by ignoring every protection of the smaller ladybird. The survival of Ms against C7 species in a food-free environment is not guaranteed by the presence of dorsal spikes. However, the absence of these spines and spikes may lead to increased incidences of IGP of these coccinellids.[32] Some of an intraguild predator’s hostile behaviour may be explained by its ability to detoxify heterospecific chemical compounds after consumption.[33] Hippodamia variegata (Goeze)[34] and A. bipunctata were preyed upon by C7 larvae.[35] C7 frequently preys on Hippodamia convergens Guerin and Coccinella transversoguttata Richardsoni.[36] Due to a higher attacking rate, cannibalism prevails more in C7 larvae between the third and fourth instars, as reported by Khan and Yoldas.[26] Food scarcity and size disparity between the cannibal and the victim are the main causes of larval cannibalism.[37,38]. High intensity of cannibalism by the fourth instars was largely due to their greater body-weights. The victim larvae’s relative inferiority in terms of body size, weight, and walking speed also contributed to a higher rate of cannibalism.[39] However, in the present study M. sexmaculatus exhibited decreased cannibalism rate due to low assault rates and high escape rates. In comparison to C7, it suggested that conspecific interactions were less frequent, which leads to decreased risk to its survival. Both Ms and C7 exhibited decreased levels of cannibalism suggesting that they are better potential intraguild predators than cannibals, due to which they dominate the guild. However, in the scarcity of extraguild prey, C7 mostly dominates the guild and may attack the inferior stages of Ms for its survival. However, with its escape strategies, Ms may often escape from the attacks of C7, thereby reducing the risk of IGP. We conclude that (i) both Ms and C7 had similar attacking rates, (ii) C7 larvae had a greater ability to escape than those of Ms, and (iii) higher incidence of cannibalism and intraguild predation by C7 larvae male them potential cannibals and intraguild predators during food scarcity. Despite being successfully attacked and eaten by C7, the inferior Ms larvae didn’t suffer much loss during the intraguild combat due to their armoured morphological features in the form of spines and rough texture. This is the reason why Ms still co-exists as the second most commonly occurring ladybird in the field.
**Author contributions**

Conceptualization, AP; methodology, AP; software, M; validation, M; formal analysis, M; investigation, M; resources, M; data curation, M; writing—original draft preparation, AP; writing—review and editing, AP; visualization, M; supervision, AP; project administration, AP; funding acquisition, AP. All authors have read and agreed to the published version of the manuscript.

**Conflicts of interest**

The authors declare no conflict of interest.

**References**


