Comparative growth and development of zigzag ladybird beetle (*Cheilomenes sexmaculata*) fed with black bean aphids (*Aphis fabae*) and green peach aphids (*Myzus persicae*)

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ABSTRACT

Background and Objective: *Cheilomenes sexmaculata*, a biological control predator found in the Philippines, is not fully adopted to naturally control various aphid species in most cultivated crops. For rearing purposes, it is important to identify its preferred natural diet to have a basis for successful mass rearing and augmentation. Thus, a study was conducted on *C. sexmaculata* to assess its growth and development when fed with different aphid species.

Methodology: Under laboratory conditions, the study was arranged in a completely randomized experimental design. *Myzus persicae* and *Aphis fabae* were the treatments of the study and supplied every morning at rates of 10, 20, 30, and 40 individuals for the 1st, 2nd, 3rd, and 4th larval instar of *C. sexmaculata*, respectively. Each treatment was replicated 30 times. All data were statistically analyzed through a two-sample t-test at $P < 0.05$.

Main Results: The results showed a significant difference in the aphids consumed at the 4th larval instar, with a mean of 39.24 ± 0.22 fed with *M. persicae* and 36.00 ± 0.75 with *A. fabae*. Regarding the developmental period, *C. sexmaculata* feeding on *M. persicae* showed a shorter developmental period (12.21 ± 1.84 days) than those fed with *A. fabae* (15.34 ± 1.98 days). Similarly, the body length of *C. sexmaculata* larvae fed with *M. persicae* reached the longest body at the 2nd and 4th instar stages with average body lengths of 2.87 ± 0.04 mm and 6.33 ± 0.09 mm, respectively.

Conclusions: *Myzus persicae* can be considered a potential natural diet for mass-rearing *C. sexmaculata*.

Keywords: *Cheilomenes sexmaculata*, growth, development, aphids, biological control

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INTRODUCTION

The use of synthetic-based pesticides has both positive and negative effects on agricultural industries. Although they have proven effective in controlling pests and increasing crop yields, their excessive use has resulted in negative impacts. The cost of synthetic-based pesticides is relatively high, which can burden all farmers, especially small-scale farmers. According to a report by UNCTAD (2013), small-scale farmers can spend up to 60% of their income on synthetic pesticides. The persistence of synthetic pesticides in the environment can lead to soil, water, and air contamination and loss of biodiversity, leading to adverse effects on human and animal health (UNEP, 2013; Sánchez-Bayo and Wyckhuys, 2019). Pesticide exposure has been linked to various health issues, including

cancer, reproductive problems, and developmental disorders. A systematic review and meta-analysis found that pesticide exposure increases the risk of Parkinson's disease and childhood leukemia (Infante-Rivard *et al*., 1999; Tanner *et al*., 2011). An estimated 200,000 deaths per year globally are due to acute pesticide poisoning (UNEP, 2013). However, farmers get caught on the treadmill, forced to use increasingly toxic chemicals to control insects and weeds that develop pesticide resistance.

Biological control is a practical option for suppressing pest populations. It is easy and safe to use and is a cost-effective and environmentally sound pest control method, especially when compared to the broad-spectrum pesticides often used. Once established, populations are self-sustaining and target-specific. Important insect predators, including ladybird beetles, are generalists in nature. A generalist predator feeds on a wide range of prey species rather than specializing in a particular type of prey (Sanchez and Gillespie, 2022). Generalist predators are often more resilient than other biological control agents under changing environmental conditions such as climate change, habitat destruction, and pesticide use (Martin *et al*., 2019). They can switch to different prey species or habitats as needed. This makes them an attractive option for farmers and other land managers who want to control pests while minimizing pesticide use.

Among generalist predators, *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae), also known as the zigzag ladybird beetle and a member of the family Coccinellidae, is an aphidophagous predator. It is known for its voracious appetite for various soft-bodied insects such as aphids, mites, and scale insects (Abbas *et al*., 2020). In terms of habitat preference, *C. sexmaculata* is commonly found in a wide range of habitats, including agricultural fields, gardens, meadows, and forests. They are often found on plants, hunting for prey, and laying eggs. Coccinelids such as *C. sexmaculata* have a broad diet, and their prey preferences may vary depending on the food availability in the environment (Chaudhary *et al*., 2016). Like other ladybird beetles, *C. sexmaculata* is considered an important biocontrol agent in agricultural settings where it controls insect pest populations (Hodek and Honěk, 1996).

Although *C. sexmaculata* is a generalist predator that existed in the Philippines, specifically in Central Visayas, its use as a biological predator was not known due to farmers' lack of awareness (Iftikhar *et al*., 2018). While *C. sexmaculata* can be reared in captivity, it requires careful handling as it is challenging to rear the insects due to the need for rearing facilities, appropriate food sources, and specific environmental conditions (Reznik *et al*., 2022). The growth and development of biological control predators are important factors to consider to fully utilize them as a biological control for most high-value crops in the country. The type of prey species affects the growth and development of ladybird beetles (Omkar and Srivastava, 2003). As black bean aphids *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) and green peach aphids (*Myzus persicae*) (Sulzer) (Hemiptera: Aphididae) are the most common and abundant aphid species of most vegetables in the country, this study utilized them as a natural diet to comparatively assess which of the two aphid species has significant effects to the growth and development of *C. sexmaculata* for mass rearing potential.

MATERIALS AND METHODS

Collection of *C. sexmaculata*, *A. fabae* and *M. persicae*

Twenty adults of *C. sexmaculata* were manually collected using a pooter or aspirator in the vegetable farm of Barangay Lunas, Asturias Cebu, Philippines. *C. sexmaculata* adults were initially identified through visual observation based on the presence of three black zigzag lines in their elytra (Singh *et al*., 2016). It was further verified using Seek Insect Identification, a mobile application that has a >90% accuracy rate (Manderfield, 2022). *M. persicae* and *A. fabae* adults were collected in the infested eggplant leaves and string bean leaves using a sharp scissor, estimating 300 pieces of aphids per leaf. The collected adults of *C. sexmaculata* and adults of two different aphid species were placed separately in a 7 × 9 × 15 cm (length ×

width × height) rectangular microwavable plastic box at Brgy, Lunas, Asturias, Cebu, Philippines (latitude 10°38'9.08" N, longitude 123°46'55.57" E). All collected insects were reared at the Crop Protection Laboratory and Plant Nursery Facility of the Cebu Technological University (CTU) – Tuburan Campus.

Rearing of Collected *C. sexmaculata* from the Field

The collected twenty adults of *C. sexmaculata* (10 males and 10 females) were transferred in an 8 × 13 × 24 cm (length × width × height) rearing box. *C. sexmaculata* adults were reared on an *ad libitum* supply of mixed aphid species (string bean leaf with *A. fabae* and leaf of eggplant with *M. persicae*). The rearing box was lined with filter paper for oviposition. After two days, the laid eggs in filter paper were separated from the rearing box and placed in a 35 × 15 mm petri dish layered with a moistened cotton pad per cluster for hatching. After hatching, the 1st instar larvae were reared again to become adults (2nd generation). The eggs produced from the 2nd generation of *C. sexmaculata* were collected and placed in petri dishes (35 × 15 mm) for the experiment.

Rearing of *A. fabae* and *M. persicae*

The two collected aphids *(Myzus persicae* and *Aphis fabae*) were reared on their respective host plants. The potted eggplant (*Solanum melongena*) for *M. persicae* and string beans (*Phaseolus vulgaris*) for *A. fabae* were grown in the CTU–Tuburan Campus Plant Nursery Facility. One hundred *A. fabae* per plant were inoculated in the string beans three weeks after seed emergence and *M. persicae* were inoculated in an eggplant two weeks after transplanting. Host plants were placed in different cages covered with mesh nets (0.6 mm diameter). The host plants were maintained in their cages along with their respective aphid species and allowed to reproduce until 2nd generation. The 2nd generation of reared aphids was used in the experiment as a natural diet for *C. sexmaculata*.

Experimental Area

The experiment was conducted in the Crop Protection Laboratory of CTU–Tuburan Campus, Tuburan, Cebu, from November 7, 2022 to December 30, 2022. The light duration of the laboratory room was maintained by a 12-hour light/12-hour dark cycle; the average temperature and the humidity level of the laboratory were 28 °C and 63.3%, respectively.

Experimental Set-up

The experiment involved two sample groups arranged in a completely randomized design (CRD). Each group served as a treatment consisting of 30 replications of the 1st instar larvae of *C. sexmaculata* separated singly per petri dish. The first sample group was fed with *M. persicae*, while the second group was fed with *A. fabae*. Several reared aphids from eggplant and string bean leaves were cut and placed on moistened cotton pads layered in each petri dish (60 × 15 mm). One first-instar larva of *C. sexmaculata* per petri dish was inoculated using a camel-hair brush and sealed using parafilm. As per treatment, the 3rd nymphal stage of aphid species was supplied daily (morning) at a rate of 10, 20, 30, and 40 for the 1st, 2nd, 3rd, and 4th larval instar stages, respectively. The nymphal stage of aphids used was verified through visual observation using a stereomicroscope having an average size of 0.21 mm, vivid body color, and prominent antennae (Begum *et al*., 2018).

Gathered Data

Data gathered during the experiment were the number of aphids consumed, the developmental period of different life cycle stages of *C. sexmaculata*, the body length of different larval stages, and the survival percentage per life cycle stage of *C. sexmaculata*. The number of aphids consumed was determined by counting all the aphids (*A. fabae* and *M. persicae*) consumed by *C. sexmaculata* within the first 24 hours per larval stage. The developmental period of different life cycle stages of *C. sexmaculata* was recorded by counting the interval days from each developmental stage to the next, specifically from eggs until adulthood. The body length of different

larval stages of *C. sexmaculata* was measured from the head of each larval stage up to the tip of its abdominal end using a ruler. Lastly, the survival percentage per life cycle stage of *C. sexmaculata* was computed using the formula:

$$\text{Survival percentage (\%)} = \frac{\text{Number of alive } C.sexmaculata}{\text{Number of } C.sexmaculata \text{ used in the experiment}} \times 100$$

Statistical Analysis

All data were analyzed through a two-sample *t*-test using the Minitab ® 17.1.0 statistical software to check for significant differences between the two sample groups of *C. sexmaculata* fed with *M. persicae* and *A. fabae* at $P < 0.05$ confidence level with assumed equal variances.

RESULTS AND DISCUSSION

Number of Aphids Species Consumed by *C. sexmaculata*

The results show no significant difference in the number of aphids consumed for *A. fabae* and *M. persicae* in the 1st, 2nd, and 3rd instar larval stages of *C. sexmaculata* (Table 1). However, a statistically significant difference ($P = 0.00$) was observed during the 4th instar larval stage of *C. sexmaculata*, wherein each larva consumed more *M. persicae* with a mean of 39.24 ± 0.22 compared to those fed with *A. fabae* (36.00 ± 0.75).

The results of the 1st, 2nd, and 3rd larval instar stages of *C. sexmaculata* can be credited to the limited size of the prey that the larvae can consume and the relatively similar nutritional requirements (carbohydrates and fats) of larvae at different developmental stages (Kraus *et al*., 2019). On the contrary, the 4th instar larvae of *C. sexmaculata* devour the highest, particularly on *M. persicae*. Ladybird beetles go through several instar stages, and how they feed may change over time. The study of Dixon (2000) supported such a result on the ladybird beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in which the 4th instar larvae fed more effectively on larger aphids than the earlier instar larvae. Similarly, the 4th instar larvae of *C. septempunctata* had higher consumption rates when fed with pea aphids than the early instars. Such a trend can be attributed to their large body size which requires a higher energy and is stored for future development from pupation (Kumar *et al*., 2014).

Furthermore, *C. sexmaculata* were more attractive to feed on *M. persicae*, specifically during the 4th instar, than *A. fabae*. Such a result is due to the aphid palatability owing to the aphid species' differences in chemical contents, morphology, and defensive behavior (Omkar and Mishra, 2005). As support, the 4th instar larvae of *C. undecimpunctata* consumed significantly more aphids when *M. persicae* was the prey compared to *A. fabae* (Cabral *et al.,* 2006). It was found that *M. persicae* induced more types of plant-derived volatile organic compounds (VOCs) than *A. fabae* (Staudt *et al.,* 2010). These compounds are attractive to natural enemies of aphids, including ladybird beetles. Herbivory-induced plant volatile compounds (HIPVs) are released when herbivores damage plants (Badra *et al*., 2021).

Table 1 The number of *Aphis fabae* and *Myzus persicae* consumed by *Cheilomenes sexmaculata* after every first twenty-four (24) hours at each larval instar stage

Aphid species	Number of aphid species			
	1st instar	2nd instar	3rd instar	4th instar
Aphis fabae	5.83 ± 0.26	15.50 ± 0.35	25.45 ± 0.46	36.00 ± 0.75[b]
Myzus persicae	6.40 ± 0.20	16.73 ± 0.52	26.62 ± 0.56	39.24 ± 0.22[a]
P-value	0.089	0.056	0.119	0.00

Note: [a,b] Means with in the same column with different superscripts differ ($P < 0.05$)

Development Period of Different Life Cycle Stages of *C. sexmaculata*

Based on the results in Table 2, significant differences in the developmental periods per life cycle stage of *C. sexmaculata* were only observed from the 1st instar to the 3rd instar larval stages (Figure 1). However, no significant differences have been observed between the two sample groups from the 3rd instar larval stage up to adult. The developmental period of *C. sexmaculata* from the 1st to 2nd instar larval stage and 2nd to 3rd instar larva fed with *M. persicae* was 1.50 ± 0.14 and 1.19 ± 0.09 days, while 2.53 ± 0.16 and 2.23 ± 0.17 days feeding on *A. fabae*, respectively. Moreover, a significant difference in the total developmental period of *C. sexmaculata* when fed with *M. persicae* and *A. fabae* was observed, wherein the developmental period of *C. sexmaculata* fed with *M. persicae* was shorter (12.21 ± 1.84 days) compared to those fed with *A. fabae* (15.34 ± 1.98 days).

During the 1st to 3rd instars, *C. sexmaculata* larvae undergo significant physiological and morphological changes requiring substantial energy and resources. The variation in the larval developmental period can be attributed to the quality and quantity of prey (Rocca *et al*., 2020). As reported, *M. persicae* had a higher number of amino acids than *A. fabae* with 20 and 18 amino acids, respectively (Douglas *et al*., 2001; Wu *et al*., 2020). In addition to functioning as protein building blocks, amino acids perform various regulatory functions in cells. These nutrients are essential for animal and insect growth, development, and health (Wu *et al*., 2014). The total developmental period of *C. sexmaculata* was shorter when fed with *M. persicae* (12.21 days) and longer with *A. fabae* (15.34 days). The result of this study has been supported by the study of Ramzan *et al*. (2023) and Mahyoub *et al*. (2013), wherein the total developmental time of *Coccinella septempunctata* (Linnaeus) (Coleoptera: Coccinellidae) was shortest when fed on *M. persicae* (18.6 days) rather than *A. fabae* (21 days).

Table 2 The developmental period (day) of each life cycle stage of *Cheilomenes sexmaculata* when fed with *Aphis fabae* and *Myzus persicae*

Developmental stage of *C. sexmaculata*	Developmental period (day)		P-value
	Aphis fabae	*Myzus persicae*	
Egg – 1st instar	2.00 ± 0.00	2.00 ± 0.00	-
1st instar – 2nd instar	2.53 ± 0.16[a]	1.50 ± 0.14[b]	0.000
2nd instar – 3rd instar	2.23 ± 0.17[a]	1.19 ± 0.09[b]	0.000
3rd instar – 4th instar	1.58 ± 0.18	1.28 ± 0.09	0.144
4th instar – Prepupa	2.78 ± 0.19	2.33 ± 0.16	0.079
Prepupa – Pupa	1.00 ± 0.00	1.00 ± 0.00	-
Pupa – Adult	3.22 ± 0.15	2.91 ± 0.09	0.093
Total developmental stage	15.34 ± 1.98[a]	12.21 ± 1.84[b]	0.032

Note: [a,b] [a,b] Means with in the same row with different superscripts differ ($P < 0.05$)

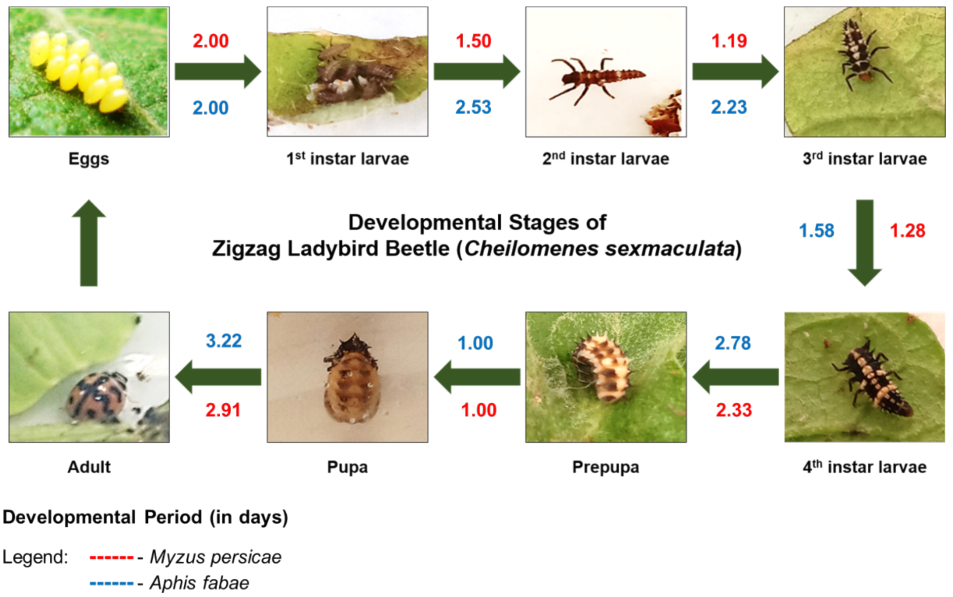

Figure 1 The developmental period (day) of different life cycle stages of *Cheilomenes sexmaculata* when fed with *Aphis fabae* and *Myzus persicae*

Body Lengths of Different Larval Stages of *C. sexmaculata*

Table 3 shows no significant differences in their body lengths in the 1st and 3rd larval instars of *C. sexmaculata*. However, significant differences in body lengths were observed during the 2nd and 4th instar larvae of *C. sexmaculata* among the two sample groups that feed differently with *A. fabae* and *M. persicae*. The average body lengths of the 2nd and 4th instar larvae fed on *M. persicae* were 2.87 ± 0.04 mm and 6.33 ± 0.09 mm, while 2.62 ± 0.06 mm and 5.77 ± 0.08 mm on *A. fabae*, respectively.

The finding perhaps has a connection to the quantity and quality of prey consumed per larval instar stages. The longer body length (size) of the 4th instar larvae fed with *M. persicae* could be due to the significantly higher consumption of its prey compared to those fed with *A. fabae*. The variation of this body size is attributed to the variation also of aphid species (Skouras and Stathas, 2015). The higher voracity of the 4th instar larvae fed on *M. persicae* is possibly due to the higher energy intake requirements for growth and for maintaining a significant body size for pupation (Hodek and Honěk, 1996).

Table 3 Body length (mm) of *Cheilomenes sexmaculata* at different larval stages when fed with *Aphis fabae* and *Myzus persicae*

Aphid species	Body length of *C. sexmaculata* (mm) per larval stages			
	1st instar	2nd instar	3rd instar	4th instar
Aphis fabae	1.00 ± 0.00	2.62 ± 0.06[b]	4.33 ± 0.09	5.77 ± 0.08[b]
Myzus persicae	1.45 ± 0.03	2.87 ± 0.04[a]	4.40 ± 0.09	6.33 ± 0.09[a]
P-value	-	0.001	0.600	0.00

Note: [a,b] Means with in the same column with different superscripts differ (P < 0.05)

Survival Percentage Per Life Cycle Stages of *Cheilomenes sexmaculata*

There were no significant differences in the survival percentage of *C. sexmaculata* as per different life cycle stages between the two sample groups fed differently with *A. fabae* and *M. persicae*. As presented in Table 4, the 1st and 2nd instars had the same survival percentage whether fed on *M. persicae* or *A. fabae* of 100%. However, the succeeding instars (3rd and 4th) until adult had a decreasing survival percentage. The study's result implies that the survival of insect larvae and other insects tends to be lower and often declines as they age. It is because as larvae grow and molt through different instars, they may become more susceptible to certain types of stress predation. Similarly, *H. variegata* (Goeze) (Coleoptera: Coccinellidae) has an immature survival percentage of 49.1% reared on *M. persicae*, which is 25.32% higher than the result's survival percentage of *C. sexmaculata* (adult emergence) reared on *M. persicae* (Lanzoni *et al*., 2004). On the other hand, the decline of the immune system is another reason for the decreasing survival percentage in older ladybirds. It was reported that older ladybirds had a weaker immune response than younger ladybirds, making them more susceptible to diseases and infections (Knapp *et al*., 2022).

Table 4 Survival percentage at each developmental stage of *Cheilomenes sexmaculata* when fed with *Aphis fabae* and *Myzus persicae*

Developmental stage of *C. sexmaculata*	Survival percentage (%) of *C. sexmaculata* per life cycle stages		P-value
	Aphis fabae	*Myzus persicae*	
1st instar	100.00 ± 0.00	100.00 ± 0.00	-
2nd instar	100.00 ± 0.00	100.00 ± 0.00	-
3rd instar	73.33 ± 8.21	90.00 ± 5.57	0.099
4th instar	63.33 ± 8.95	83.33 ± 6.92	0.083
Prepupa	60.00 ± 9.10	80.00 ± 7.43	0.094
Pupa	40.00 ± 9.10	60.00 ± 9.10	0.125
Adult	30.00 ± 8.51	36.67 ± 8.95	0.591

CONCLUSIONS

Based on the study's results, *C. sexmaculata* preferred to feed on *M. persicae*, specifically during the 4th instar stage of their life cycle. Moreover, *M. persicae*, as an aphid diet, enhances the larval body length of *C. sexmaculata* larvae at the 2nd and 4th larval stages. The shorter developmental period of *C. sexmaculata* on *M. persicae* proved that it is the most potential aphid diet in mass-rearing *C. sexmaculata*, even though its survival rate was not statistically significant compared to those fed with *A. fabae*.

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