

REFLEX OF PREY RESOURCE FLUCTUATION ON FEEDING BEHAVIOUR OF A COCCINELLID PREDATOR, *MENOCHILUS SEXMACULATUS* (FABRICIUS)

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Abstract

Learning in insects tends to adapt them to their current environment. However, if learned information is integrated into prey searching process, rate of encountering prey by insect predators is increased. In present study, we considered prey resource fluctuation (cowpea aphid, *Aphis craccivora* Koch) as a stimulus and assessed its response on associative learning of a coccinellid predator, *Menochilus sexmaculatus* (Fabricius). For this, we abruptly changed larvae and adults of *M. sexmaculatus* from their rearing condition of scarce/optimal (control)/abundant prey to five testing conditions, i.e. extremely scarce/scarce/sub-optimal/optimal (control) or abundant; and observed effect of sudden prey changeability on their predation attributes. Results revealed significant influence of resource fluctuation on behavioural plasticity of *M. sexmaculatus* larvae and adults in terms of their modulated consumption rates, conversion efficiencies and growth rates. Lower consumption rates but higher conversion efficiencies and growth rates of fourth instars/adult females on scarce prey and *vice versa* on abundant prey rearing conditions exemplified that probably reflexes of scarce prey are much stronger to mask their usual feeding behaviour. They, therefore, learn more quickly to compensate for a shortage of food by accelerating their conversion efficiencies and growth rates on scarce prey. However, field based studies are further needed to strengthen present findings.

Keywords: Coccinellids; Aphids; Learning; Biocontrol; Resource fluctuation

Introduction

Insects respond to diverse external cues while in search of food, shelter, oviposition sites or mates (Dukas, 2008). The signals emitted by plants, prey or conspecifics are detected by vision, olfaction or other sensory systems in insects. However, response to many of these cues are innate though some may be learned during an insect's lifetime (Remen, 2004; Giurfa, 2013, 2015). Such behavioural flexibility of insect species over their genetically determined behaviour is favoured by environmental unpredictability (Volk, 2001; Dejean *et al.*, 2003; Borenstein *et al.*, 2008). Thus, learning tends to adapt an insect to its current environment. Nevertheless, if learned information is integrated into prey searching process the rate of encountering prey by insect predators may be increased (Papaj, 1993). Although there are different types of learning; but associative learning is quite common in predaceous insects (Remen, 2004; Dawson *et al.*, 2013; Giurfa, 2007, 2015). Such learning involves establishment, through experience, of an association between a stimulus and a response

(Papaj and Prokopy, 1989; Hollis and Guillette, 2015). There are also many cases of insect learning corresponding to habituation, a non-associative type of conditioning in which individual responses decrease when a stimulus is repeated (Dejean *et al.*, 2003; Watanabe and Mizunami, 2007; Vinauger *et al.*, 2013).

The speed, reversibility and mechanism of learning may be related to the frequency with which insects have to switch resources in their lifetime (Vet and Dicke, 1992). Therefore, the ability to learn differs among insect taxa, and some predaceous groups are more 'prepared to learn' certain stimuli than others. Since generalist predators are more flexible in their prey selection behaviour than specialists, there may be a difference in learning between the generalist and the specialist species (Papaj and Prokopy, 1989; Jones and Agrawal, 2017). Although, the specialist species are more likely to have innate responses to stimuli that are directly related to their host or host habitat; however the generalist species are believed to form an association through close temporal and spatial pairing of the stimulus and the response (Waage, 1979).

Coccinellids are an important group of predatory insects with considerable potential to be used as biocontrol agents of aphids and other pest species (Hodek and Honek, 1996; Hodek *et al.*, 2012). However, despite few attempts (Ettifouri and Ferran, 1993; Mondor and Warren, 2000; Ninkovic *et al.*, 2001; Ninkovic and Pettersson, 2003; Remen, 2004; Boivin *et al.*, 2010; Glinwood *et al.*, 2011), their ability to learn has not been studied extensively. While the coccinellids are best at exploiting aphids (Pettersson *et al.*, 2005; Evans, 2008), but owing to their short life span and patchy distribution, availability of aphids is quite uncertain, which may sometimes result in coccinellids starving in fields. In the present study we have considered prey resource fluctuation (cowpea aphid, *Aphis craccivora* Koch; Hemiptera: Aphididae) as a stimulus and assessed its response on the associative learning of a generalist coccinellid predator, *Menochilus sexmaculatus* (Fabricius). For this, we abruptly changed larvae and adults of *M. sexmaculatus* from their rearing condition of scarce/optimal (control)/abundant prey to five testing conditions, i.e. extremely scarce/sparse/sub-optimal/optimal (control) or abundant; and observed effect of sudden prey changeability on their predation attributes.

Menochilus sexmaculatus is an aphidophagous coccinellid of Oriental region with a wide prey range and a greater competitive reproductive ability (Agarwala and Yasuda, 2000; Omkar *et al.*, 2005). *Aphis craccivora* is a serious pest of leguminous crops and is most suitable prey of this coccinellid (Kumar *et al.*, 2013). We hypothesized that during rearing conditions the predatory stages (fourth instars and adult females) of *M. sexmaculatus* would learn ways to associate their energy budget according to the available prey resources. Based on their experiences predatory stages would subsequently regulate their feeding behaviour to utilize the immediate prey resources maximally. Thus, the predatory stages that are reared on scarce prey would exhibit higher predation attributes than those reared on optimal/abundant prey when instantly tested on low prey densities. Because the scarce prey conditions would allow

predatory stages to learn ways to compensate for a shortage of food and to overcome the stress of development. Henceforth, the present study would not only provide information on the energy budget of the concerned predatory stages for various biological activities, but would also help in assessing their ability to learn.

Materials and methods

1. Stock maintenance

Adult stages of *M. sexmaculatus* were collected from the agricultural fields of Varanasi, India (25°20'N, 83°0'E) and paired randomly in plastic Petri dishes (9.0 × 1.5 cm²). They were reared under constant environmental conditions (27±2°C; 65±5% relative humidity; 14h light: 10h darkness) in B.O.D. Incubator (NSW-152; Narang Scientific Works Pvt. Ltd., New Delhi, India) on daily replenished supply of *A. craccivora* Koch (Homoptera: Aphididae) reared on cowpea, *Dolichos lablab* Linnaeus (Fabaceae). The eggs laid were collected every 24 h and subsequent first instars were used for further experimentation.

2. Experimental design

The experiments were divided into two parts. The first part considered the optimisation of different food conditions for life stages of coccinellid species, as suggested earlier by Chaudhary *et al.* (2016). The second part of the experiment evaluated the predation attributes (consumption rate, conversion efficiency and growth rate) of the most voracious predatory stages of coccinellids, i.e. fourth instars and 10-day old virgin females (Mishra *et al.*, 2012; Omkar *et al.*, 2014), under fluctuating prey conditions.

2.1. Optimisation experiment

Individual fourth instars were provided with aphid biomass ranging from 1 to 15 mg (i.e., 1, 2, 5, 10, and 15 mg) intermediate (second and third) instars of prey for 24 hours in ten replicates (biomass measured using Analytical Balance: RA-200, Roy Electronics, Varanasi, India). The minimum biomass at which no prey was left and the larvae survived was referred to as scarce condition. The minimum biomass at which prey was left (1.00 ± 0.50 mg) in some replicates and the larvae survived was referred to as optimal condition (considered as control in the present study). The replicates in which a minimum biomass of 4.00 ± 1.00 mg of prey was left were referred to as abundant condition. Scarce, optimal (control), and abundant biomasses for *M. sexmaculatus* were 2, 10, and 15 mg of prey per larva per day, respectively.

2.2. Evaluation of predatory attributes

The first instars of *M. sexmaculatus* were reared under scarce (n = 50), optimal (n = 50), and abundant (n = 50) prey supply up to the third instar under abiotic conditions similar to that of stock culture. After instars moulted to fourth instars, they were weighed prior to the experiment. The fourth instars from each prey supply were divided into five groups of 10 each. They were kept singly in Petri dishes (size mentioned

above) and provided with either an (i) extremely scarce (1 mg second/third instars of prey), (ii) scarce (2 mg second/third instars of prey), (iii) sub-optimal (5 mg second/third instars of prey), (iv) optimal (10 mg second/third instars of prey) or (v) abundant (15 mg second/third instars of prey) supply of prey for the next 24 h and kept in the above mentioned abiotic conditions. After 24 h, the larvae and the prey left were weighed in each Petri dish. Thereafter, the food of the larvae was switched back to their respective rearing conditions till the larvae reached the adult stage. The newly emerged adults were also fed on the diet similar to the rearing conditions *vide supra*. When the adults reached the sexual maturity (i.e. 10-day-old), females were selected and used for further experimentation.

The 10-day-old unmated adult females from each prey supply were divided into five groups of 10 each, giving a total of 150 females; and shifted to extremely scarce, scarce, sub-optimal, optimal or abundant prey conditions under the above abiotic conditions. Rest of the experimental protocol remained the same as above. The females were weighed before and after the experiment and the biomass of aphids left after 24 h was also recorded.

The natural reduction in aphid biomass in the absence of predators, if any, was analyzed by keeping 1, 2, 5, 10 and 15 mg of prey in five replicates each, under similar abiotic conditions for 24 h and reweighed. Thus, the average loss in prey biomass, if any, was determined. Using the average loss in prey biomass in absence of predators, the loss in biomass of the remaining prey left by the individual predator (larva/adult) after 24 h was determined (using unitary method of mathematics). Now this value was deducted from the prey biomass consumed by that predator after 24 h to obtain the actual consumption rate of the predator.

Consumption rate, conversion efficiency, and growth rate of the fourth instars and the adult females were calculated using the following formulae:

1. Consumption rate ($\text{mg}\cdot\text{day}^{-1}$) =
$$\frac{\text{Aphid biomass consumed by the larval instar adult female (mg)}}{\text{Feeding duration of the larval instar adult female (days)}}$$
2. Conversion efficiency =
$$\frac{\text{Increased biomass of the larval instar adult female (mg)}}{\text{Aphid biomass consumed by the larval instar adult female (mg)}}$$
3. Growth rate (day^{-1}) =
$$\frac{\text{Fresh mass gain of larval instar adult female (mg)}}{[(\text{Feeding duration (days)}) \times (\text{Mean biomass of adult female larval instar (mg)})]}$$

Where, mean body biomass is average of the initial (prior to experimentation) and final (after experimentation) biomass.

Statistical analysis

The distributions of data sets obtained in this study were checked for normality using the Kolmogorov-Smirnoff test. Means were separated using Tukey's test when

data were normally distributed and variances were homogeneous (Bartlett's test for equal variances).

Predation parameters (dependent factors), *viz.* consumption rate, conversion efficiency and growth rate, were subjected to general MANOVA followed by Tukey's post hoc comparison of means, considering stage (4th instar/ 10-day old female), rearing conditions (scarce/ optimal/ abundant), testing conditions (extremely scarce/ scarce/ sub-optimal/ optimal and abundant) and their interaction as independent factors during the analysis.

Moreover, consumption rate of the coccinellid predator was individually regressed with its conversion efficiency and growth rate per rearing condition and per stage; and scattered plot graphs were extrapolated. All statistical analyses were performed using MINITAB 16 (Minitab Inc., State College, Pennsylvania, United States of America).

Results

In the present study, MANOVA revealed significant influence of the testing conditions ($F= 672.94$; $P < 0.0001$; $df= 4, 299$) on the consumption rate of *M. sexmaculatus*. However neither the stage ($F= 0.82$; $P= 0.375$; $df= 1, 299$) and rearing conditions ($F= 1.43$; $P= 0.242$; $df= 2, 299$), nor the interaction between the three independent factors ($F_{\text{interaction}}= 1.21$; $P= 0.290$; $df= 8, 299$) significantly affected the consumption rate of the coccinellid predator. Irrespective of the rearing conditions, fourth instars and adult females had highest consumption rates on the optimal/abundant prey and lowest on the extremely scarce prey testing conditions (Table 1).

The results of MANOVA further revealed that the conversion efficiency was significantly influenced by the rearing conditions ($F= 3.26$; $P= 0.040$; $df= 2, 299$), the testing conditions ($F= 27.86$; $P < 0.0001$; $df= 4, 299$), and the interaction between the three independent factors ($F_{\text{interaction}}= 2.22$; $P= 0.026$; $df= 8, 299$). However the influence of stage ($F= 1.29$; $P= 0.257$; $df= 1, 299$) on the conversion efficiency of *M. sexmaculatus* was not significant. Comparison of means revealed that the scarce prey reared fourth instars/adult females had higher conversion efficiencies than the optimal or abundant prey reared individuals on the five testing conditions. Further, the conversion efficiencies were recorded highest on the optimal/abundant prey and lowest on the extremely scarce prey testing conditions per rearing condition (Table 1).

Moreover, the growth rate was significantly affected by all the three independent factors, *viz.* the stage ($F= 10.38$; $P= 0.001$; $df= 1, 299$), the rearing conditions ($F= 8.28$; $P < 0.0001$; $df= 2, 299$) and the testing conditions ($F= 59.66$; $P < 0.0001$; $df= 4, 299$). However, the interaction between the three independent factors was insignificant ($F_{\text{interaction}} = 1.69$; $P= 0.101$; $df= 8, 299$). Tukey's *post-hoc* comparison of means revealed higher growth rates of scarce prey reared fourth instars/adult females over the optimal/abundant prey reared individuals on the five prey testing conditions. Further, the growth rates were recorded highest on the optimal/abundant prey and lowest on the

extremely scarce prey testing conditions per rearing condition. In addition, the fourth instars exhibited higher growth rates than the adult females on the three rearing and five testing conditions (Table 1).

The scattered plot graphs of conversion efficiency/ growth rate regressed individually with the consumption rate revealed that with increase in consumption rate, both the conversion efficiency and growth rate of fourth instars/ adult females were increasing; and irrespective of the rearing conditions, higher conversion efficiencies and growth rates were achieved at higher consumption rates (Fig. 1). However, the predation attributes were recorded highest under the scarce prey, followed by the abundant prey and lowest under the optimal prey rearing conditions (Fig. 1; Table 1).

Discussion

In the present study, prey consumption rates of predatory stages of *M. sexmaculatus* were not affected by rearing conditions, but were significantly influenced by the testing conditions when there were fluctuations in the prey availability. Although being reared on scarce, optimal and abundant prey, the fourth instars and adult females consumed equivalent prey biomass. However, irrespective of the rearing conditions, predatory stages had highest consumption rates on optimal/abundant prey and lowest on extremely scarce/scarce prey testing conditions.

While earlier studies have shown that coccinellids have suboptimal and optimal consumption rates when reared consistently on scarce and abundant prey (Hodek *et al.*, 2012; Bista and Omkar, 2014). However, constancy in consumption rates of predatory stages of *M. sexmaculatus* under three rearing conditions and variability under five testing conditions, probably suggests that the predatory stages change their consumption rates according to immediate prey conditions as in case of sudden fluctuations in prey resource availability. Possibly, during rearing conditions the larvae and adults of *M. sexmaculatus* learn to correlate their energy budget or metabolic costs with the prey availability, which subsequently regulates their consumption rates to utilize available prey resource maximally.

Since behavioural flexibility of insects is encouraged by environmental unpredictability (Volk, 2001; Dejean *et al.*, 2003); the immediate prey conditions (testing conditions) possibly act as stimuli for the predatory stages of *M. sexmaculatus* to develop some instantaneous compensatory abilities to overcome prey resource fluctuations. But the lower consumption rates of *M. sexmaculatus* stages under extremely scarce or scarce prey testing conditions further substantiate that food availability is a limiting factor under extremely scarce/ scarce prey testing conditions. However, substantial influence of rearing and testing conditions on conversion efficiency and growth rate of larvae and adults of *M. sexmaculatus* in the present study further demonstrate that despite having lower consumption rates on extremely scarce/ scarce prey testing conditions, larvae/adults of *M. sexmaculatus* compensate for a shortage of food by accelerating their conversion efficiencies and growth rates, thereby converting and using higher prey biomass than usual when suddenly encountering

fluctuating prey resources (testing conditions). In contrast, optimal or abundant prey conditions possibly signal the predatory stages to learn to consume the prey biomass maximally but less efficiently. The results are in conformity with those reported earlier (Chaudhary *et al.*, 2016).

The scattered plot graphs of conversion efficiency/ growth rate regressed individually with the consumption rate also reveal that irrespective of the rearing conditions the conversion efficiency and growth rate of predatory stages are increasing with increasing consumption rates. However, the higher predation attributes of predatory stages under the scarce prey rearing conditions reveal that probably the reflexes of scarce prey are much stronger to mask the genetically determined behaviour; and the predatory stages may learn more quickly to compensate for a shortage of food by accelerating their conversion efficiencies and growth rates. Thus, the increase in conversion efficiency and growth rate under prey scarce condition are amongst the several mechanisms displayed by coccinellids to overcome the shortage of trophic resource, as suggested earlier by Schüder *et al.* (2004) and Chaudhary *et al.* (2016) in the larvae of *Adalia bipunctata* (L.) and *M. sexmaculatus*, respectively.

In the present study, fourth instars exhibited higher growth rates than the adult females on the three rearing and five testing conditions. Higher growth rate of larvae over adult females may possibly be due to overcome the stress of pupation and further metamorphosis. Similar results have also been reported earlier in coccinellids, both on constant (Mishra *et al.*, 2011; Kumar *et al.*, 2013) and fluctuating (Chaudhary *et al.*, 2016) prey resources.

The results of the present study therefore affirm our hypothesis and suggest that trophic unpredictability influences the behavioural flexibility of *M. sexmaculatus*; and the predatory stages that are reared under scarce prey conditions learn more quickly to compensate for a shortage of food than those reared under optimal/ abundant prey conditions. Owing to this intrinsic advantage, *M. sexmaculatus* has the potential to establish itself earlier in an area of introduction over the other coexisting ladybird species; as also reported earlier by Chaudhary *et al.* (2016). However, field based studies are still needed to further strengthen the present findings.

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Figure legends

- Fig. 1.** The scattered plot graphs of conversion efficiency and growth rate of *M. sexmaculatus* regressed individually with the consumption rate per rearing condition.

Table 1. Predation attributes of the fourth instar larvae and adult females of *M. sexmaculatus* under different rearing and testing conditions.

Stage	Rearing condition	Testing condition	Consumption rate (mg day ⁻¹)	Conversion efficiency	Growth rate (day ⁻¹)
Fourth instar	Scarce	Extremely scarce	1.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Scarce	2.00±0.00 ^{bA(a)}	0.16±0.08 ^{bB(b)}	0.05±0.03 ^{bB(b)}
		Sub Optimal	5.00±0.00 ^{cA(a)}	0.15±0.06 ^{bB(b)}	0.13±0.04 ^{cA(b)}
		Optimal	8.69±0.84 ^{dA(a)}	0.23±0.04 ^{bB(a)}	0.31±0.05 ^{dC(a)}
		Abundant	10.09±1.11 ^{dA(a)}	0.23±0.05 ^{bA(a)}	0.29±0.07 ^{dA(a)}
	Optimal	Extremely scarce	0.92±0.08 ^{aA(a)}	0.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Scarce	1.83±0.17 ^{bA(a)}	0.02±0.02 ^{bA(a)}	0.01±0.00 ^{aA(a)}
		Sub Optimal	4.51±0.21 ^{cA(a)}	0.15±0.05 ^{bB(a)}	0.09±0.03 ^{cA(a)}
		Optimal	9.24±0.34 ^{dA(a)}	0.08±0.01 ^{bA(a)}	0.11±0.02 ^{cA(a)}
		Abundant	9.73±0.44 ^{dA(a)}	0.18±0.03 ^{bA(a)}	0.21±0.05 ^{cA(a)}
	Abundant	Extremely scarce	1.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Scarce	2.00±0.00 ^{bA(a)}	0.10±0.04 ^{bB(b)}	0.03±0.02 ^{bA(b)}
		Sub Optimal	4.50±0.21 ^{cA(a)}	0.24±0.04 ^{bB(a)}	0.14±0.03 ^{cA(a)}
		Optimal	8.19±0.27 ^{dA(a)}	0.21±0.04 ^{bB(b)}	0.20±0.03 ^{cB(b)}
		Abundant	9.15±0.41 ^{dA(a)}	0.34±0.04 ^{cB(b)}	0.35±0.05 ^{dB(b)}
Adult female	Scarce	Extremely scarce	1.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Scarce	2.00±0.00 ^{bA(a)}	0.02±0.01 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Sub Optimal	4.62±0.18 ^{cA(a)}	0.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Optimal	8.97±0.27 ^{dA(a)}	0.38±0.12 ^{cB(a)}	0.32±0.08 ^{dB(a)}
		Abundant	10.74±0.68 ^{dA(a)}	0.25±0.05 ^{bB(a)}	0.28±0.06 ^{dB(a)}
	Optimal	Extremely scarce	1.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Scarce	2.00±0.00 ^{bA(a)}	0.02±0.01 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Sub Optimal	4.42±0.31 ^{cA(a)}	0.15±0.06 ^{bB(a)}	0.07±0.03 ^{bB(a)}
		Optimal	7.71±0.28 ^{dA(a)}	0.19±0.03 ^{bB(b)}	0.13±0.02 ^{cA(a)}
		Abundant	10.00±0.61 ^{dA(a)}	0.20±0.05 ^{bA(b)}	0.16±0.04 ^{cA(a)}
	Abundant	Extremely scarce	1.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Scarce	2.00±0.00 ^{bA(a)}	0.02±0.01 ^{aA(a)}	0.00±0.00 ^{aA(a)}
		Sub Optimal	4.62±0.22 ^{cA(a)}	0.28±0.11 ^{cB(a)}	0.11±0.04 ^{cB(a)}
		Optimal	9.10±0.35 ^{dA(a)}	0.12±0.01 ^{bB(a)}	0.10±0.01 ^{cA(a)}
		Abundant	10.54±0.61 ^{dA(a)}	0.16±0.02 ^{bA(a)}	0.14±0.02 ^{cA(a)}

*Small letters, large letters and small letters in parentheses represent comparison of means amongst rearing conditions, amongst testing conditions and between predatory stages, respectively.

