PREFERENCE OF THE APHIDOPHAGOUS LADYBIRD PROPYLEA DISSECTA FOR TWO SPECIES OF APHIDS REARED ON TOXIC HOST PLANTS

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ABSTRACT

We investigated prey preference of adult male and female *Propylea dissecta* (Mulsant) when fed on the aphids, *Aphis craccivora* and *Lipaphis erysimi*, which sequester toxic allelochemicals from their host plants. Both the male and female adults of *P. dissecta* prefer to consume *L. erysimi* in most mixed diet treatments (cafeteria experiment). This was well supported by significantly high values of the β and C prey preference indices. However, when provided with these aphids separately, the adults showed no significant difference in aphid consumption, regardless of the species of aphid and sex of the adult ladybird. We conclude that host plant allelochemicals/toxicants have a direct effect on prey preference of ladybirds. Host plant toxic constituents can alter the biochemical composition of the most preferred prey and make them the least preferred. Mixing two toxic similar diets can make one diet more suitable than the other.

Keywords: Coccinellidae, *Aphis craccivora*, *Aphis nerii*, *Ranunculus sceleratus*, *Brassica*, diet

Introduction

Dietary habits of predaceous ladybirds (Coleoptera: Coccinellidae) have fascinated many workers (Evans and Gunther 2005; Michaud 2005; Provost et al. 2006; Honek et al. 2008a, b; Sloggett 2008a, b; Hodek and Evans 2012). Ladybirds' food is classified as either essential (supports development and reproduction) or alternative (supports only survival) (Hodek and Evans 2012). Much focus has been given recently to diets that are nutritious or toxic for ladybirds (Guroo et al. 2017). Toxic prey can become more nutritious if ladybirds are fed on them for several generations (Rana et al. 2002). Mixing toxic and nutritious diets could be more beneficial than providing a suitable monotypic diet (Soares et al. 2004; Ferrer et al. 2008; Nedved and Salvucci 2008). Toxicity of aphids is directly dependent on host plant toxicants or allelochemicals, which are sequestered by aphids (Pratt et al. 2008) as a means of defence against predators and may reduce their growth rates (Noriyuki et al. 2012). Hence, prey suitability is directly related to the host plants and the same prey could be either nutritious or toxic depending on its host plant. Wu et al. (2010) found that the aphid, *Aphis gossypii* (Glover) reared on five different host plants have different effects on the population parameters of its predator, *Hippodamia variegata* (Goeze).

Aphids are the primary food of aphidophagous ladybirds, however, their suitability is dependent on various factors (Rana et al. 2002; Soares et al. 2004). Hodek and Evans (2012) consider the black bean aphid, *Aphis craccivora* (Koch) to be toxic due to allelochemicals and/or toxicants, *viz*. amines canavanine and ethanolamine (Obatake and Suzuki 1985) that it sequesters from its host plant (Hukusima and Kamei 1970). The buttercup, *Ranunculus sceleratus* L. is a toxic plant with global wide distribution and contains toxic allelochemicals that may

be easily sequestered by aphids, particularly *A. craccivora* (Gupta and Singh 1983; Aslam et al. 2012). It is likely that *A. craccivora* raised on this host plant will be toxic for predators that prefer this aphid when feeding on other hosts. For instance, *A. craccivora,* infesting *Dolichos lablab*, is palatable for the predatory ladybird, *Propylea dissecta* (Mulsant) and is preferred over other aphids (Omkar and Pervez 2001; Chaudhary et al. 2016) and is highly nutritious (Pervez and Omkar 2004; Omkar and Mishra 2005).

Mustard aphid, *Lipaphis erysimi* (Kaltenbach) infesting *Brassica* plants, especially *Brassica campestris* L., sequesters allelochemicals that lower their palatability and nutritive value (Ahuja et al. 2010). This results in slower development and reduced oviposition in many of ladybirds (Pervez and Omkar 2004; Omkar and Mishra 2005) that feed on such aphids. Hence, it would be interesting to quantify the effect of a preferred aphid-prey raised on a toxic host plant with that of a well-established toxic prey raised on a toxic host. Giorgi et al. (2009) review the evolution of food preferences in ladybirds. Previous studies on food preferences of predaceous ladybirds used single species of aphid separately (Omkar et al. 1997, 1999; Omkar and Bind 1998; Omkar and Pervez 2001) and recorded their effect on development and reproduction (Pervez and Omkar 2004; Omkar and Mishra 2005). However, providing several food choices in the same microhabitat, *i.e.* a cafeteria setup, could be more accurate in quantifying prey preference in ladybirds (Ferrer et al. 2008; Nedved and Salvucci 2008; Šenkeříková and Nedvěd 2013; Guroo et al. 2017).

Propylea dissecta is an aphidophagous ladybird occurring in agro-ecosystems in North India and have a high aphid biocontrol potential (Omkar and Pervez 2004a; Pervez and Omkar 2011). Both adults and larvae of *P. dissecta* prefer *A. craccivora* reared on *D. lablab* over *L. erysimi*

reared on *Brassica campestris* (Pervez and Omkar 2004; Omkar and Mishra 2005). Comparative demographics of this predator attacking five species of aphids indicates that its abundance is more synchronized with *A. craccivora*, while the advent of *L. erysimi* marks its decline in the field (Omkar and Pervez 2004b). Comparison of the functional responses of *P. dissecta* reveals its better at attacking and handling *A. craccivora* than other aphids (Pervez and Omkar 2005). The question, therefore, is could a highly preferred prey in terms of ease of attack and handling, consumption and net reproduction be rejected or less preferred if cultured on a toxic plant? Considering the fact that plant allelochemicals have a major role in the preference and suitability of prey, we hypothesize that *A. craccivora* reared on the toxic *R. sceleratus* will be less preferred when mixed in different proportions with *L. erysimi*. Hence, we performed laboratory experiments to investigate the prey preference of *P. dissecta* for aphids reared on toxic plants.

Materials and Methods

Stock maintenance

We collected adults of *P. dissecta* in the suburbs of Kashipur, Uttarakhand, Northern India (30.2937°N, 79.5603°E) and brought them to the laboratory. They were sexed and paired in Petri dishes (2.0 × 9.0 cm diameter) containing an *ad libitum* supply of the aphids, *A. craccivora* and *L. erysimi* infesting pieces of leaves/twigs of *R. sceleratus* and *B. campestris*, respectively (n = 10), and then kept in an Environmental Test Chamber (*REMI Instruments*, India) at 27 ± 1 °C, 65 ± 5% R.H and 12L : 12D. We monitored mating pairs daily and any eggs they laid were transferred to a new Petri dish (size as above). On hatching, we transferred the neonates to muslin covered beakers (1 litre) and provided them with an *ad libitum* diet until they completed their larval development. We sexed the emerging F_1 adults, which were then isolated and raised on their parental diets.

Prey preference in a cafeteria setup

Prey preference was studied by offering the aphids, *A. craccivora (Ac)* and *L. erysimi (Le)* to adult male and female *P. dissecta* in three ratios, *i.e. Ac : Le* 50 : 100, 75 : 75 and 100 : 50. For this purpose, a 12-hour starved 10-day-old adult male *P. dissecta* was placed in a glass beaker containing the two aphids, *Ac* and *Le* (50 : 100 respectively) infesting plant twigs of their respective host plants (as mentioned above). The beaker was covered with muslin fastened with a rubber band and kept in the Environmental Test Chamber (*REMI Instruments*, India) at 27 ± 1 °C, 65 ± 5% R.H and 12L : 12D. Similar treatments were carried using the other two mixtures of aphids (*Ac: Le* = 75 : 75 and *Ac : Le* = 100 : 50). After 24 hours, the beaker was taken out and the number of unconsumed aphids counted to quantify the number of aphids consumed. The experiment was replicated ten times (n = 10).

The experiment was repeated using adult female *P. dissecta* as the predator. We calculated Manly's preference index (Manly 1972) for each treatment using the formula, $\beta = \log (N_A / r_A) / [\log (N_A / r_A) + \log (N_B / r_B)]$, where N_A and N_B are the number of prey A and prey B offered to the ladybird and r_A and r_B are the numbers of unconsumed prey. This index overcomes the error associated with prey depletion, i.e. it is applicable in those experiments, where killed prey items are not replaced (Cook 1978; Sherratt and Harvey 1993). If β is close to 1, the predator prefers prey A and if close to 0, prey B. An index value close to 0.5 indicates no preference. We tested the β obtained for each treatment for significant difference from a value of (0.5) using a one sample t-test and statistical software, SAS Version 9.0. Prey preference was also analyzed using the *C* index ($C = (E_A \times N_B) / (E_B \times N_A)$) (Sherratt and Harvey 1993), where E_A and E_B are the number of prey A and prey B consumed. A *C* value between 0 and 1 indicates a preference for prey B and a value more than 1 indicates preference for prey A. We subjected the *C*-index for each treatment to a one sample t-test to determine whether it was significantly different from a value of 1.0 using SAS 9.0. We subjected data on the number of prey consumed to Wilcoxon's matched-pairs signed rank test and the proportion of each prey consumed in a cafeteria setup to a two sample t-test in SAS Version 9.0. We subjected the proportions of aphids, *A. craccivora* and *L. erysimi* consumed by adult male and female *P. dissecta* to a two-way ANOVA using 'aphid species' and 'sex' as independent variables and 'proportion of prey consumed' as a dependent variable using SAS 9.0.

Response to monotypic diet

Adult male and female *P. dissecta* were each provided with a monotypic diet of *Ac* or *Le* to determine their feeding propensity. For this purpose, an adult male was kept in a glass beaker containing 100 third instars of *Ac* infesting a twig of the respective host plant (as above). The beaker was covered with a muslin cloth fastened with a rubber band and kept in the Environmental Test Chamber (*REMI Instruments*, India) at 27 ± 1 °C, 65 ± 5% R.H and 12L : 12D. After 24hours, the beakers were taken out and the number of live aphids counted to quantify the number of aphids consumed (n = 10). The experiment was repeated using adult female *P. dissecta* as the predator. We tested the data on prey consumption for normality using the Kolmogorov–Smirnoff test and homogeneity of variance using Bartlett's test in statistical software, SAS Version 9.0. The data on the consumption of aphids when provided with a monotypic diet were subjected to a two sample t-test using SAS 9.0 and the means compared. We subjected the data on the number of the two species of aphids consumed by the adult male and female *P. dissecta* to a two-way ANOVA using 'aphid species' and 'sex' as independent variables and 'prey consumed' as dependent variable in SAS 9.0.

Results

Prey preference in a cafeteria setup

Adult male *P. dissecta* preferred the aphid, *L. erysimi* over *A. craccivora* at all three ratios of the mixed diet tested, *i.e.* at 50 : 100 (*Ac : Le*) ratio ($Z_{(1, 18)} = -2.701$; P = 0.0069), 75 : 75 (*Ac : Le*) ratio ($Z_{(1, 18)} = -2.803$; P = 0.005) and 100 : 50 (*Ac : Le*) ratio ($Z_{(1, 18)} = -2.701$; P = 0.0069; Fig. 1a). However, adult females only preferred *L. erysimi* over *A. craccivora* when an equal proportions of the aphids were provided (75*Ac* : 75*Le*) ($Z_{(1, 18)} = -2.701$; P = 0.0069) and at the 100 *Ac* : 50*Le* ratio ($Z_{(1, 18)} = -2.599$; P = 0.009). The proportion of prey consumed at the 50*Ac* : 100*Le* ratio by female *P. dissecta* was not statistically significant ($Z_{(1, 18)} = -2.599$; P = 0.009 ; Fig. 1b). Two-way ANOVA revealed a significant main effect of 'aphid' species on the proportion of prey consumed (F = 44.20; P < 0.0001; d.f. = 1). The main effect 'sex' was not found to be statistically significant (F = 44.20; P < 0.0001; d.f. = 1). The interaction between 'sex' and 'aphid' did not differ significantly (F = 2.64; P = 0.107; d.f. = 1). Both β and C indices had significantly negative t-values in all the combinations except for adult females provided with the aphid proportion 50*Ac* : 100*Le* (Table 1).

Response to a monotypic diet

Both adult male (t = −0.22; P = 0.83; d. f. = 1, 9) and female (t = 0.92; P = 0.383; d. f. = 1, 9) *P. dissecta* consumed the aphids, *A. craccivora* and *L. erysimi* raised on toxic hosts, equally (Fig. 2). The two-way ANOVA reveals that the main effects 'aphid species' (F = 0.11; P = 0.745; d.f. = 1) and 'sex' (F = 0.18; P = 0.713; d.f. = 1), along with the interaction 'aphid species' x 'sex' (F = 0.43; P = 0.516; d.f. = 1, 39) were not statistically significant.

Table 1 Mean values of the β and *C* indices of *P. dissecta* provided with diets made of different proportions of the aphids *A. craccivora* and *L. erysimi*. The predator prefers *A. craccivora* if β is close to 1 and *L. erysimi* if β is close to 0; β close to 0.5 indicates no preference. *C* index more than 1 indicates a preference for *A. craccivora*, while *C* index between 0 and 1 indicates a preference for *L. erysimi*.

Life stage	Prey ratio	β index	t-value	C index	t-value
Adult Male	50:100	0.44 ± 0.02	t = −3.57; P = 0.006	0.84 ± 0.03	t = −3.87; P = 0.004
	75:75	0.37 ± 0.02	t = −6.98; P < 0.0001	0.67 ± 0.03	t = −8.14; P < 0.0001
	100:50	0.37 ± 0.03	t = −5.54; P < 0.0001	0.69 ± 0.04	t = −5.92; P < 0.0001
Adult Female	50:100	0.46 ± 0.03	t = −1.06; P = 0.318	0.95 ± 0.06	t = −0.40; P = 0.7
	75:75	0.42 ± 0.02	t = −4.58; P < 0.001	0.80 ± 0.04	t = −4.23; P < 0.001
	100:50	0.42 ± 0.02	t = −4.14; P = 0.003	0.78 ± 0.04	t = −4.07; P = 0.003

Discussion

Both adult male and female *P. dissecta* consumed more *L. erysimi* than *A. craccivora* in almost all mixed diet combinations. This supports our hypothesis that *A. craccivora* reared on the toxic *R. sceleratus* will be less preferred when mixed with *L. erysimi*. It is also evident from the preference (β and *C*) indices that *L. erysimi* is preferred over the toxic *A. craccivora*. Our finding agrees with that of Guroo et al. (2017) who report similar preference indices for the larvae and adults of *Coccinella septempunctata* L. for *L. erysimi* when mixed with the toxic prey, *Brevicoryne brassicae* L.. However, *C. septempunctata* prefers to consume *L. erysimi* over other species of aphid (Omkar et al. 1997;

(a)

(b)
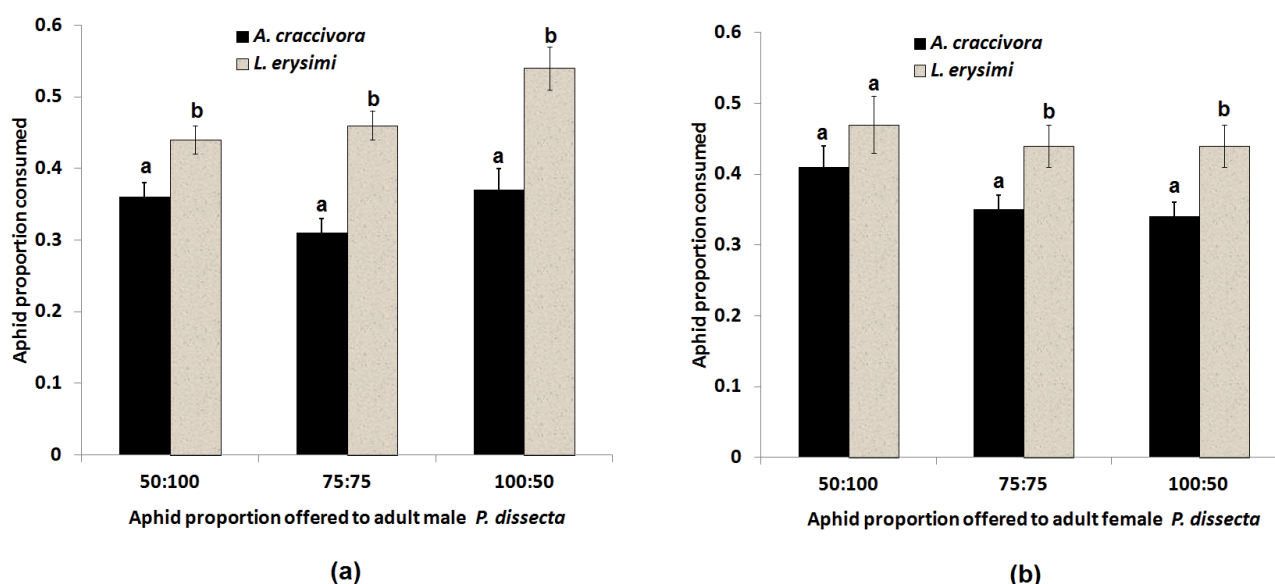
Fig. 1 Proportion of aphids (*A. craccivora*; *L. erysimi*) consumed by adult (a) male and (b) female *P. dissecta*. Error bars show Standard Error. Different letters indicate the data in the two columns differ significantly.
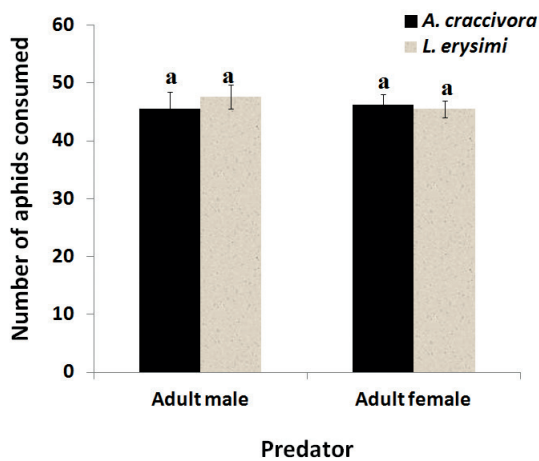
Fig. 2 Prey consumption by adult male and female *P. dissecta* when fed on a single species of aphid. Error bars denote Standard Error. Same letter indicates that data are not significantly different.

Omkar and Srivastava 2003; Ali and Rizvi 2007), whereas *P. dissecta* does not (Pervez and Omkar 2004; Omkar and Mishra 2005). It is clear that plant allelochemicals and toxicants have a direct effect on the quality of aphids in terms of their palatability for predators. *Ranunculus* sp. have secondary metabolites, like glycosides, phenolic compounds, steroids, di and tri terpenes, coumarins and flavanoids, which when sequestered by herbivores may harm predators (Hachelaf et al. 2013). This is the first laboratory study using *A. craccivora* raised on the toxic buttercup, *R. sceleratus*. Previous studies on *P. dissecta* indicate that *L. erysimi* is its least preferred and less suitable prey (Pervez and Omkar 2004; Omkar and Mishra 2005) because this aphid contains secondary metabolites and glucosinolates (Francis et al. 2002; Ahuja et al. 2010). Preference for *L. erysimi* clearly indicates that allelochemicals from *Ranunculus* sp. are more toxic and have a greater effect on the quality of *A. craccivora*.

Recently, Guroo et al. (2017) infer that ladybirds can preferentially feed on toxic or rejected prey if also provided with nutritious prey, as the latter may supply the necessary nutrients absent in the former. Hence, the possible essential nutrients lacking in *L. erysimi* might have been replaced by eating *A. craccivora* resulting in the preference for the former species by *P. dissecta*. The seven-spotted ladybird, *Coccinella septempunctata* L. eats twice as many of the toxic aphid, *Aphis sambuci* L. than of the nutritious aphids, *viz. Acyrthosiphon pisum* (Harris) and *Aphis philadelphi* (Nedved and Salvucci 2008). Šenkeříková and Nedvěd (2013) report that the Harlequin ladybird, *Harmonia axyridis* (Pallas) consumes a greater number of the toxic aphid, *A. sambuci* than of the suitable aphid, *Aphis fabae cirsiiacanthoidis (*syn. *A. philadelphi)*. Similarly, the two spotted ladybird, *Adalia bipunctata* (L.) consumes more *A. craccivora* than of the more suitable aphid, *Acyrthosiphon pisum* (Harris) (Ferrer et al. 2008). Soares et al. (2004) report that a mixed aphid diet is better than a monotypic aphid diet in certain situations (Soares et al. 2004). Continuous rearing on toxic prey for a few generations can also skew the preference of ladybirds towards toxic prey rather than the previously considered more nutritious prey (Rana et al. 2002).

We found a similar reluctant behaviour of both adult male and female *P. dissecta* when provided with monotypic diets. The main effects of 'aphid species' and 'sex' were not statistically significant, which reveals similarity in terms of biochemical contents of the two aphids, and the response of the two sexes of adults towards these aphids. This indicates that both prey were preferred equally when provided separately, and the significant preference for *L. erysimi* in the cafeteria experiment reveals that prey preference was skewed towards this prey. This furthers affirms our theory that possible vital nutrients missing in *L. erysimi* might have been supplemented by eating *A. craccivora*. We conclude that: (i) host plant allelochemicals have a direct effect on the prey preference of ladybirds, (ii) a change in host plant can result in a most preferred prey becoming less preferred, and (iii) mixing two toxic similar diets can make one diet more suitable than the other.

Acknowledgements

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