Adaptive plasticity of parasitoid *Acerophagus papayae* Noyes and Schauff on Papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink *vis-a-vis* divergent natural selection

R. Nisha*[1] and J.S. Kennedy[2]

[1]Department of crop protection, Imayam Institute of Agriculture and Technology, Thuraiyur, Trichy – 621 206
[2]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore- 641 003

Abstract: *Adaptive plasticity of the parasitoid Acerophagus papayae was estimated using the divergent natural selection process involves different host plants of papaya mealybug PMB Paracoccus marginatus like papaya, cotton, mulberry, brinjal, hibiscus, tapioca and comparatively with potato sprouts. The plasticity of parasitoid was exploited out using the infestivity and virulence studies through the parameters involved in the host selection process of the parasitoid on PMB. The results showed significant differences in parasitoid infectivity on their natal host compared with the non-natal hosts. However, parasitoids showed a similar high fitness on both natal and nonnatal hosts, thus supporting a lack of host adaptation in these introduced parasitoid populations. The result concluded the role of phenotypic plasticity in fitness related traits of parasitoids, enabling them to maximize fitness on alternative hosts. This could be used to increase the effectiveness of biological control of insect pests.*
Keywords: *Adaptive plasticity, parasitoid, Acerophagus papayae, natural selection, phenotypic plasticity, Paracoccus marginatus, papaya mealybug*

I. Introduction

The host selection process in parasitoid involves a sequence of phases mediated by physical and chemical stimuli from the host, the substrate, and/or associated organisms, eventually leading to successful parasitism (Vinson, 1985 and Godfray, 1994). Because parasitoid foraging time is limited and the potential cues available are numerous, the parasitoid faces the need to optimize exploitation of available cues and discriminate those most reliable in indicating the presence of a suitable host (Hilker and Meiners, 2006). The use of alternative hosts imposes divergent selection pressures on parasitoid populations. In response to selective pressures, these populations may follow different evolutionary trajectories. Divergent natural selection could promote local host adaptation in populations, translating into direct benefits for biological control, thereby increasing their effectiveness on the target host. Alternatively, adaptive phenotypic plasticity could be favored over local adaptation in temporal and spatially heterogeneous environments, Zepeda Paulo *et al*. (2013). This study focused on understanding the mechanism of local adaptations *ie*. adaptive plasticity in the parasitoid *A. papayae* and its host papaya mealybug *P. marginatus* system. All this poses the need to incorporate new eco-evolutionary approaches in the selection process of biocontrol agents. In addition, our present study reaffirms the usefulness of this experimental approach to study patterns of adaptation in biocontrol agents to certain target hosts, thus making a clear distinction between infectivity (preference) and virulence (proxy of fitness) of parasitoids, because often both are camouflaged in the measures of adaptation or explicitly focused on infectivity as the main measure of host adaptation (Zepeda-Paulo *et al.*, 2013).

II. Methodology

The study was carried out in the Bio control laboratory of Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu during 2013 – 2014. The mealybug and parasitoid were reared and the development time, parasitic potential and survival probability of parasitoid was found using the following methodology. To study the response of parasitoids to different selection agents (PMB from different host plants), a reciprocal transplant experiment was conducted to determine the infestivity and virulence of parasitoid females to their natal hosts and non-natal hosts. This type of experiment has proven to be useful in the detection of adaptive patterns, studying the mean fitness shown by a set of populations or demes through a set of experimental habitats, and allowing the direct testing of the role of a particular environmental factor as a divergent selection agent (Kawecki and Ebert, 2004).

i. Mass culturing of parasitoid *Acerophagus papayae* on papaya mealybug *Paracoccus marginatus* using potato sprouts

Potato was used as food source for rearing mealybugs (Serrano and Laponite, 2002). Two month old Robin eyed healthy seed potatoes were bought and kept in a dark air conditioned room for four to five days to induce sprouting. Sprouted potatoes were washed in water and disinfected with 1% carbendazim solution. Later, two cm incision was given using a sharp blade and treated with 100 ppm gibberlic acid for half an hour. Potatoes were air dried and transferred to plastic trays (10 tubers/ tray placed at about 2 cm apart in each tray of 18'' diameter) containing solarized sand. These trays were kept in rearing room and watered gently. Eight to ten days after sowing, potato sprouts emerged and reached a height of 4 to 6 cm were used for inoculation with mealybugs. Papaya mealybugs collected from different host plants like papaya, tapioca, cotton, mulberry, brinjal and hibiscus were released on potato sprouts using camel hair brush at the rate of 3 to 5 ovisacs per potato and mealybugs en masse were obtained within 25 to 30 days of release. They were used for mass culturing of *A. papayae*. Mass culturing was also carried out in above said host plants and used for further experiments. The sprouted potatoes and infested host leaves, colonized with mealybugs were transferred to oviposition cages of 45 x 45 x 45 cm. Ten *A. papayae* adults were allowed inside the cage for parasitisation. After 10 days of release, the sprouts and leaves along with the mummified mealybugs were removed from the potatoes using a fine scissor and collected separately in the plastic containers. The emerged parasitoids were collected by an aspirator and observed for development time and parasitic potential.

ii. Experiment on development and parasitic potential of *Acerophagus papayae* on mealybugs from different host plants

Each assay was conducted in an experimental arena of 25-cm-diameter mud pots. The mealybugs reared in different host crops and potato sprouts were used in this study. The effect of different host crops on the development time of *A. papayae* was assessed. Twenty *A. papayae* were released per plant infested with mealybugs and covered with a mylar film cage. The mealybugs reared from the potato sprouts were also taken as another source of treatment in the plastic basins. The experiment was conducted in a completely randomized design (CRD) with four replications. One week after releasing the parasitoids, the sample leaves and sprouts were taken from each plant and potato sprout respectively. They were transferred to plastic containers of 10 cm diameter covered with a muslin cloth. The containers were checked daily for parasitoid emergence and from this data, the development period and the duration of different life stages of *A. papayae* on mealybugs reared on different hosts were worked out. Two months after releasing the parasitoids, the parasitism rate was calculated in second and third instars and adult female mealybugs separately using the formula,

$$\text{Parasitisation rate of } A.\,papayae = \frac{\text{Number of parasitised melybugs}}{\text{Total number of mealybugs offered}} \times 100$$

The differences in the parasitisation rate of *A. papayae* on the mealybugs from different hosts and different stages were recorded. Based on the above method, the bioecology of *A. papayae* was studied. To study variation in the reproductive success of the parasitoids on mealybug from different host plants, survival percentages of parasitoid on mealybugs of different host plants was compared. Survival alone is not a sufficient to estimate the reproductive success. Therefore developmental time, adult longevity and lifetime fertility were also studied on parasitoids emerging on mealybugs from different host plants.

iii. Survival probability

To examine the survival probability of *A. papayae* on *P. marginatus* from different host plants, 10 individuals of second instar papaya mealybugs were collected from different hosts papaya, tapioca, cotton, hibiscus, mulberry, brinjal and potato sprouts and placed in the plastic containers with host leaves and sprouts. Mealybugs were placed on the leaves and sprouts 24 hours before the experiment to allow them to settle. One individual of *A. papayae* for each replication was released. The containers were covered with black cloth secured with rubber band. Honey solution and water soaked cotton were renewed once in two days upto 6 days. After 10 days of release, the leaves and sprouts were transferred to another transparent plastic containers. The containers were examined daily for the emergence of parasitoids. After 1 week of the final emergence of the parasitoid, unemerged parasitoids were counted.

iv. Parasitoid infestivity

Parasitoid infestivity was described through the recording of a suit of behaviors. Previous observations and published studies (Wang and Keller 2002; Araj *et al*., 2011) were revisited to choose relevant behavioral traits of the parasitoid females. The handling time, recognition time and data for relative encounter rate were observed in this experiment. Infestivity experiments were carried out on mealybug from six different environments like papaya (natal host of mealybug), cotton, tapioca, mulberry, brinjal, hibiscus (mealybug from

non natal hosts) and potato sprouts for comparison of results. Experimental arena consisted of a modified glass Petri dish (2 cm. diameter) containing one second instar of each natal and non natal mealybug host onto a small piece of leaf. Second instars were chosen as they represent a high-quality resource for *A. papayae* being normally preferred over other nymphal stages. After 5 min of settling of the mealybug on the leaf, one single-mated naive female parasitoid per assay was placed inside the experimental arena and behaviors were recorded during 10 min. Female parasitoids were used only once. Behavioral observations were done under an light microscope with a diffuse light source under the experimental arena. Each test was repeated at least 10 times, renewing the experimental arena for every new test (Petri dish, plant leaves, mealybug, and test parasitoid). The proportion of time spent for each of the behavioral traits was estimated. A static foraging model with handling and recognition time rewritten as per Hughes (1979), was used here to calculate the optimal decisions. The profitability of host type is expressed as the survival probability of eggs in larvae of this type. Parasitoids should specialize on papaya mealybug instar if the encounter rate with this host species is larger than the certain value.

$$\lambda_{crit} = \frac{-SP_2}{SP_1.t_R + SP_2.t_H + R.SP_2.t_R - SP_1.t_H}$$

where,

λ_{crit} = certain value of parasitoid encounter rate

SP_1 = survival probability of parasitoid on highly preferred host,

SP_2 = survival probability of parasitoid on poorly preferred host,

t_R = recognition time of parasitoid (seconds)

t_H = handling time of parasitoid (seconds)

v. Parasitoid virulence

Parasitoid virulence was studied using the development time and parasitisation potential on the host species. From the data obtained from the parasitic potential of *A. papayae*, it was inferred that the parasitoid had the highest efficiency on second instar mealybug and so it was taken to be correlated with total developmental time of parasitoid. The correlation and linear regression analysis was done on the observation to see the virulence potential of the parasitoids.

III. Results and Discussion

Parasitoid virulence: Correlation among parasitoid developmental days and parasitic potential by host plants

The table 1 inferred that the *A. papayae* had the highest efficiency on second instar *P. marginatus*. Hence correlation was made between development time of parasitoid. The parasitisation rate decreased as development time increased and vice versa. The overall correlation analysis and linear regression analysis comparing total developmental time with parasitisation rate resulted in the negative correlation coefficient (r2 = -0.905) and the equation y = y = -3.399x + 122.0 (R² = 0.819 and P= 0.009) (Fig. 1). Therefore, the percentage of parasitism was significantly affected by the total developmental time of parasitoid as it was influenced by the host plants. The lowest level of parasitism (67.3 %) occurred at 16.5 days of development in tapioca reared PMB and the highest (87.7 %) at 10.7 days of development in papaya reared PMB (Table 1).

Table 1. Total development time and parasitisation rate of *Acerophagus papayae* on *Paracoccus marginatus* from different host plants

Plant host	Total development time (days)	Mean parasitisation rate on second instar mealybug (%)
Papaya	10.7[a]	87.7[a]
Cotton	11.8[b]	84.3[b]
Tapioca	16.5[f]	67.3[e]
Mulberry	12.3[c]	81.0[c]
Brinjal	12.9[d]	80.0[c]
Hibiscus	14.2[e]	71.3[e]
Potato	11.9[b]	75.7[d]

* Means followed by the same alphabets are not significantly different at 5 % level by DMRT

Fig.1. Line fit plot of regression analysis using total developmental time and parasitism rate of *A. papayae* on *P. marginatus* from different host plants
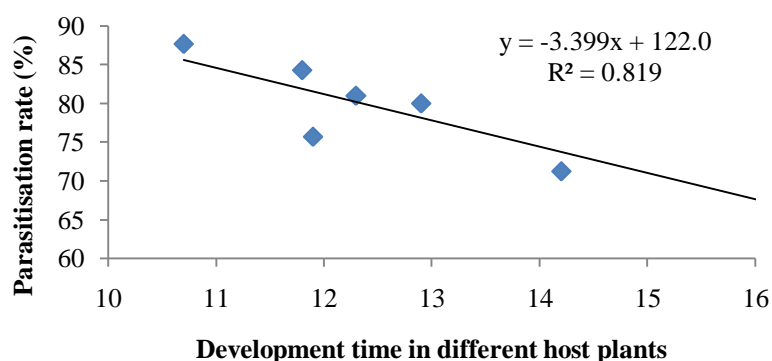
There was statistically significant correlation among parasitoid developmental days and parasitic potential (p<0.05) in cotton (r= -0.95), mulberry (r= 0.66), potato (r= -0.73) and tapioca (r= 0.58). Negative correlation with cotton and potato implied that as parasitoid development time (days) increase the parasitic potential decrease. When assumed a linear relationship among two variables (only for the host plants that had statistically significant correlation) (Table 2).

Table 2.Correlation among parasitoid developmental days and parasitic potential by host plants

Host Plant	Pearson Correlation (r)	p value	R squared (Linear Relationsip)
Papaya	0.356	0.312	-
Cotton	-0.947	<0.001*	0.90
Tapioca	0.583	0.077*	0.34
Mulbery	0.655	0.040*	0.43
Brinjal		.	
Hibiscus	0.352	0.318	-
Potato	-0.732	0.016*	0.54

Correlation among parasitoid female mealybug development days and egg hatching in different host plants

There was no statistically significant correlation among female mealybug developmental days and egg hatching in any of the host plants (Table 3).

Table 3. Correlation among female development days and egg hatching by host plants

Host Plant	Pearson Correlation (r)	p value
Papaya	-0.33	0.356
Cotton	-0.17	0.629
Tapioca	0.26	0.461
Mulbery	-0.09	0.797
Brinjal	-	.-
Hibiscus	0.26	0.474
Potato	-0.12	0.749

Correlation among parasitoid male mealybug development days and egg hatching in different host plants

There was no statistically significant correlation among male mealybug developmental days and egg hatching in any of the host plants (Table 4).

Table 4. Correlation among male mealybug developmental days and egg hatching by host plants

Host Plant	Pearson Correlation (r)	p value
Papaya	-0.19	0.896
Cotton	0.18	1.000
Tapioca	0.18	0.707
Mulbery	-0.05	0.745
Brinjal	-	-
Hibiscus	-0.12	0.619
Potato	0.14	0.595

Correlation between mealybug development days and parasitoid developmental days in different host plants

There was no statistically significant correlation among mealybug developmental days and parasitoid developmental days in any of the host plants (Table 5). When the developmental time of a parasitoid is shorter than the developmental time of the host, there is an advantage for the parasitoid. Later in the season with overlapping host generations, it can produce its progeny at a faster rate than the host and can parasitize the host populations in a shorter time. It is in agreement with findings of Powell and Bellows, 1992; Sengonca *et al*. 1998, who reported that developmental period of *Eretmocerus* sp. depends on the developmental period of its host insect, thus a short developmental period of *P. myricae* on a certain host plant induced a short developmental period of the parasitoid. Determining developmental time of a parasitoid is necessary to determine its efficiency in controlling the host. Generally, the developmental time of a biological control agent should be shorter than the developmental time of the host (Greathead, 1986).

Table 5. Correlation among mealybug development days and parasitoid development days in host plants

Host Plant	Male Pests		Female Pests	
	Pearson Correlation (r)	p value	Pearson Correlation (r)	p value
Papaya	-0.05	0.896	-0.33	0.356
Cotton	0.0	1.000	-0.17	0.629
Tapioca	0.14	0.707	0.26	0.461
Mulbery	-0.12	0.745	-0.09	0.797
Brinjal	-	-	-	-
Hibiscus	0.18	0.619	0.26	0.474
Potato	-0.19	0.595	-0.12	0.749

Infestivity of parsitoid Acerophagus papayae

The parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae; Aphidiinae) is commonly used in biological control and one of the best-studied parasitoid model systems in ecology and evolution (Henry *et al*., 2010). On this basis, the current study was undertaken study to determine the infestivity of parasitoids using the reciprocal experiment. The study revealed that, handling time, recognition time and survival probability of parasitoid was vary accordingly with host plants. Handling and recognition time was maximum in tapioca (38.8 and 8.8 sec) and minimum in papaya (20.4 and 4.8 sec), while survival probability was in reverse to this trend. It was higher in papaya (88.0 %) and lower in tapioca (39.8 %). It showed a significant negative correlation between survival probability and handling time of parasitoids. Whenever handling and recognition time increased, there was a decrease in the survival probability of parasitoid in the different host plants. Results are discussed in the context of host-affiliated ecological selection as a potential source driving diversification in parasitoid communities and the influence of host species heterogeneity on population differentiation and local adaptation. Similar results were obtained by Henry *et al*. (2008) on the aphid parasitoid, *A. ervi* that maintain a high level fitness on an ancestral and novel host, suggested a genetic basis for the host utilization. The reason might be trade-offs associated with utilization of different hosts are important mechanism generating genetic diversity among populations of insects and encourages local adaptation when combined with limited gene flow (Kawecki and Ebert, 2004).

Recognition time, handling time and survival probability

The variables recognition time, handling time and survival probability followed normal distribution as established by Kolmogrov Smirnov test – p- 0.548, 0.211 and 0.216 respectively. A horizontal stacked bar diagram was constructed in order to depict the adaptability process of the parasitoids by host plants. One way ANOVA with Duncan's post hoc test to identify homogenous hosts was performed to compare mean adaptability pattern. To study the parasitoid infestivity on mealybug from different host plants, handling time and recognition time of parasitoids were recorded. The study revealed that, handling time, recognition time and survival probability of parasitoid was vary accordingly with host plants. Handling time of *A. papayae* was statistically

significantly different among host plants (F – 74.1, p<0.0001). Tapioca and hibiscus had similar handling time, while Brinjal, Mulberry and Cotton were grouped together. Papaya had significantly lower handling time of 20.4 seconds, tapioca had 38.8 seconds and the rest of the plants brinjal, hibiscus and tapioca were not different (Table 6). Similarly, recognition time of *A. papayae* was statistically significantly different among all the host plants, tapioca took the longest time to be recognized (mean 8.8 sec) followed by hibiscus (mean 8.4 sec). Results are discussed in the context of host-affiliated ecological selection as a potential source driving diversification in parasitoid communities and the influence of host species heterogeneity on population differentiation and local adaptation. Similar results were obtained by Henry *et al.* (2008) on the aphid parasitoid, *A. ervi* that maintain a high level fitness on an ancestral and novel host, suggested a genetic basis for the host utilization. The reason might be trade-offs associated with utilization of different hosts are important mechanism generating genetic diversity among populations of insects and encourages local adaptation when combined with limited gene flow (Kawecki and Ebert, 2004).

Table 6. Infestivity of parsitoid *Acerophagus papayae* showing handling and recognition of parasitoid

| Host plants | Mean infestivity time (Seconds)* | | | | Survival probability (percentage) | |
| | Handling Time | | Recognition Time | | | |
	Mean	SD	Mean	SD	Mean	SD
Papaya	20.4[a]	0.9	4.8[a]	1.1	88.0[a]	1.9
Cotton	25.2[b]	2.9	6.0[b]	0.7	71.6[b]	2.2
Tapioca	38.8[d]	1.8	8.8[c]	0.8	39.8[e]	1.3
Mulbery	29.2[c]	1.1	6.8[b]	0.4	66.2[c]	1.5
Brinjal	37.0[d]	1.9	6.4[b]	0.5	57.4[d]	1.8
Hibiscus	37.4[d]	2.4	8.4[c]	0.5	41.0[e]	1.2
One Way ANOVA	F -74.1 p<0.0001		F-21.3 p<0.0001		F-610.6 p<0.0001	

*Mean of ten replications

Papaya had the shortest recognition time (4.8 sec), handling time (20.4 sec) and recorded higher survival percentage (88.0 %) among the host plants. On the other extreme on tapioca, parasitoids took more time for recognition (8.8 sec) and handling (38.8 sec) and ended up with a lower survival percentage (39.8 %). It may be observed that the more the recognition or handling time, lesser is the survival percentage (Table 6).

Encounter and acceptance rate of parasitoid in different host plants
Encounter rate of parasitoids

Plasticity is a major component of phenotypic variation and has recently attracted much attention as an important factor in evolution. It is clear that plasticity can have a genetic basis, can be adaptive and may be altered by natural selection. Our study demonstrated the adaptive plasticity of *A. papayae* on different host plants using the encounter rate and acceptance rate of parasitoid and showed the difference in all the host plants. The Generalized Linear Model is an extension of the General Linear Model to include response variables that follow any probability distribution in the exponential family of distributions. The encounter data followed poisson distribution (Kolmogrov Smirnov, p value-0.305). Encounter of the parasitoid was appropriately analyzed as a Poisson random variable within the context of the Generalized Linear Model (Table 7). The recognition time was included in the model as covariates. The dependent variable defined for the model was encounter and independent variable being the host. The objective of the analysis was to see if the encounter of the insects was different among the host plants given the time taken for recognition.

Table 7. Nornmality distribution of encounter rate by parasitoids

Parameter	B	p value
Papaya	0.523	<0.001
Cotton	0.425	<0.001
Tapioca	-	-
Mulberry	0.295	0.010
Brinjal	0.133	0.262
Hibiscus	-0.046	0.711

Out of 50 mealybugs provided as feed for parasitoids revealed different rate of encountering on different host plants (Table 8). On mealybugs from papaya, parasitoid encountered 45.2 ± 0.8 per cent mealybugs followed by cotton (41.0 ± 1.0 per cent). Lowest encounter was recorded on hibiscus (25.6 ± 0.5 per cent) and tapioca (26.8 ± 1.1 per cent). The present findings are supported by Zepeda-paulo *et al.* (2013) who reported that the *A. pisum*-alfalfa parasitoid population showed a high frequency and proportion of time spent attacking on their natal host, when compared to other non-natal hosts. In contrast to the result, they also reported that the virulence assay showed a high plasticity for traits related to fitness. The three different parasitoid populations studied (from both *A. pisum* races and *S. avenae*) showed a similar high virulence (parasitism rate, survival, and productivity) on natal and non- natal hosts, thus providing evidence for the absence of local host adaptation.

Table 8. Encounter rate of parasitoids on *Paracoccus marginatus* from different host plants

Host	Parasitoid Encounter rate (per cent)			
	Mean	SD	Minimum	Maximum
Papaya	45.2	0.8	44	46
Cotton	41.0	1.0	40	42
Tapioca	26.8	1.1	26	28
Mulberry	36.0	1.2	35	38
Brinjal	30.6	0.9	30	32
Hibiscus	25.6	0.5	25	26

Acceptance rate of parasitoids

Acceptance proportion was calculated by counting the number of parasitoids that were accepted by the host and dividing it by the number of parasitoids that successfully encountered. For comparison of proportion of parasitoids between the hosts, the data were mathematically transformed into arcsine of the proportions (Kolmogrove Smirnov, p value-0.632). The transformed values were subjected to analysis of variance and then Duncan's post hoc test. Table 9 revealed the difference in the rate of acceptance of encountered mealybugs by parsitoids on different host plants. The parasitoid accepted 89.9 per cent mealybugs on papaya, that is on par with mealybugs from cotton (86.9 %) and mulberry (86.2 %). Lowest acceptance was recorded on tapioca (77 %), which was not significantly different from hibiscus (78.1 %) and brinjal (78.5 %).

Table 9. Acceptance rate of parasitoids on *Paracoccus marginatus* from different host plants

Host	Parasitoid acceptance rate (%)				ANOVA*
	Mean	SD	Minimum	Maximum	
Papaya	89.9[b]	3.6	87.0	95.5	
Cotton	86.9[b]	4.1	83.3	92.5	
Tapioca	77.0[a]	5.8	71.4	84.6	
Mulberry	86.2[b]	4.8	81.6	91.4	F – 7.57
Brinjal	78.5[a]	5.4	71.0	83.3	P<0.0001
Hibiscus	78.1[a]	2.1	76.0	80.8	

*ANOVA and Post hoc Duncan based on arcsine transformed data

Adaptability of parasitoids on prey from different host plants

Non parametric correlations were calculated using Spearman's rho. There was a statistically significant correlation between the variables when the correlation was made among the hosts plants considerably all variables together (Table 10).

Table 10. Overall correlation among recognition time, handling time and survival rate among the host plants

Spearman's rho	Recognition Time	Handling Time	Survival
Recognition Time	1	0.763[*]	-0.875[*]
Handling Time		1	-0.887[*]
Survival			1
			*statistically significant at p<0.0001

There was a significant positive correlation (r^2: 0.11, p<0.001) between the number of mealybugs encountered and accepted by parasitoids (Table 11)..

Table 11.Correlation between encounter rate and acceptance rate of parasitoid among the host plants

	Encounter rate	Acceptance rate
Encounter rate	1	
Acceptance rate	0.11*	1
	*statistically significant at p<0.0001	

There was a significant negative correlation (Spearman's rho: -0.85, p<0.001) between the number of mealybugs encountered by parasitoids and the time taken for recognition of mealybug by parasitoid (Table 11). Negative correlation was revealed between the host (mealybug) acceptance rate of parasitoid and time taken for handling of pest (Spearman's rho: -0.66, p<0.001).

Host acceptance and host encounter are usually correlated, but females in several species are known to accept mealybugs that are unsuitable for immature development (Griffiths, 1960). Thus, acceptance is insufficient evidence of host suitability, and rejection does not indicate that a candidate host is in fact unsuitable. Moreover, some hosts may be suitable and available but not susceptible to parasitism. The host selection process in the current study showed the order sequence in the manner of "host encounter- host recognition- host acceptance- host handling". There was a positive correlation between acceptance and encounter rate of parasitoids and a negative correlation between the recognition time and encounter rate (r^2: -0.85) and between handling time and acceptance rate (r^2: -0.66) of the parasitoid in all the host plants. It is concluded here that, The current study concluded that, plasticity of parasitoid well revealed in the natal host (papaya) of mealybug than non natal hosts. Followed by the natal host, parasitoid showed plasticity on mealybug from cotton and potato sprouts and marginally adapted on mulberry. The adaptive plasticity was quite pronounced on hibiscus and tapioca. The reason were already given earlier in the discussion (Section 5.2.1 and 5.2.2) on the basis of plant characters influencing the response and behavior of mealybug that will indirectly influencing the efficiency of parasitoid. It was supported by Bowers (1990), Nishida (2002) and Hartmann (2004), who reported a more complex evolutionary response to plant chemical defense in herbivores that have co-opted the toxins for their own benefit by sequestering them and using them as a defense against natural enemies. The host plants seemed to play an important role in the host recognition and acceptance behaviour of parasitoids in the present study. The result was supported by the findings of Braimah and van Emden (1994), who reported that the cereal aphid specialist *Aphidius rhopalosiphi* attacked the host species *Sitobion avenae* significantly more, when the aphids were presented together with a wheat plant. The parasitoid also showed greater response to the non-host *Myzus persicae*, when this was presented with wheat leaves than when it was presented with Brussels sprouts leaves, indicating the role of plant-derived synomones in aphid-parasitoid interaction. In another study Powell and Wright (1992) observed more oviposition by *A. rhopalosiphi* in *Acyrthosiphon pisum*, a non-host aphid, when wheat leaves were present. Similar trends in host preference behaviour of generalist aphid parasitoid, Praon volucre as influenced by host plants were also observed by Rehman (1999).

It has also been demonstrated that oviposition may be a matter of experience and that female parasitoids with a wide range of hosts often prefer the host species from which they have evolved (Eijsackers and van Lenteren, 1970; Rehman, 1999). It would not be surprising to find that a female's preference for a particular host or a host-plant complex, after being determined by the proper stimuli for habitat and host location and recognition, is strongly influenced by prior exposure and success. Preference for a particular host may be influenced by both genetic factors and conditioning (Rehman, 1999; Poppy and Powell, 2004; Poppy *et al.*, 2008). Prior oviposition experience of *Aphidius pisivorus* on *A. pisum* affected the attack rate on *Macrosiphum creelii* but did not change its innate order of host preference (Chow and Mackauer, 1992). The findings of the present study is in conjunction with the above that the efficiency of parasitoids was higher in the mealybug from the natal host (papaya) than non natal hosts in all the manner.

Table 11.Correlation between recognition and handling time with encounter and acceptance rate of parasitoid among the host plants

Spearman's rho	Encounter rate	Acceptance rate
Recognition Time	-0.85*	-
Handling Time		-0.66*
	*statistically significant at p<0.0001	

Optimal foraging model of parasitoid *Acerophagus papayae* on *Paracoccus marginatus* from different host plants

The results from the above study on the sensitivity of survival probability, handling time, recognition time, acceptance and encounter rate were used to promote a static optimal foraging model of parasitoid. A static foraging model with handling and recognition time rewritten as per Hughes (1979), was used here to calculate the optimal decisions of the parasitoids. The profitability of host type is expressed as the survival probability of eggs in larvae of this type. Parasitoids were specialized on papaya mealybug instar if the encounter rate with this host species is larger than the certain value, when comparing the between the host plants.

The parameters taken for estimating optimal decisions of the parasitoids were, survival probability, handling time, recognition time, encounter rate (Table 12).

Table 12. Parameters taken for estimating optimal decisions of the parasitoids

Host	Survival probvability (%)	Handling time (Sec)	Recognition time (Sec)	Encounter rate (%)
Papaya	88	20.4	4.8	45.2
Cotton	71.6	25.2	6	41
Tapioca	39.8	38.8	8.8	26.8
Mulberry	66.2	29.2	6.8	36
Brinjal	57.4	37	6.4	30.6
Hibiscus	41	37.4	8.4	25.6

In the paired combination of the plants, the good host and the bad host were selected by the mean value of parasitoids attraction and the relative encoiunter rate (R) was estimated using the following formula,

$$Relative\ encounter\ rate\ (R) = \frac{encounter\ rate\ in\ bad\ host}{encounter\ rate\ in\ good\ host}$$

By this, optimal decisions of the parasitoids were estimated with the combination of the plants and good host and bad host from the outcome of 'Y' tube olfactometer experiment. Table 13 revealing the mean parasitiods attracted to each host entries when they paired. The mean value highest in the paired combinations are bold.

Table 13. Correlation matrix on attraction of parasitoids in 'Y' tube olfactometer using combination of infested host leaves

Host leaves	T1 Papaya	T2 Tapioca	T3 Cotton	T4 Mulberry	T5 Brinjal	T6 Hibiscus
T1 Papaya	*	**7.8**	**8.2**	**7.2**	**8.8**	**8.6**
T2 Tapioca	1.6	*	1.6	1.2	0.4	**2.6**
T3 Cotton	1.8	**6.0**	*	**6.2**	**6.2**	**6.8**
T4 Mulberry	1.6	**5.8**	2.6	*	**6.0**	**6.6**
T5 Brinjal	1.2	**4.2**	3.6	3.2	*	**4.6**
T6 Hibiscus	0.8	1.4	3.2	3.2	4.4	*

- Upper diagonal- attraction of parasitoids to the first treatment of permutations
- Lower diagonal - attraction of parasitoids to the second set of permutations

Table 14 exhibits the λ_{crit} certain value of parasitoid encounter rate and relative encounter rate of the parasitoid in each combination of plants. The value was higher than the 'R' value, only in the T1T5 combination (Papaya *vs* Brinjal) and in the T5T6 combination (Brinjal *vs* Hibiscus). It revealed that parasitoid was specialized on mealybug from papaya when papaya combined with brinjal, and specialized on mealybug from brinjal when brinjal combined with hibiscus. Other combinations did not show any trend of optimal decisions of specialization. The successful development of the parasitoid depends on the selection of a suitable host, and it is directly related to host nutrition, intraspecific larval competition, the host's immunity response and the host's endocrine balance. Different host species may differ in their suitability. Some authors distinguish host suitability (Vinson and Iwantsch, 1980a) and host regulation (Vinson and Iwantsch, 1980b) as separate criteria of host selection by a parasitoid. For clarity, Mackauer *et al.* (1996) distinguish between host suitability, host quality, and host value. They suggested that host suitability and quality are assessed by means of innate responses to the host species and the host individual, respectively. The response of the host species is regulated by the host plants it evolved.

Table 14. Optmial decision of parasitoid showing λ $_{crit}$ certain value and relative encounter rate of parasitoid

Host combination	λ $_{crit}$ value		Relative encounter rate (R)	Good host*
	A*	B*		
Papaya * Tapioca	0.09	0.04	0.59	TI
Papaya * Cotton	-0.18	-0.14	0.91	TI
Papaya * Mulberry	-0.29	-0.21	0.80	TI
Papaya * Brinjal	3.75**	0.18	0.68	TI
Papaya * Hibiscus	0.10	0.05	0.57	TI
Tapioca * Cotton	0.11	-0.07	0.65	T3
Tapioca * Mulberry	0.22	0.33	0.74	T4
Tapioca * Brinjal	-0.31	0.66	0.88	T5
Tapioca * Hibiscus	-0.06	-0.06	0.96	T2
Cotton * Mulberry	-0.10	-0.09	0.88	T3
Cotton * Brinjal	-0.17	-0.28	0.75	T3
Cotton * Hibiscus	0.22	0.13	0.62	T3
Mulberry * Brinjal	-0.11	0.14	0.85	T4
Mulberry * Hibiscus	0.47	0.29	0.71	T4
Brinjal * Hibiscus	2.06**	-0.26	0.84	T5

A = λ $_{crit}$ value, when handling time and recognition time is taken from the first pair of the combination
B = λ $_{crit}$ value, when handling time and recognition time is taken from the second pair of the combination
*Good host – Selected by the mean value of parasitoid attraction shown in the correlation matrix

The present findings are in a line with Zepeda-paulo *et al*. (2013) who reported the adaptive evolution *A. ervi* parasitoid on pea aphid from four different host plants. The results showed significant differences in parasitoid infectivity on their natal host compared with the non-natal hosts. However, parasitoids showed a similar high fitness on both natal and non natal hosts, thus supporting a lack of host adaptation in these introduced parasitoid populations. This process of local adaptation was thought to inherently destabilize generalization and promote specialization, even if trade-offs are absent (Bull *et al*., 1987). When combined with a trait under hard selection, such as parasitoid virulence, fitness trade-offs associated with the use of different host species have the potential to rapidly progress populations toward specialization on different hosts as alternate stable states (Fry, 1996). However, *A. papayae* may counteract this process by the use of a highly sensitized host location mechanism or through the "hitchhiking" of parasitoid larvae within parasitizing mealybug. Furthermore, local adaptation could be diluted, if constant migration from other hosts occurs, although migration between host species could slow evolution but not prevent a response to selection or diffuse coevolution between species (Henter, 1995).

However, local adaptation has not been detected in a number of natural populations of other, widely studied parasitoid systems exhibiting similar traits (Hufbauer 2001; Kraaijeveld *et al*. 2002; Dupas *et al*. 2003) but has been detected in others (Vaughn and Antolin 1998; Althoff and Thompson 2001; Morehead *et al*. 2001; Antolin *et al*. 2006; Hayward and Stone, 2006). It should be noted that the insects and hosts studied to date represent only a fraction of the tremendous diversity of parasitic relationships that exist. Only now are we acquiring evidence that suggesting the host-associated differentiation especially for the insect parasitoids (Stireman *et al*., 2006). The Same host asscociated difference was carried out in the *A. ervi* by Henry *et al*. (2010). Although traits facilitating directional selection and specialization exist in many parasite systems as a means to constantly improve fitness or counteract host defenses, the evolution and maintenance of differentiation between populations requires specific environmental conditions, such as stable host populations and limited gene flow between host-affiliated populations. Host–parasite systems that are prone to disturbances or those that experience extensive gene flow may have selection slowed or disrupted. Finally the present study accomplished that the development time of parasitoid inversely correlated with the parasitic potential. It might be due to the energy conservation process by parasitoid that it conserves more energy when developed early and uses the same energy for parasitisation process. This "trade-off" hypothesis suggests that negative fitness correlations can lead to specialization on different hosts as alternative stable strategies. In this study we demonstrated a trade-off in the ability of the parasitoid, *A. papayae*, to maintain a high level of fitness on an natal and non natal host, which suggested a plant biochemical basis for host utilization that may limit host-range expansion in parasitoids. Furthermore, behavioral evidence suggested the mechanisms that could promote specialization through induced host fidelity.

Our work has demonstrated the potential for a single population of *A. papayae* parasitoids to differentiate based strictly on host species utilization. The information gathered from this study will be important in the management of papaya mealybug *P. marginatus* in an efficient manner. However, to gain a better understanding of how these traits function in nature, detailed studies are required that link mechanisms that drive differentiation to the genetic structure of natural parasitoid populations. In order to establish an

efficient method for using parasitoids as biological control agents against *P. marginatus*, the properties of *A. papayae* adults and host plants should also be studied.

IV. Conclusion

The results emphasized that *A. papayae* does not equally use its potential host range, showing a low preference and virulence to *P. marginatus* on different host plants, which suggests an effect of host phylogeny on the traits studied. In addition, the infectivity of *A. papayae* shows host preferences mediated through host fidelity of some populations, demonstrated by significant differences in infectivity across the different hosts studied. In this respect, host fidelity has been observed to have an effect on parasitism rates in mass-reared *A. papayae* on a novel host. And it also highlighted the role of phenotypic plasticity in fitness related traits of parasitoids, enabling them to maximize their fitness on alternative hosts. This could be used to increase the effectiveness of biological control programme with phenotypic plasticity of parasitoid. In addition, *A. papayae* females showed significant differences in infectivity and virulence across the tested host range, thus suggesting a possible host phylogeny effect for those traits. Future research should be focused on the potential of phenotypic plasticity as an adaptive mechanism in generalist parasitoids living in changing environments, determining the effect of high plastic parasitoids on the efficiency of pest control, and quantifying the relative frequency and dynamics of these *A. papayae* and *P. marginatus* -host populations in the field. This will become especially relevant, as practices of biological control will need to adopt new strategies for choosing agents and their release under the present climate change.

References

[1]. Althoff, D.M. and J. N. Thompson. 2001. Geographic structure in the searching behaviour of a specialist parasitoid: combining molecular and behavioural approaches. J. Evolution. Biol., **14:** 406–417.
[2]. Antolin, M. F., T. A. Bjorkstein and T. T. Vaughn. 2006. Host-related fitness trade-offs in a presumed generalist parasitoid, Diaeretiella rapae (Hymenoptera: Aphidiidae). Ecol. Entomol., **31:**242–254.
[3]. Araj, S. E., S. Wratten, A. Lister, H. Buckley and I. Ghabeish. 2011. Searching behavior of an aphid parasitoid and its hyperparasitoid with and without floral nectar. Biol. Control., **57:**79–84.
[4]. Bowers, M. D. 1990. Recycling plant natural products for insect defense. In: Insect Defenses. Hogerth press, London, pp. 353-386.
[5]. Braimah, H. and H. F. Van Emden. 1994. The role of the plant in host acceptance by the parasitoid Aphidius rhopalosiphi (Hymenoptera: Braconidae). Bull. Entomol. Res., **84:** 303-306.
[6]. Bull, J. J., C. Thompson, D. Ng and R. Moore. 1987. A model for natural selection of genetic migration. Am Nat., **129**(1): 143-157.
[7]. Chow, F. J and M. Mackauer. 1992. The influence of prior ovipositional experience on host selection in four species of aphidiid wasps (Hymenoptera; Aphidiidae). J. Insect Behav., **5:** 99-108.
[8]. Dupas, S., Y. Carton and M. Poirie. 2003. Genetic dimension of the coevolution of virulence-resistance in Drosophila–parasitoid wasp relationships. Heredity, **90:** 84 - 89.
[9]. Eijsackers, H.J.P. and J. C.Van Lenteren. 1970. Host choice and host discrimination in Pseudeucoila bochei. Neth. J. Zool., **20:** 414.
[10]. Fry, I. 1996. On the Biological Significance of the Properties of Matter: L. J. Henderson's Theory of the Fitness of the Environment. J. Hist. Biol., **29** (2):155 – 196.
[11]. Godfray, H.C.J. 1994. Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton, New Jersey.
[12]. Greathead, D. J. 1986. Parasitoids in classical biological control. In: Insect parasitoids. Waage, J. & Greathead, D. (Eds). London. 289-318.
[13]. Griffiths, D.C. 1960. The behaviour and specificity of Monoctonus paludum Marshall (Hymenoptera: Braconidae), a parasite of Nasonovia ribis-nigri (Mosley) on lettuce. Bull. Entomol. Res., **51:** 303-319.
[14]. Hartmann, T. 2004. Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. Planta, **219:** 1–4.
[15]. Hayward, A. and G. N. Stone. 2006. Comparative phylogeography across two trophic levels: the oak gall wasp Andricus kollari and its chalcid parasitoid Megastigmus stigmatizans. Mol. Ecol., **15:**479–489.
[16]. Henry, L. M., B. D. Roitbergand and D. R. Gillespie 2008. Host-range evolution in Aphidius parasitoids: fidelity, virulence and fitness tradeoffs on an ancestral host. Int. J. Org. Evol., **62:**689–699.
[17]. Henry, L. M., N. May, S. Acheampong, D. R. Gillespie and L. D. Roitberg 2010. Host-adapted parasitoids in biological control: Does source matter?. Ecol. Appl., **20:**242–250.
[18]. Henter, H.J. 1995. The potential for coevolution in a host-parasitoid system, 2: Genetic variation within a population of wasps in the ability to parasitize an aphid host. Evolution, **49:**439–445.
[19]. Hilker, M. and T. Meiners. 2006. Early herbivore alert: insect eggs induce plant defense. J. Chem. Ecol., 32: 1379– 1397.
[20]. Hufbauer, R. A. 2001. Pea aphid-parasitoid interactions: have parasitoids adapted to differential resistance?. Ecology, **82:**717-725.
[21]. Hughes, R. N. 1979. Optimal diets under the energy maximization premise: the effects of recognition time and learning. Am. Nat., **113:**209-221.
[22]. Kawecki, T. D. and D. Ebert 2004. Conceptual issues in local adaptation. Ecology Letter, **7:**1225–1241.
[23]. Kraaijeveld, A.R., J. Ferrari and H.C.J. Godfray. 2002. Costs of resistance in insect–parasite and insect–parasitoid interactions. Parasitology, **125:** 71–82.
[24]. Mackauer, M., J. P. Michaud and W. Völkl. 1996. Host choice by aphidiid parasitoids (Hymenoptera: Aphidiidae): host recognition, host quality, and host value. Can. Entomol., **128:** 959-980.
[25]. Morehead, S.A, J. Seger and D.H. Feener. 2001. Evidence for a cryptic species complex in the ant parasitoid Apocephalus paraponerae (Diptera: Phoridae). Evol. Ecol. Res., **3:** 273–284.
[26]. Nishida, R. 2002. Sequestration of defensive substances from plants by Lepidoptera. Annu. Rev. Entomol., **47:** 57–92.
[27]. Poppy, G.M. and W. Powell. 2004. Genetic manipulation of natural enemies: can we improve biological control by manipulating the parasitoid and/or the plant? In: Genetics, Evolution and Biological control, (Eds.) L.E. Ehler, R. Sforza and T. Mateille, CABI Publishing, UK, pp. 219-233.

[28]. Poppy, G.M., H. van Emden, A.P. Storeck, S. Douloumpaka, I. Eleftherianos and W. Powell. 2008. Plant chemistry and aphid parasitoids (Hymenoptera: Braconidae): Imprinting and memory. Eur. J. Entomol., **105**: 477-483.

[29]. Powell, D. P. and T. S.JR. Bellows. 1992. Adult longevity, fertility and population growth rates for Bemisia tabaci (GENN.) (Hom., Aleyrodidae) on two host plant species. J. Appl. Ent., **113:** 68-78.

[30]. Powell, W. and A. F. Wright. 1992. The influence of host food plants on host recognition by four aphidiine parasitoids (Hymenoptera: Braconidae). Bull. Entomol. Res., **81:** 449–453.

[31]. Rehman, A. 1999. The host relationships of aphid parasitoids of the genus Praon (Hymenoptera: Aphidiidae) in agro-ecosystems. Ph.D., Thesis, University of Reading, Reading UK, p.291.

[32]. Sengonca, Ç., N. Uygun, M.R. Ulusoy and U. Kersting. 1998. Population dynamics of Parabemisia myricae (Kuwana) and its parasitoid Eretmocerus debachi Rose and Rosen (Hymenoptera, Aphelinidae) on non-Citrus host plants. J. Plant Dis. Prot., **105:** 146-156.

[33]. Serrano, M. S. and S. L. Laponite. 2002. Evaluation of host plants and a meridic diet for rearing Maconellicoccus hirsutus (Hemiptera: Pseudococcidae) and its parasitoid Anagyrus kamali (Hymenoptera: Encyrtidae). Florida Entomol., **85**: 417 – 425.

[34]. Stireman, J. O. I. I. I., J. D. Nason, S. B. Heard and J. M. Seehawer. 2006. Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. Proceeding of the Royal Society., **273**: 523-530.

[35]. Vaughn, T.T. and M. F. Antolin. 1998. Population genetics of an opportunistic parasitoid in an agricultural landscape. Heredity, **80:** 152–162.

[36]. Vinson, S. B. 1985. The behavior of parasitoids. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology, G. A. Kerkut and L. I. Gilbert (Eds.). Vol. 9. Pergamon Press, Elmsford, New York. pp. 417–469.

[37]. Vinson, S. B. and G. F. Iwantsch. 1980a. Host regulation by insect parasitoids. Q. Rev. Biol., **55:** 143-165.

[38]. Vinson, S. B. and G. F. Iwantsch. 1980b. Host suitability for insect parasitoids. Annu. Rev. Entomol., **25:** 397-419.

[39]. Wang, X. G. and M. A. Keller. 2002. A comparison of the host-searching efficiency of two larval parasitoids of Plutella xylostella. Ecol. Entomol., **27:**105–114.

[40]. Zepeda-Paulo, F. A., A. Sebastian, Ortiz-Mart_ınez, C. Christian Figueroa and Blas Lavandero. 2013. Adaptive evolution of a generalist parasitoid: implications for the effectiveness of biological control agents. Evolu. Appl., **6**(6): pp. 1-17.