Journal of Biological Control, 27(4): 268–271, 2013

Research Article

Selective differentiation of distinct good foragers of *Cryptolaemus montrouzieri* Mulsant for population genetic studies

P. D. KAMALA JAYANTHI*, R. RAJANIKANTH and ABRAHAM VERGHESE[1]

Department of Entomology and Nematology, Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bangalore 560 0 Karnataka, India

[1]National Bureau of Agriculturally Important Insects, P.B. No. 2491, H.A. Farm Post, Hebbal, Bangalore 560 024, Karnataka, India.

**Corresponding author E-mail* : jaiinsect@gmail.com

ABSTRACT: The present study explains an efficient method for identifying the good foragers among the *Cryptolaemus montrouzieri* Mulsant populations for genetic studies where the trait accuracy is of utmost importance. Subjecting the adult *C. montrouzieri* beetles to pre-starvation was found to be an accurate method for identifying the good foragers over continuous feeding.

KEY WORDS: *Cryptolaemus montrouzieri*, predatory coccinellid, screening, continuous feeding, pre-starvation

(Article chronicle: Received: 01-10-2013; Revised: 15-12-2013; Accepted: 20-12-2013)

INTRODUCTION

Within the natural enemy populations, the intra-specific variability of traits *viz*., feeding potential and reproductive capabilities have had impact on their population structure. According to Bellows (2001), when natural enemies are introduced into a natural system, one of three outcomes may result. First, the natural enemy may not survive or reproduce, but simply die out in the new environment. Second, the natural enemy may reproduce, but have only a limited impact in the system and remain a relatively unimportant part of the system. Third, a natural enemy may reproduce and succeed well in the new environment, demonstrating a ready ability to locate and utilize its targeted pest. The third case is the principle objective of the introduction of natural enemies into systems with adventive pests. The genetic structure of the source population can have profound effect on probability of successful initial establishment as the genotype in generation I influences the fecundity and viability of future generations in the natural environments (Remington, 1968). The accurate identification of good forager from poor one has important implications for understanding to what extent the parental traits influence the future progenies. This is further more important in predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant where continual rearing takes place under laboratory conditions for their field release. The present study outlines the accurate screening method to differentiate the good forager from the population for genetic studies.

MATERIALS AND METHODS

The *C. montrouzieri* a generalist predator of several economically important mealybug species (Kamala Jayanthi *et al.,* 2010) is a well known classical biological control agent that have been introduced in to several countries (Solangi *et al.,* 2012).

The *C. montrouzieri* samples used for the present study were collected from the laboratory established culture at Indian Institute of Horticultural Research, Bangalore (12°58'; 77° 35'E). The original culture for the experiment was obtained from NBAII, Bangalore (National Accession No. NBAII-MP-COC-01). The culture was further maintained on pink hibiscus mealybugs, (*Maconellicoccus hirsutus*) reared on round yellow pumpkins (*Cucurbita moschata*) (Kairo *et al.,*1997; http://www.nbaii.res.in/Featured% 20insects/cryptolaemus.htm).

The pupae of *C. montrouzieri* of uniform age were randomly selected from the established laboratory cultures of *C. montrouzieri* and kept for emergence.

The population was screened to assess the mean feeding rate by providing 10 mealybugs/day. The experiment was conducted in two sets, each set consisting of ten adult *C. montrouzieri* beetles. The initial screening was carried out through elaborate feeding observations for first fifteen days immediately after eclosion of adult beetles in two different ways. Firstly, after emergence, the beetles were kept individually in separate Petriplates and provided with fixed number of prey (10 mealybugs/ day) continuously without any intermittent starvation to study their feeding potential. Secondly, after finishing the first experiment, the same batch of beetles were pre-starved and provided with fixed number of prey as mentioned above. However, here the pre-starvation was given on regular basis intermittently i.e., every 24 hr feeding observation was followed by similar period of pre-starvation. Observations on number of mealy bugs consumed were recorded on daily basis. The data on feeding rate were subjected to analysis of variance (ANOVA), regression analyses (linear/ non-linear) and also compared using paired t test (Little and Hills, 1978).

RESULTS AND DISCUSSION

Evaluation of individual adult beetles of *C. montrouzieri* both by pre-starving and continuous feeding showed that pre starving is the effective way of isolating the good foragers with poor ones as shown in the Fig. 1 and Table 1. The pre-starved adults recorded feeding rate of 2.92 (set I) and 5.12 (set II) mealybugs/day compared to continuously fed adults which recorded 1.60 (set I) and 1.66 (set II) mealybugs/day. The feeding rate of pre-starved adults was found to be highly significant ($p<0001$, paired t test) compared to continuously fed adults. The pre-starvation method was found to be very critical in differentiating the good feeder from the poor feeder as depicted in Fig. 1. Here, in particular, the adult beetles 6 and 7 could almost feed similarly (1.44 mealybugs/day) under continuous feeding. However, under pre-starvation the beetle 6 can stretch its feeding potential enormously (3.60 mealybugs/day) compared to beetle 7 (1.73 mealy-bugs/day) (Fig. 1). Thus, the beetles which can be grouped as of similar feeding potential under continuous feeding are actually not similar. The subtle and authentic difference in the feeding potential which is of utmost importance for a predator was clearly brought out by pre-starvation method. The correlation coefficients showed that the feeding in continuously fed adults was significantly and negatively correlated as the days progress (Table 1). However, in pre-starved adults in set I though the relationship was found negative, was not significant. Similarly, in set II the relationship was found to be positive, though insignificant. The regression analysis further supported this (Fig. 2). Under pre-starvation method, the variability in the feeding rate was explained to the tune of 45% through linear ($y = –0.1716x + 5.7708$, $R^2 = 0.4585$) and 58% through polynomial order (2) ($y = –0.0189x^2 + 0.1874x + 4.5742$, $R^2 = 0.5771$) equations. However, under continuous feeding, the variability in the feeding rate can be explained only to the tune of 27% through linear equation ($y = –0.0299x + 1.916$, $R^2 = 0.2661$). Thus, the maximum variability in the feeding rate can be explained only through pre-starvation method.

The distinction between phenotype and genotype is fundamental to understand the heredity of traits of interest. The genotype of an organism is determined by the description of the actual physical material made up of DNA that was passed to the organism by its parents. The phenotype of an organism is the class to which that organism belongs as determined by the description of

Table 1. Food consumption in prestarved and continuously fed *Cryptolaemus* adults

Treatments	Mean feeding (number of mealybugs/day)		Correlation coefficient 'r'	
	Set I (n=8)	Set II (n=10)	Set I (n=8)	Set II (n=10)
Starved adults	2.92 ± 0.19*** (R:1.73-3.60)	5.12 ± 0.29 (R:3.80-6.80)	– 0.29	0.32
Continuously fed adults	1.60 ± 0.11 (R:1.19-2.07)	1.66 ± 0.10 (R:1.19-2.07)	– 0.85**	– 0.89**

P = <0.001***; ** 0.01; R = Range

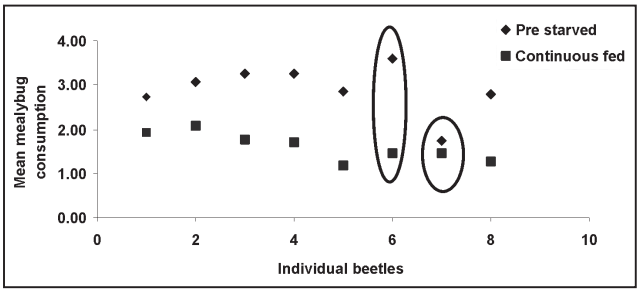

Fig. 1. Feeding differences observed between pre-starvation and continuous feeding methods; the actual difference of feeding potential of good forager expressed under pre-starvation method (in circles)

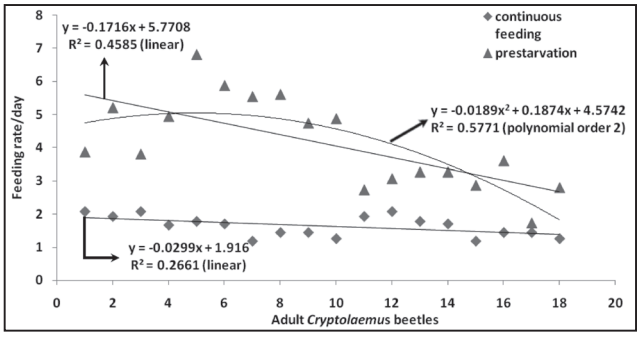

Fig. 2. Foraging trend in continuously fed and pre-starved adult *Cryptolaemus montrouzieri*

the physical and behavioral characteristics of the organism, for example its size and shape, its metabolic activities and its pattern of movement (Allen, 1979). In other terms, the phenotype is the descriptor of the phenome that manifest physical properties of the organism, its physiology, morphology and behavior. In practice the phenotypic variations are not total but partial, restricted to some subset of the characteristics of the organism that is regarded as relevant for a particular experimental purpose. The aim of many genetic mapping studies is to identify reliable phenotypic variation (polymorphism) of trait of interest to understand the underlying genes that can be correlated with phenotypic variation (Sean *et al*., 2009). Therefore, the individual insects are crossed to generate a mapping population in which the trait quantification is known. This concept remains an important parameter of population genetic studies of *C. montrouzieri* too, where the potential feeders should be isolated from the rest of the population to study the genetics underlying the foraging behaviour.

In the present study, significantly high feeding rate was observed with pre-starved adult *C. montrouzieri* beetles compared to the continuously fed adult beetles (Table 1). This may be due to the fact that under continuous food supply the beetles may attain quick satiation leading to lesser and lesser feeding. This type of rapid satiation that was noticed in *C. montrouzieri* in earlier studies (Magro *et al*., 2002) may explain the differential feeding rates under continuous and pre-starvation conditions. Further, this elaborate on limited gut capacity of *C. montrouzieri* compared to other aphidophagous coccinellids with slower rate of food digestion might partially explain the rapid satiation of *C. montrouzieri*. In the present study, the pre-starvation was found to trace out the actual difference between good and poor forager as observed in Fig 1. For insect population genetic studies, identifying the experimental mapping population with authentic genetic variation for the quantitative traits of interest (feeding potential as in the present case) is the basic need. Therefore, the present study indicates that the accurate method to separate the authentic good forager among the *C. montrouzieri* population is through pre-starvation method. Identification of true phenotype-genotype differences in feeding potential may well lead to a better understanding of foraging behavior and have important implications for population genetic studies of this predatory coccinellid.

ACKNOWLEDGMENTS

The authors thank the Director, Indian Institute of Horticultural Research, Bangalore for providing the research facilities and ICAR, New Delhi for financial assistance through ICAR AP Cess fund scheme.

REFERENCES

Allen G. 1979. Naturalists and Experimentalists: The genotype and the phenotype. *Studies Hist Biol.* **3**: 179–210.

Bellows TS. 2001. Restoring population balance through natural enemy introductions. *Biol Control* **21**: 199–205.

Kairo MTK, Cross AE, Lopez VF, Peterkin DD, Ram P. 1997. *Biological control of the hibiscus mealybug: Rearing the hibiscus mealybug, Maconellicoccus hirsutus, and the parasitoid Anagyrus kamali Moursi*; International Institute of Biological Control, Trinidad. 33 pp.

Kamala Jayanthi PD, Sangeetha P, Abraham Verghese. 2010. Does food adaptation influences prey choice of a generalist predator, *Cryptolaemus montrouzieri* Mulsant? *Curr Sci.* **99**: 1520–1522.

Solangi GS, Lohar MK, Abro GH, Buriro AS. 2012. Biology and release of exotic predator, *Cryptolaemus montrouzieri* Mulsant on mealybug, *Phenacoccus solenopsis* Tinslely at Tandojam. *Sarhad J Agric.* **28**: 429–435.

Little TM, Hills FJ. 1978. *Agricultural experimentation design and analysis*. Wiley, New York.

Magro A, Hemptinne JL, Codreanu P, Grosjean S, Dixon, AFG 2002. Does the satiation hypothesis account for the differences in efficacy of coccidophagous and aphidophagous ladybird beetles in biological control? A test with *Adalia bipunctata* and *Cryptolaemus montrouzieri. Biocontrol* **47**: 537–543.

Remington CL. 1968. The population genetics of insect introduction. *Ann Rev Ent.* **13**: 415–426.

Sean M, Jason Peiffer, Patrick J. Browen, Elhan S Ersoz, Zhiwu Zhang, Denise E Costich, Edward S Buckler. 2009. Association mapping: critical considerations shift from genotyping to experimental design. *Pl Cell* **21**: 2194.