Influence of Polyandry on Clutch Size of the Predatory Coccinellid, Cryptolaemus montrouzieri (Coleoptera: Coccinellidae)

Authors: Kamala Jayanthi, P. D., Sangeetha, P., and Verghese, Abraham

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INFLUENCE OF POLYANDRY ON CLUTCH SIZE OF THE PREDATORY COCCINELLID, *CRYPTOLAEMUS MONTROUZIERI* (COLEOPTERA: COCCINELLIDAE)

P. D. Kamala Jayanthi*, P. Sangeetha and Abraham Verghese
Division of Entomology and Nematology, Indian Institute of Horticultural Research, Bangalore 560089, India

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

Abstract

The influence of different polyandrous situations on clutch size was determined in the predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant, as a measure of female reproductive fitness. The study revealed that single female beetles each held with 3 mates under a continuous mating situation laid significantly more eggs (6.93/ day) than females each similarly held either with 2 males or with 1 male. However, correlation analysis revealed a highly significant negative correlation (r = -0.60**) between the progression of days and clutch size with 3 mates. The number of multiple matings and clutch size also decreased over time compared to other treatments. The implications of these results are discussed.

Key Words: egg mass, fecundity, fitness, predatory coccinellid, reproductive success

Resumen

Se determinó la influencia de las diferentes situaciones poliandras sobre el tamaño de los grupos de huevos puestos por el depredador coccinélido, *Cryptolaemus montrouzieri* Mulsant, como una medida de la capacidad reproductiva femenina. El estudio reveló que las hembras de los escarabajos, mantenida separada con 3 machos en una situación de apareamiento continuo, pusieron un número significativamente mayor de huevos (6.93/ día) que las hembras mantenidas similarmente con 2 o 1 macho. Sin embargo, el análisis de correlación reveló una correlación negativa altamente significativa (r = -0.60**) entre la progresión de días y el tamaño del grupo de huevos puestos para las hembras aisladas con 3 machos. El número de apareamientos múltiples y el tamaño de los grupos de huevos también disminuyeron con el tiempo en comparación con los otros tratamientos. Se discuten las implicaciones de estos resultados.

Palabras Clave: masa de huevos, fecundidad, capacidad reproductiva, depredadores coccinélidos, éxito reproductivo

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The predatory coccinellid, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) lays eggs singly or in clutches near the host mealybug egg masses. The grubs hatch and feed on the mealybugs until pupation. When host resources are fixed, there will be an optimum clutch size for mother to ensure the maximum number of good quality offspring. A mated female of *C. montrouzieri* starts laying eggs about 5 days after emerging as an adult and lays up to 10 eggs per day totalling to as many as 500 eggs in her life time (60-70 days) (McPartland et al. 2000). Polyandry (multiple mating) is very common in females of this predatory coccinellid. Male insects often have several mating strategies to maximize reproductive fitness and one measure of their reproductive success is related to the number of mates (Thornhill & Alcock 1983; Arnold & Duvall 1994). However, female reproductive fitness is measured by overall lifetime fecundity, which may not be directly correlated with the number of matings (Fortes & Fernando 2011). Increased matings may lead to reduced foraging and increased energy expenditure (Daly 1978; Watson et al. 1998; Harshman & Zera 2007) resulting in an increased cost for female reproduction. Nevertheless, despite the costs that mating imposes to female reproductive fitness, polyandry in coccinellids is quite common. In coccinellids, the pre-oviposition period decreases with mating duration and is positively correlated with paternity success and fertility (De Jong et al. 1998; Omkar et al. 2006). Multiple matings often occur in *C.montrouzieri,* and the spermatheca may contain the sperm from 3-4 males leading to genetically diverse progeny (Kaufmann 1996). Hodek & Ceryngier (2000) showed that sperm precedence does not occur in *C. montrouzieri*.

The objective of the present study was to understand the influence of multiple matings of female *C. montrouzieri* on their clutch size as a measure of female reproductive fitness.

Materials and Methods

The studies were carried out at the Indian Institute of Horticultural Research, Bangalore, India. Laboratory cultures of *C. montrouzieri* were continuously maintained on the pink hibiscus mealy bug, *Maconellicoccus hirsutus* Green (Pseudococcidae: Heteroptera) with mature pumpkin fruits (*Cucurbita moschata* Duchesne ex Poir.; Cucurbitales: Cucurbitaceae) (Kairo et al. 1997; http://www.nbaii.res.in/Featured%20insects/cryptolaemus.htm) as a laboratory host. Pupae of *C. montrouzieri* of uniform age were randomly selected from the established laboratory cultures and kept separately in individual petri dishes until adult eclosion. After adult eclosion, the beetles were first sexed as per standard procedure (http://www.nbaii.res.in/Featured%20insects/cryptolaemus.htm) and marked. Each adult beetle was placed in an independent Petri dish (9 cm diam. approximately 64 cm^2) lined with Whatman filter paper (90 mm diam). Adult female *C. montrouzieri* beetles involved with different numbers of mates as well as different numbers of matings constituted the experimental treatments. The treatments were as follows: i) single female with single male –allowed to mate once; ii) single female with single male –allowed to mate multiple times; iii) single female with 2 males - allowed to mate multiple times; and iv) single female with 3 males –allowed to mate multiple times. Each treatment was replicated 3 times and all treatments were provided with a fixed number of uniform age second instar of *M. hirsutus* mealybugs. Except for treatment (i), the remaining 3 treatments were carefully observed for occurrence and confirmation of multiple matings (data not shown) between 9 AM to 4 PM. Observations were made on the number of eggs laid on each day (= clutch size) by the female beetle for 15 days continuously. The data were subjected to ANOVA, paired *t*-test, correlation, linear and curvilinear analyses (Little & Hills 1978).

Results and Discussion

There was no significant difference between single male (single time mating), single male (multiple mating) and 2 males (multiple mating) treatments for clutch size (Table 1). However, the treatment with a single female with 3 mates differed significantly from the other treatments with respect to clutch size and the maximum number of eggs laid (6.93 + 0.96 per day). The total number of eggs laid during the experimental period was greatest in the treatment with 3 mates (104 eggs), followed by 2 mates (75 eggs), single mate - single mating (60 eggs) and single mate - multiple matings (56 eggs) (Fig. 1).

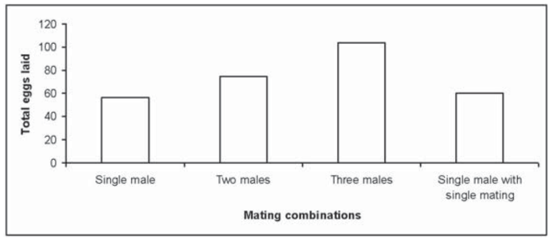

Fig. 1. Numbers of eggs laid per female *Cryptolaemus montrouzieri* in the different treatments during the study period (data from 3 replicates).

Correlation analysis revealed a highly significant negative correlation (r = -0.60**) between progression of days and clutch size with 3 mates and multiple matings. The correlation between progression of days and clutch size with other treatments was not significant (r = -0.17 and -0.25 for single mate and multiple matings and single mate and single mating respectively). In case of the 2 mates treatment, the correlation was non-significant (r = 0.15, NS).

Regression analysis explained the variability in the clutch size as days progressed in the 3 mates and multiple matings treatment up to 71% ($y = 1.198x + 2.5918$; $R^2 = 0.7107$; $F = 22.52$, $P = 0.001$) (Fig. 2). The polynomial order (2) could enhance the reliability of R^2 up to 0.91 ($y = 0.1723x^2 – 3.3665x + 20.047$; Fig. 2). It was further observed that the maximum difference in the 3 mates and multiple matings treatment was contributed by the eggs from the first day only, as the maximum number of eggs were laid during the first few days. Mated females had an almost similar oviposition pattern across the treatments. Also the average egg clutch size was comparatively smaller in the 3 mates and multiple mating treatment compared to other treatments as shown in Fig. 3 where the

Table. 1. Influence of mate number per female on clutch size *of Cryptolaemus montrouzieri* .

Treatment	Oviposition/day (Mean ± SE)
i. Single ♀ with single ♂ (single time mating)	4.00 + 0.64 (2.00 - 8.00)
ii. Single ♀ with single ♂ (multiple matings)	3.73 + 0.41 (1.00 - 15.00)
iii. Single ♀ with 2 ♂'s (multiple matings)	4.97 + 0.47 (1.00 - 7.00)
iv. Single ♀ with 3 ♂'s (multiple matings)	6.93 + 0.96 (1.00 - 8.00)
CD (P = 0.05%)	1.26

Figures in parenthesis show the minimum and maximum number of eggs laid in each treatment.

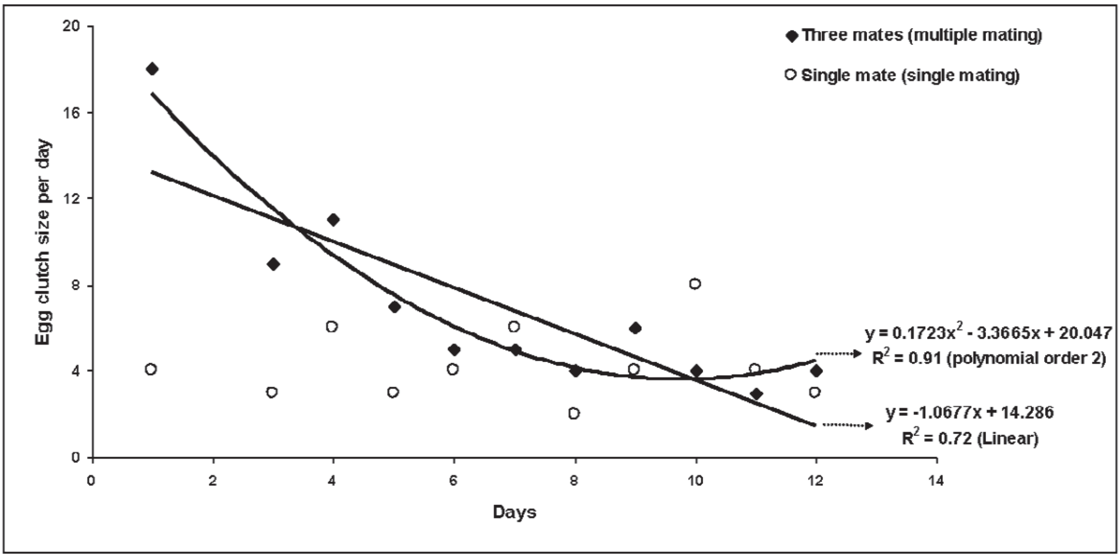

Fig. 2. Relationships between progression of days and a single male versus 3 males as mating partners for a single *Cryptolaemus montrouzieri* female and average clutch size of females.

bubble size of each day represents the total number of eggs laid, i.e., clutch size.

Since each mating offers an opportunity to father off-spring, males can generally increase their fitness by mating with many females, and high mating rates are thus typically associated with high male reproductive success. Females, in contrast, maximize reproductive success by maximizing the number of viable eggs produced. Thus, one or a few matings are sufficient for females to maximize their reproductive success. Nevertheless, multiple matings are common in females of the majority of animal species, most often with different males (polyandry) but also with the

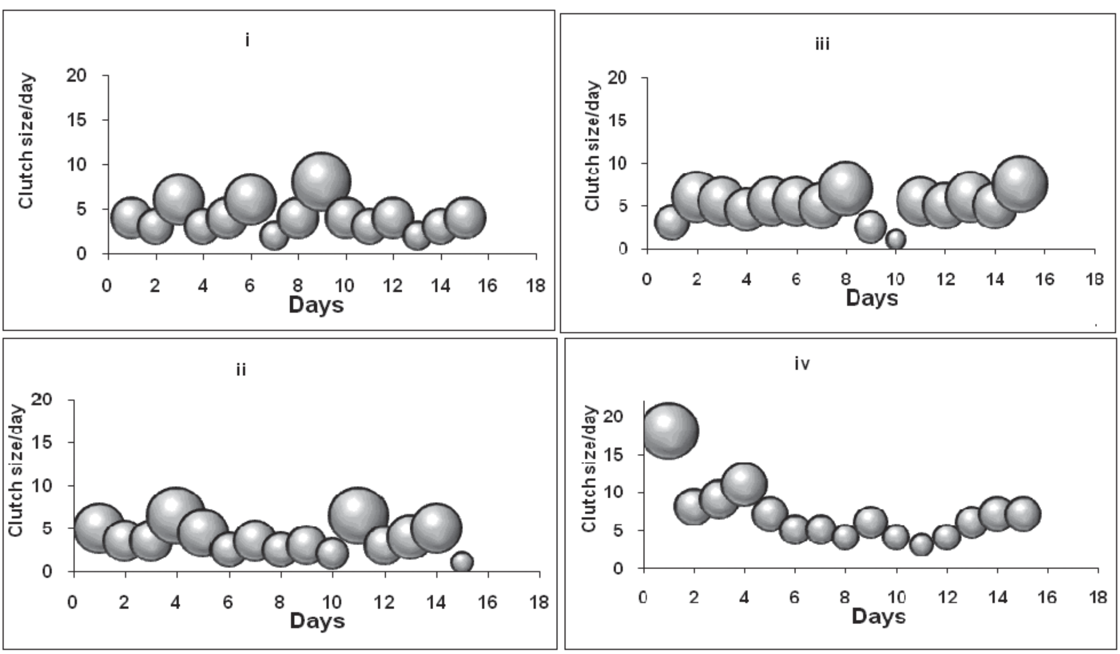

Fig. 3. Relationship between the egg laying period and the clutch size in different mating combinations of *Cryptolaemus montrouzieri* (the size of the bubble represents the relative clutch size).

same male (repeated matings) (Goran & Tina 2000; Fortes & Fernando 2011).

A single female beetle caged with 3 mates laid significantly the greatest number of eggs per female per day (6.93) compared to other treatments. There was no significant difference in the total number of eggs laid per female under single mate (single mating), single mate (multiple mating) and 2 mates (multiple mating) treatments. Theoretical studies have suggested that polyandry may affect female fitness indirectly, by providing various genetic benefits that increase off-spring fitness (Yasui 1998). However, the ecological costs of mating include time and energy costs (Daly 1978; Thornhill & Alcock 1983; Watson et al. 1998). These costs may decrease both female life span and egg production rate (Goran & Tina 2000). A study by Tregenza & Wedell (1998) showed that an ample and diverse supply of sperm may also increase female fertility. However, an excess of sperm may affect both egg production rate (Nilakhe 1977) and fertility negatively (Eberhard 1996; Goran & Tina 2000), and this was observed in the present study in which egg production exhibited a highly significant negative relationship with age in the treatment with 3 mates, and clutch size decreased over time compared to other treatments.

It is clear from our study that in the predatory coccinellid, *C. montrouzieri,* a single mating usually does not maximize female beetle fitness similar to many insect species (Goran & Tina 2000). Multiple matings in *C. montrouzieri* can contribute to maximum female egg production in spite of negative effects such as small clutch size. However, further detailed studies on the life span of female *C. montrouzieri*, the sexual receptivity of female beetle, the percent hatchability of eggs laid and progeny survival in relation to mate number are likely to provide additional inputs to understand the exact impact of polyandry in *C. montrouzieri*.

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