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INCREASED PAUSE BETWEEN CONSECUTIVE MATINGS IMPROVE REPRODUCTION AND PROGENY FITNESS IN *Coccinella septempunctata* L. (Coleoptera: Coccinellidae)

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Abstract: *In predaceous ladybird beetles, the intensity with which males exhibit courtship and frequency with which they mate are key components of male reproductive success. Present study has therefore been designed to investigate effects of male mating frequency on reproductive attributes of females; and their offspring development and survival, using four separate experimental treatments done at 27±1ºC; 65±5% RH and 14L:10D photoperiod in ETC on daily replenishment of ad libitum supply of bean aphid. In these treatments, viz. A, B, C, and D, 15-day old unmated adults of Coccinella septempunctata L. were paired and subjected to 3-successive matings within 1-day (control); 3-days (single mating/day); 5-days (single mating on first, third and fifth day); and 10-days (single mating during first, fifth and tenth day), respectively. Daily oviposition and egg hatching were recorded for next 20 days; and newly hatched instars were randomly selected per treatment for rearing until adult emergence. Experimental data were checked for normal distribution using Bartlett's test; and thereafter analyzed using one-way ANOVA followed by Tukey's post hoc comparison of means. Results revealed maximum fecundity and egg viability in females having 3-matings in 10-days (treatment-D); and minimum in females having 3-matings in 1-day (treatment-A). Moreover, parents' C. septempunctata when mated thrice in 10-days had better offspring development and survival. Probably, spacing of matings across 10-days allows replenishment of energy, sperm and seminal fluids, thereby allowing increased reproductive attributes and short immature development. Present results may be utilized for producing better offspring in laboratory for augmentative biocontrol of aphid pests.*

Keywords: *Ladybirds, Aphid, Mating frequency, Reproductive attributes, Temperature.*

Introduction: The frequency of mating in insects is often an important determinant of female reproductive output and male sperm competition. Generally, males mate with as many females as possible to increase their fitness as each mating offers an opportunity to father offspring [1]. The intensity with which males exhibit courtship and the frequency with which they mate are key components of male reproductive success [2-3]. Males can achieve high reproductive success and father significantly more offspring even under intense competition if they are able to monopolize access to females and their ova. They can do this through increasing their investment in premating traits, such as courtship, which may then lead to higher mating frequency. They can also invest more in post-mating traits, such as sperm transfer and mechanisms to promote sperm precedence and prevent female re-mating [4]. However, despite the realization that many traits can influence male reproductive success, until recently, few studies have investigated the relative contribution of pre- and post-mating traits to a male's overall paternity [5-6].

Inspite of obtaining enough sperm from a single mating to fertilize all of their eggs [1], females mate multiply to acquire (i) sufficient, or fresh sperm [7], (ii) gonadotropins derived from the male seminal fluid [8], (iii) diverse genetic material to increase reproductive output, fitness of offspring and avoid genetic incompatibility [9] and (iv) nutritional benefits [10-12]. In contrast, multiple mated females of geometrid moth, *Mnesampela privata* (Guenee), showed a trend toward decreasing rather than increasing female reproductive output [13].

Most of the work pertaining to mating frequency is on various social insects, like ants [14, 15] and bees [16, 17]. Besides, studies on mating frequency have been conducted on western corn rootworm, *Diabotrica virgifera* LeConte [18]; cabbage white fly, *Artrogeia* (*Pieris*) *rapae crucivora* Boisduval [19];

pecan nut casebearer, *Acrobasis nuxvorella* Neunzig [20]; Mexican fruitfly, *Anastrepha ludens* (Loew) [21]; field crickets, *Gryllus integer* Scudder [22]; navel orangeworm, *Amyelois transitella* (Walker) [23]; *Amphipyra* moths [24]; mite, *Caloglyphus berlesei* (Michael) [25]; pentatomid, *Edessa meditabunda* (Fabricius) [26]; and emerald ash borers, *Agrilus planipennis* (Fairmaire) [27]. All such studies suggest that at high mating rates, the costs of mating becomes substantial and decreases female lifespan and egg production, suggesting that females should mate at an intermediate rate to maximize fitness. In contrast, frequently mated queens in ant species, *Formica truncorum* Fabricius had a 37% fitness advantage over singly mated queens [28].

There are also possibilities that species with multiple benefits and high costs to mating can show several alternative fitness peaks. A previous study has shown that when varying the number of matings to females of bean weevil [29], *Callosobruchus maculatus* (Fabricius), their fitness was maximized at two alternative mating rates, i.e. high and low; while females mated at intermediate mating rates had lower offspring production. Thus, females mating at low rates were efficiently minimizing the cost of mating, while females mating at high rates were maximizing the benefits of mating. While studying the mating behaviour of butterfly, *Heliconius* Kluk, it was observed that besides protein in the spermatophore [30], males increased gift quality by providing females with cyanide, which contributed towards the protection of female eggs, and both spermatophore weight and cyanide content were correlated with mating frequency.

Recently, effects of multiple matings on reproductive attributes and progeny fitness of predaceous ladybird beetles have received the greatest attention by researchers, because of their wide significance in integrated pest management. Numerous studies have evaluated the effects of multiple matings on reproductive performance of ladybirds [31-43]. However, all such matings took place within a single day; and the studies, therefore, did not investigate the effects of increased pause between consecutive matings on the reproductive attributes and progeny fitness. In this regards, the present study has been designed to investigate the benefits of multiple matings by varying the number of days between each consecutive mating, in terms of reproductive performance and offspring development and survival, using *C. septempunctata* as the experimental model. *Coccinella septempunctata*, though of Palearctic origin, is ubiquitous, with wide tolerance of different climatic conditions and able to forage in wide range of habitats [44-45]. It is a large species, prominent in Indian agroecosystems where it considered to be an indigenous species; and is found feeding on numerous natural enemies of agricultural pests, *viz*. aphids, coccids and mites [46-47]. It is expected that the results would be helpful for producing better offspring in laboratory for augmentative biocontrol of aphid pests.

Materials and Methods

Establishment of Stock: Adult *C. septempunctata* (n=100) were collected from agricultural fields around the city of Lucknow, India (26°50'N, 80°54'E) and brought to laboratory to establish the stock in environmental test chamber at 27±1°C; 65±5% relative humidity and 14L:10D light:dark hour photoperiod. They were supplied with *A. craccivora* infested on bean (*Dolichos lablab* Linnaeus; Fabaceae) plants reared in greenhouses (at 28±1°C; 65±5% relative humidity and 14L:10D light:dark hour photoperiod). The adults were paired in plastic Petri dishes (14.5×1.5 cm). The aphid supply was replenished every 24 hours and the oviposition, if any, was recorded, after which mating pairs were transferred to new dishes. The Petri dishes containing eggs were observed for hatching and the newly emerged larvae were reared in beakers (14.5×10.5 cm) on daily replenishment of an exclusive *ad libitum* supply of the aphid species provided to parents.

Experimental Design: Newly emerged adults of *C. septempunctata* were separated from the stock culture and kept isolated in plastic Petri-dishes. On attainment of sexual maturity, both the unmated males and virgin females were paired in Petri dishes at 1000 hrs and observed for mating till 1800 hrs. Prior to formation of experimental setups, preliminary investigations were undertaken to determine the possible number of matings within a single day (from 1000hrs to 18000hs) in *C. septempunctata*.

To study the effects of male mating frequency on reproductive attributes of females and their offspring development and survival, four separate experimental treatments were formed. In treatment (A), 15-day old unmated adults of *C. septempunctata* were paired and subjected to three successive matings in a day (control). In treatment (B), 15-day old unmated adults of *C. septempunctata* were subjected to three matings within three days (one on each day). In treatment (C), 15-day old unmated adults of *C. septempunctata* were subjected to three matings within five days (single mating on

first, third and fifth day). In treatment (D), 15-day old unmated adults of *C. septempunctata* were subjected to three matings within ten days (single mating during the first, fifth and tenth day). The pairs were not changed between mating. Females of each treatment were separated and reared individually in Petri dishes with *ad libitum* supply of *A. craccivora*. Daily oviposition and daily egg hatching were recorded for next 20 days, after the completion of requisite number of matings. The experiments were conducted in 10 replicates.

For the purpose of evaluating male mating frequency on offspring development and survival, eggs from each treatment (A, B, C and D) were randomly selected and allowed for hatching. The newly hatched 100 instars were randomly selected and were reared in beakers (15.0 × 12.0 cm) at 27 ± 1ºC; 65 ± 5 % RH and 14L: 10D photoperiod in ETC on daily replenishment of *ad libitum* supply of *A. craccivora* until adult emergence, recording the development and survival of each immature stage side by side.

Total developmental period was calculated by adding incubation period, durations of all four instars, pre-pupal and pupal periods. Percent pupal survival, percent adult emergence, growth index, sex ratio and developmental rate were calculated as:

$$\text{1. Percent Pupation} = \frac{\text{Number of pupae}}{\text{Number of first instars}} \times 100$$

$$\text{2. Percent Adult Emergence (\%)} = \frac{\text{Number of adults}}{\text{Number of first instars}} \times 100$$

$$\text{3. Growth Index} = \frac{\text{Percent pupation}}{\text{Mean larval duration}}$$

$$\text{4. Sex Ratio} = \frac{\text{Total number of females}}{\text{Total number of adults}}$$

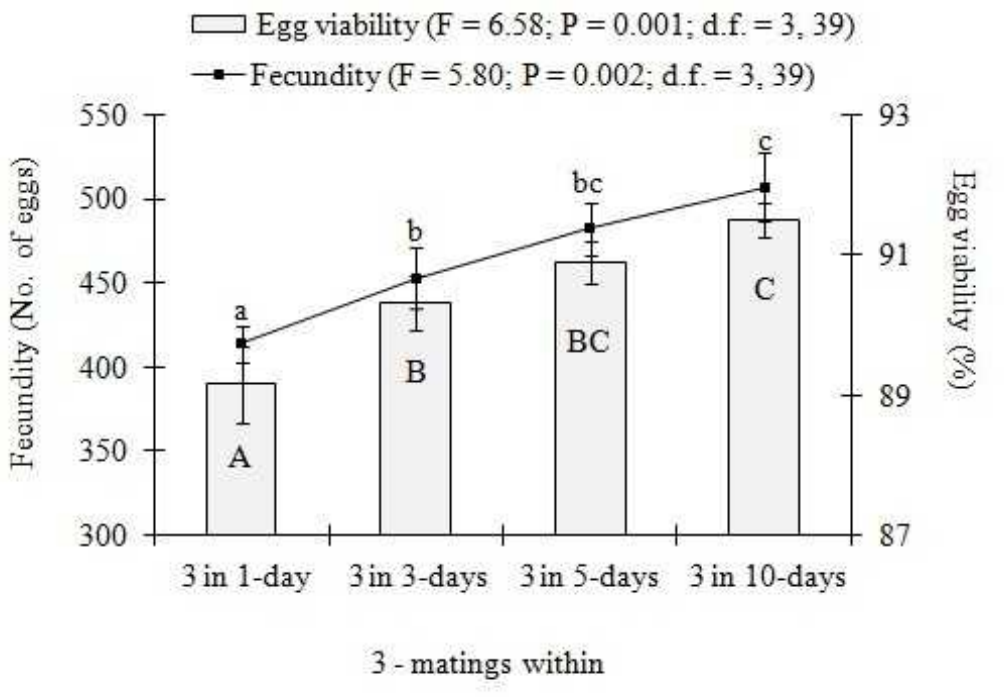
$$\text{5. Developmental rate} = \frac{1}{\text{Developmental duration}}$$

Statistical Analysis: Data on fecundity and percent egg viability were subjected to one-way ANOVA followed by *post hoc* Tukey's test of significance for comparison of means. The influence of number of matings on different developmental attributes and survival parameters of offspring, *viz.* total developmental duration, percent pupation, percent adult emergence, growth index, developmental rate and sex-ratio, was also assessed using one-way ANOVA followed by *post hoc* Tukey's test of comparison. All data were checked for normal distribution using Bartlett's test for normality and percent data were subjected to arcsine square root transformation prior to ANOVA. All analyses were done using statistical software MINITAB 16.

Results

In the present study, fecundity was maximum when females were subjected to three matings in ten days (mating treatment D) and minimum when subjected to three matings in one day (mating treatment A). Significant ($F = 5.80$; $P = 0.002$; d.f. = 3, 39) effect of different day mating frequency was observed on the fecundity that increased significantly ($r = 0.538$; $P < 0.0001$) from 3 matings in 1 day to 3 matings in 10 days. Individual means were insignificant between 3 matings in 5 days and 3 matings in 10 days. The egg viability also increased significantly from 3 matings in 1 day to 3 matings in 10 days ($r = 0.551$; $P < 0.0001$) and was significantly different ($F = 6.58$; $P = 0.001$; d.f. = 3, 39). Individual means were insignificant between 3 matings in 5 and 10 days. Thus, the results suggested that parents of *C. septempunctata* when subjected to three matings in ten days had higher fecundity and egg viability (Figure 1).

Figure 1: Effect of mating frequency (different day) on fecundity and egg viability of *C. septempunctata*.



Values are Mean± S.E.; Values followed by different alphabets show significant differences

Results further revealed that the total developmental period differed significantly (F = 19.06; P < 0.0001; d.f. = 3, 39). It was longest (15.24±0.07 days) for 3 matings in 1 day and shortest (14.57±0.08 days) for 3 matings in 10 days. The total development period for 3 matings in 3 and 5 days was 14.86±0.09, 14.67±0.04 days, respectively. Individual means between 3 matings in 5 and 10 days were insignificant. Both the percent pupation (F = 2.29; P = 0.095; d.f. = 3, 39) and percent adult emergence (F = 0.96; P = 0.423; d.f. = 3, 39) varied insignificantly. The growth index in different mating treatments was statistically significant (F = 6.41; P = 0.002; d.f. = 3, 39). However, Individual means were insignificant between 3 matings in 5 and 10 days. The variations in developmental rate were significant (F = 20.76; P < 0.0001; d.f. = 3, 39) in different mating frequencies; while the sex ratio varied insignificantly (F = 1.60; P = 0.210; d.f. = 3, 39). Thus, the results suggested that parents' *C. septempunctata* when subjected to three matings in ten days caused better growth, development and immature survival of offspring (Table 1).

Table 1: Effect of mating frequency (different day) on immature development and survival of *C. septempunctata*.

Mating frequency	Percent pupation (%)	Percent adult emergence (%)	Growth index (day^{-1})	Developmental rate (day^{-1})	Sex ratio
3-matings in 1 day	78.00±2.00^{a}	73.00±3.00^{a}	8.90±0.24^{a}	0.066±0.000^{a}	0.54±0.02^{a}
3-matings in 3 days	81.00±1.80^{a}	76.00±2.69^{a}	9.50±0.18^{b}	0.067±0.000^{b}	0.53±0.03^{a}
3-matings in 5 days	83.00±1.53^{a}	77.00±2s.13^{a}	9.88±0.20^{c}	0.068±0.000^{c}	0.57±0.03^{a}
3-matings in 10 days	84.00±1.63^{a}	79.00±2.77^{a}	10.06±0.19^{c}	0.069±0.000^{d}	0.60±0.04^{a}
F-value	2.29^{NS}	0.96^{NS}	6.41*	20.76*	1.60^{NS}

Values are Mean± S.E.
** denotes F-values to be significant at P<0.01 (d.f. = 3, 39)*
NS denotes F-values to be non-significant at P>0.05
Values followed by different alphabets show significant differences

Discussion

Results revealed that the females of *C. septempunctata* when subjected to multiple matings within one, three, five and ten days, showed improved reproduction and offspring development and survival when the matings were spread over different days, with maximum effect when over 10 days.

Both, fecundity and egg viability were least when females of *C. septempunctata* underwent requisite matings within a single day. However, the reproductive attributes were highest when requisite matings occurred within ten days. Such an enhanced

reproductive performance with the requisite number of matings relative to the number of days may be explained in terms of: (i) the mating frequency, (ii) the body movements involved during the mating process, and/or (iii) the age of mating pairs. Highest fecundity and egg viability amongst the subsets of the present study show that a single mating may not provide sufficient stimulants, hormones and/or nutrients to facilitate maturation and oviposition of entire eggs in ovarioles [48] and thus, the female has to re-mate to fertilize all of her mature ova. Spacing of the matings across ten days probably allows the replenishment of energy, sperm and seminal fluids, thereby allowing increased fecundity and percent egg viability.

It is further expected that the maternal age may also affect reproductive performance [49-53]. Studies have revealed that early age matings may be suboptimal for females [54]. In instances where matings were spread out over 10 days, the females had most likely reached their best reproductive age, causing enhanced reproductive output.

The shortest developmental duration and highest survival attributes of offspring of *C. septempunctata* females that were subjected to multiple matings spread across 10 days may also be attributed to the replenished ejaculate size and accessory gland proteins. In ladybird, *A. cardoni* too, offspring of females mated multiple times/increased mating frequencies had lower developmental durations [42]. Also, age based studies have revealed that the offspring of middle aged pairs develop faster and survive in greater numbers than old [55, 56, 53] and young pairs; and that appropriate/ middle age may be achieved in the ten day treatment leading to enhancement in offspring development and survivability.

In brief, females of *C. septempunctata* that have requisite mating frequencies within ten days have greater reproductive success and their offspring have high fitness levels than those having requisite matings within single day.

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Conflict of interest

The authors have no potential conflict of interest in relation to this article.

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