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Influence of mating duration on fecundity and fertility in two aphidophagous ladybirds

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Abstract: The influence of different mating durations on fecundity and fertility has been studied in two aphidophagous ladybirds, *Cheilomenes sexmaculata* and *Coelophora saucia*. Pre-oviposition period decreased while fecundity and fertility increased with increase in mating duration in both the ladybird species. The increase in fecundity with longer mating durations is probably due to the female response because of cryptic female choice. Mating of 10-s duration resulted in oviposition but of unviable eggs probably because of lack of sperm transfer. A minimum duration of 1 min of mating was probably essential for fertilization of the eggs. Absence of spermatophore in the reproductive tract of females is indicative of direct sperm transfer in both the ladybird species. Trend of fertility (similar fertility at 1 and 5 min followed by increase at 60 min which was again similar to that at complete mating) indicates probable sperm transfer in spurts in *C. sexmaculata* while continuous transfer in *C. saucia* is indicated by continuous increase in fertility with increase in mating duration.

Key words: *Cheilomenes sexmaculata*, *Coelophora saucia*, Coccinellidae, fecundity, fertility, mating duration

1 Introduction

Predaceous ladybirds (Col., Coccinellidae) are potential predators of phytophagous insect and acarine pests and could be used as biocontrol agents (Hodek and Honek 1996). Bulk information is available on their feeding, predator–prey interactions and biocontrol releases (Dixon 2000; Omkar and Pervez 2003). This has led to little attention being paid to studies of the sexual activities at least in predaceous ladybirds (Hodek and Ceryngier 2000). Such studies could be of direct relevance to biocontrol as a better understanding of sexual activity could lead to the maximization of reproduction in ladybirds (Omkar and Mishra 2005a). It has been shown that multiple mating and promiscuity are essential for maximizing both fecundity and fertility (Omkar and Mishra 2005a; Srivastava and Omkar 2005). Moreover, we know that optimal number of matings in a ladybird's life history result in maximization of progeny (Omkar et al. 2005), even if it might also have certain costs such as reduced longevity, increased risk of injury, predation and disease transmission, (Daly 1978; Majerus 1999; Omkar and Mishra 2005b).

Sperm transfer is a component of sexual activity and directly associated with mating. There are two basic sperm transfer mechanisms in ladybirds: direct sperm transfer which lacks spermatophore, as in *Cheilomenes sexmaculata* (Fabricius) (Omkar 2004) and *Propylea dissecta* (Mulsant) (Omkar and Pervez 2005) and, indirect sperm transfer that occurs after bouts and lateral movements aided by spermatophore, as in *Chilocorus* sp. (Fisher 1959), *Harmonia axyridis* (Pallas) (Obata 1987) *Coccinella septempunctata* Linnaeus (Omkar and Srivastava 2002) and *Coccinella transversalis* Fabricius (Omkar and James 2005). In the ladybird *H. axyridis*, mating interrupted after 30 min of genital contact did not result in fertile eggs (Obata 1987), indicating that sperm is probably not transferred during the early phase of mating (complete mating lasting 119.08 ± 16.82 min) in this species (Obata and Johki 1991).

Though in a number of insects, mating behaviours (whole copulation duration including interruptions, copula repetitions and movements displayed) have been explained in terms of sperm competition theory and cryptic female choice theory (Eberhard 1996), this has not yet been worked upon in ladybirds.

The three important questions likely to be answered are: (1) what should be the minimum mating duration to produce fertile progeny?, (2) is sperm-transfer continuous?, and (3) does prolonged mating increase fertility?

Thus, to address the above questions, two aphidophagous ladybirds, viz. *C. sexmaculata* and *Coelophora saucia* (Mulsant) were selected as experimental tools. The first species has a wider prey range and has been studied extensively (Agarwala and Yasuda 2000; Omkar and Bind 2004; Omkar and Pervez 2004), while the second ladybird has been reported from colonies of

aphid *Cervaphis rappardi indica* Basu on *Cajanus cajan* (Shantibala et al. 1997) but further information on its ecology is lacking.

2 Materials and Methods

2.1 Stock maintenance

Adults of *C. sexmaculata* and *C. saucia* were collected from bean fields (*Dolichos lablab*) of Lucknow preying on aphid, *Aphis craccivora* Koch and used for stock culture in the laboratory. They were paired in plastic Petri dishes (9.0 × 2.0 cm) and observed for oviposition providing above prey under laboratory conditions (28 ± 2°C; 65 ± 5% RH; 10L : 14D photoperiod). Eggs that were laid were further observed for hatching and the neonates were reared in plastic beakers (12 × 9.5 cm) till adult emergence.

2.2 Experimental design

Two conspecific 10-day-old virgin adults of the opposite sex were paired in a Petri dish (size and prey as above). They were observed for the start of mating (establishment of genital contact). Thereafter, the mating was interrupted after durations of either 10 s, 1 min, 5 min or 60 min. The adults were separated and the females provided with *ad libitum* supply of *A. craccivora* for the next 20 days during which daily oviposition and egg viability (fertility) was recorded twice each day. The adults were used only once during the course of the experiment and not reused in other replicates or sets. In the control set-up, the adults were allowed to mate for their complete natural mating duration. The control and each set-up were replicated 10 times ($n = 10$). Though all the five set-ups were designed to study the sperm transfer mechanism, the initial two, viz. 10 s and 1 min were designed to evaluate the minimum duration required for start of sperm transfer. In each of the above experimental set-ups, five additional replicates were set up and the females dissected immediately after the requisite mating duration under a stereoscopic binocular microscope (40×) to observe the presence of a spermatophore in the reproductive tract of female, if any.

The data on percentage fertility were subjected to arcsine square root transformation analysis prior to further analysis. The data on pre-oviposition period (i.e. duration between mating and first oviposition), fecundity and fertility (dependent variables) were subjected to two-way ANOVA using 'species' (two levels) and 'mating duration' (five levels) as the independent variables followed by *post hoc* Tukey's test of comparison at 5% levels. Thereafter, to study the main effects of mating duration on fecundity and fertility (%) of each species, one-way ANOVA relative to the mating duration was performed followed by *post hoc* comparisons (Tukey's test).

3 Results

All the five groups of mated females produced eggs, but those laid by the females interrupted after 10 s were unviable. Two-way ANOVA revealed that interaction of the two independent variables (mating-duration × species; $F = 5.53$, $P < 0.001$; d.f. = 1) significantly affected pre-oviposition period. The main effect of mating duration was also significant ($F = 3.70$, $P < 0.001$; d.f. = 4) but that of 'species' was not significant ($F = 1.53$; $P > 0.1$; d.f. = 1). As the interaction level was significant, one-way ANOVA was conducted, which revealed that the pre-oviposition period decreased significantly with increase in mating duration in *C. sexmaculata* ($F = 3.04$; $P < 0.05$) and *C. saucia* ($F = 32.37$, $P < 0.001$; fig. 1).

Interaction effect of the two independent variables was significant ($F = 21.38$, $P < 0.001$; d.f. = 4) on fecundity. Significant main effect of 'mating duration' ($F = 94.86$, $P < 0.001$; d.f. = 4) was observed on effect but it was not significantly influenced by 'species' ($F = 0.04$, $P > 0.1$; d.f. = 1). Fecundity increased with increase in mating durations up to 60 min in *C. sexmaculata*, while it was almost constant after 60 min of mating duration and complete mating. However, in *C. saucia*, fecundity increased with increase in mating duration but it was almost static after 1 and 5 min of mating duration. One-way ANOVA revealed significant effect of mating duration on fecundity ($F = 68.43$, $P < 0.0001$; $F = 32.37$, $P < 0.001$) in *C. sexmaculata* and *C. saucia*, respectively (fig. 2).

Fertility was significantly influenced by the interaction of the two independent variables ($F = 150.23$, $P < 0.001$; d.f. = 4). It was also significantly affected by the mating duration ($F = 110.77$, $P < 0.001$) while not affected by the species ($F = 1.31$, $P > 0.1$; d.f. = 4). The fertile eggs were obtained after only at least 1 min of mating. One-way ANOVA revealed significant increase in fertility with increase in mating

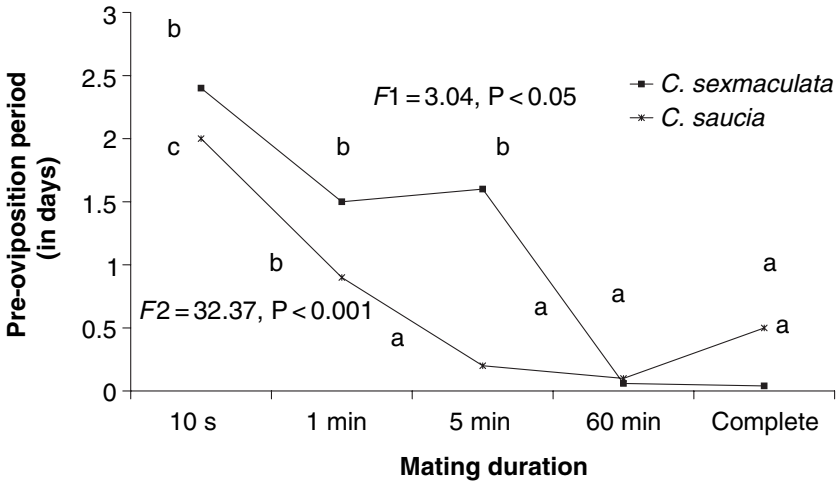

Fig. 1. *Effect of mating durations on pre-oviposition period in* Cheilomenes sexmaculata *(F1) and* Coelophora saucia *(F2); Tukey's test (range = 4.02; d.f. = 4, 45). Means followed by different letters denote statistically significant data*

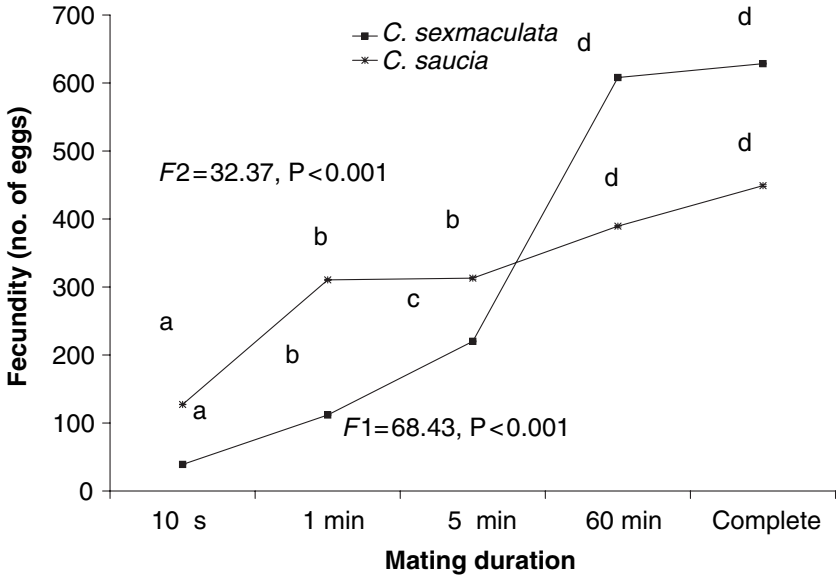

Fig. 2. *Effect of mating durations on fecundity in* Cheilomenes sexmaculata *(F1) and* Coelophora saucia *(F2); Tukey's test (range = 4.02; d.f. = 4, 45). Means followed by different letters denote statistically significant data*

duration of *C. sexmaculata* ($F = 41.36$, $P < 0.001$) and *C. saucia* ($F = 533.74$, $P < 0.001$; fig. 3).

Post hoc Tukey's comparison of the results of two-way ANOVA revealed that the individual differences in 'mating duration' within the group differed significantly between 10 s and 1min, 1 min and 5 min, and 5 min and 60 min on both fecundity and fertility. However, the differences between 60 min and complete mating treatment did not differ significantly for both fecundity and fertility. Dissections revealed the absence of any spermatophore in the reproductive tract of the females of both ladybird species.

4 Discussion

These results show that fecundity and fertility increased with the increase in mating duration in *C. sexmaculata* and *C. saucia*, while the pre-oviposition period decreased. The decrease in pre-oviposition period is probably indicative of the fact that prolonged mating triggers females to expedite egg production and oviposition. This could be a result of either increased sperm content, increased stimulation indicative of male potential or increased access to certain contents of male ejaculates, which could be nutritional or stimulatory in nature. Male ejaculate content excluding sperm is known to increase the egg production and female fitness in insect groups (Savalli and Fox 1999). Pre-oviposition period decreases while magnitude of fecundity increases with increasing mating duration. It is, thus, inferred that mating is essential for increased and viable oviposition at least in these two ladybird species. In, *P. dissecta*, the eggs sired by older males which experience a long copulation were more fertile than those of younger males mated shortly (Pervez et al. 2004), which is indirectly supported by the present finding.

Fecundity was also found to follow an overall increasing trend relative to the mating duration. The higher fecundity under subjected conditions of 60 mins and/or complete matings is probably the outcome of females displaying cryptic choice: longer matings may

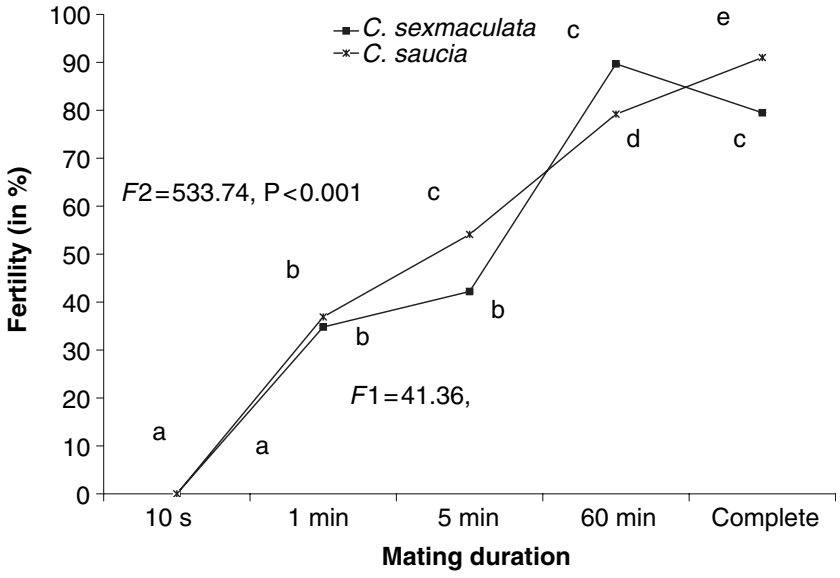

Fig. 3. *Effect of mating durations on fertility in* Cheilomenes sexmaculata *(F1) and* Coelophora saucia *(F2); Tukey's test (range = 4.02; d.f. = 4, 45). Means followed by different letters denote statistically significant data*

possibly indicate the increased fitness levels of the mating males thereby stimulating the females to lay more number of eggs.

Overall fertility was found to increase with mating duration, though no fertile eggs were laid when the females were subjected to matings of only 10 s. The results indicate that at least 1 min of mating was required to produce fertile eggs. This suggests that the sperm transfer might have occurred beyond 10 s after start of mating. We did not find any ejection of spermatophore after mating of any duration. Absence of spermatophore in the genital tract of females of both species and the relatively high fertility after a minute of mating probably indicate a strong likelihood of direct sperm transfer in the two ladybirds that were studied. In *H. axyridis* (Obata 1987) and *C. septempunctata* (Omkar and Srivastava 2002) where bouts are observed, the sperm transfer occurs via the spermatophore, which occurs after 30 min of genital contact in the former ladybird (Obata 1987).

The increase in fertility with increase in mating duration beyond a certain limit could probably be attributed to continuous sperm transfer/multiple ejaculations during a mating. Probable large ejaculate size in prolonged mating appears to fertilize greater quantity of eggs and thus increases the fertility. The males of *Adalia bipunctata* (Linnaeus) repeat mating cycle twice, thrice or even more during a single mating and this could lead to multiple ejaculations (Majerus 1999). The sperm transfer in *C. sexmaculata* seemed to occur in spurts with almost insignificantly different fertility after 1 and 5 min matings. This was, however, not true in *C. saucia* where the fertility showed a continuous increase with increase in mating duration. Thus, there is likelihood that in *C. sexmaculata* the sperm transfer took place in parts while in *C. saucia* the transfer was perhaps continuous.

Similar effects of mating duration in *C. sexmaculata* and *C. saucia* provide broader inference that mating duration enhances the progeny production in ladybirds. Apart from that, prolonged matings also ensure an increased sperm transfer along with increase in the possibility of oviposition soon after its termination (Garćia-Gonźalez and Gomendio 2003). There was no significant species-specific behaviour in sperm transfer in *C. sexmaculata* and *C. saucia*, which indicates a close phylogenetic similarity between them.

Mating duration in insects is also shaped by sperm competition, where mating may serve other functions apart from the obvious sperm transfer (Parker 1970; Simmons 2001). Prolonged matings are assumed to be costly because they can be energetically expensive, increase the risk of predation, decrease the time devoted to other activities, such as feeding, egg laying and the search for other suitable partners. There is also the risk of suffering an interruption before the completion of sperm transfer (Daly 1978; Dickinson 1997). There is a mating-longevity trade-off, which reveals that mating reduces the longevity in ladybirds (Mishra and Omkar 2006). However, the successful evolution of prolonged mating in these ladybirds signifies that its benefits must have outweighed the associated costs. It might be a strategy to enhance fertilization success due to sperm competition. Under scramble competition, it is an attempt of the male to prevent the female from mating again before laying the eggs in order to prevent or reduce the risk of sperm competition (Alonso-Pimentel and Papaj 1996). It also provides phoresis and can also increase the fertilization success of the male (Eberhard 1996). The possibility of paternity increases when male spends more time *in copula*. In the eucalyptus snout beetle, *Gonipterus scutellatus* Gyllenhal, prolonged matings play a role in the cryptic female choice (Carbone and Rivera 1998). Thus it can be concluded that in *C. sexmaculata* and *C. saucia*: (1) an increase in mating duration increases the fecundity and fertility, and decreases the duration between mating and oviposition, (2) minimum 1 min mating is needed for viable egg production, and (3) the sperm transfer is possibly direct and continuous.

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