Optimal number of matings in two aphidophagous ladybirds

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Abstract. 1. The present study was designed to identify the optimal number of matings required for maximum fecundity and egg viability in two aphidophagous ladybirds, *Cheilomenes sexmaculata* and *Propylea dissecta*.

2. For this purpose, ladybirds were subjected to different numbers of matings and the reproductive responses were recorded thereafter.

3. The Gompertz model was used to draw asymptotic graphs for fecundity and per cent egg viability in both ladybird species. Ninety-five per cent and 50% of maximum theoretical fecundity and per cent egg viability were predicted from the model.

4. Ninety-five per cent maximum theoretical fecundity was obtained after 13.25 and 12.95 matings in *C. sexmaculata* and *P. dissecta*, respectively; and 8.95 and 11.25 matings were required for 95% maximum theoretical per cent egg viability in *C. sexmaculata* and *P. dissecta*, respectively.

5. The results of these experiments clearly support the existence of an optimal number of matings in these two ladybird species leading to maximum adult fitness.

Key words. Fecundity, ladybirds, optimal matings, per cent egg viability.

Introduction

Multiple matings or specifically female re-matings occur frequently in insects (see Arnqvist & Nilsson, 2000) despite its numerous costs (Daly, 1978). They are not only time and energy consuming (Thornhill & Alcock, 1983), but also increase the risk of predation (Arnqvist, 1989) and disease transmission (Hurst *et al.*, 1995). Yet the prominent occurrence of female multiple matings in nature indicates the presence of numerous direct and indirect benefits. The act of mating *per se* is known to directly stimulate female egg production (Opp & Prokopy, 1986); second, the mere presence of sperm in the genital tract stimulates egg production (Gromko *et al.*, 1984), and an ample and diverse supply of sperm also improves fertility (Tregenza & Wedell, 1998). Multiple matings also offer some more direct benefits, such as increased nutritional gifts, and accessory proteins, and a few indirect ones, such as the

benefit of good genes to the females or gene pool enrichment (Parker & Simmons, 1989; Boggs, 1990; Andersson, 1994; Fox *et al.*, 1995a, b; Eberhard, 1996; Wedell, 1996). However, an excess of sperm may also negatively affect the egg production (Nilakhe, 1977) and fertility due to polyspermy (Eberhard, 1996).

Multiple matings are beneficial to the males because their fitness increases by siring more offspring, but the same is not true for females as their fitness is governed by viable progeny and rarely by the number of matings (Bateman, 1948) except for some odd instances (Wedell *et al.*, 2002). This basic asymmetry between the sexes suggests that male fitness increases monotonically with increased mating rate, while one or a few matings are sufficient for females to maximise their reproductive success (Bateman, 1948). There should thus, theoretically exist an optimal mating rate for females at which their fitness is maximised. A recent meta-analysis by Arnqvist and Nilsson (2000) suggests that moderate levels of re-mating substantially increase the lifetime reproductive success of females in insects, and a similar effect has also been reported in vertebrates (Osikowski & Rafiński, 2001). Arnqvist and Nilsson (2000) suggest that the primary question is not about the

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evolutionary reasons of multiple matings *per se* but about factors determining optimal mating rate for females, and whether or not the natural mating rate diverges from that optimum. To answer this question, careful analysis of costs and benefits of multiple matings under a range of conditions is necessary. Optimal mating rates are likely to not only differ between species but perhaps also within species.

Although a few studies have been conducted on the optimal mating rates of some insects (Tregenza & Wedell, 1998; Arnqvist & Nilsson, 2000), little information on this aspect is available in ladybirds (Coleoptera: Coccinellidae). The ladybirds are potential biological control agents of numerous phytophagous insect and acarine pests. Few reproductive studies have been conducted using ladybirds (Hodek & Ceryngier, 2000); however, some have been conducted on the effect of multiple matings on the general reproductive biology of the ladybirds. Ladybirds, *Coccinella septempunctata* Linnaeus (Omkar & Srivastava, 2002), *Cheilomenes sexmaculata* (Fabricius), *Coccinella transversalis* Fabricius (Omkar, 2004; Omkar & James, 2005), and pale morph of *Propylea dissecta* (Mulsant) (Omkar & Pervez, 2005) lay increased numbers of eggs with high viability in response to increased number of matings. However, experiments have not been specifically designed to investigate the costs and benefits of the optimal mating rates in ladybirds.

The present work thus aims to calculate the optimal mating rates in Coccinellidae using two aphidophagous ladybirds, *C. sexmaculata* and *P. dissecta*, as experimental tools. These two ladybirds are found in the Oriental region and commonly found in the agricultural fields infested with aphids in the Indian subcontinent (Omkar & Pervez, 2004).

Materials and methods

Stock maintenance

Adults of the ladybirds, *C. sexmaculata* and *P. dissecta*, were collected from local agricultural fields of *Dolichos lablab* Linnaeus infested by the aphid, *Aphis craccivora* Koch. The adults were paired in Petri dishes (9.0×2.0 cm) for facilitating oviposition in the laboratory along with the prey–host-plant complex from which they were collected (25 ± 2 °C, 60 ± 5 % RH, and LD 14:10 h). Eggs laid were collected and reared from the time the larvae hatched to adult emergence in glass beakers (11.0×9.0 cm) on daily replenishment of prey. The emerging adults were sexed and separated for experiments, later performed at the above-mentioned abiotic conditions.

Experimental design

Five-day-old virgin adults of ladybirds were paired in Petri dishes at 10.00 hours with *ad libitum* prey (prey and space as above) and observed for mating until 18.00 hours. The adults were separated after termination of mating.

They were re-paired on the next day for the next mating, as required. The adults were mated daily and were subjected to the requisite number of matings with a single mating per day. Our observations of stock reared in semi-natural conditions in large cages indicate the mating rate of one per day to be realistic.

The ladybirds were subjected to 1, 3, 5, 7, 10, or 20 matings and the fecundity and egg viability recorded thereafter for a lifetime. There were 10 replicates of each mating treatment in both the ladybirds.

Statistical analysis

Data were tested for normal distribution and then subjected to one-way ANOVA followed by *post hoc* Tukey's test of significance to observe the effect of matings using the statistical package SAS (SAS Institute Inc., 2002). Curves were fitted to the mean values of fecundity and per cent egg viability of both ladybirds by using the Gompertz equation:

$$Yt = A\mathrm{e}^{-B\mathrm{e}^{(-kt)}}$$

where Yt is predicted fecundity or per cent egg viability at t matings; A is the asymptote (maximum fecundity or per cent egg viability); e is the general logarithm; k is the rate of growth (slope); B is the constant of integration, and t is the number of matings.

The maximum theoretical fecundity or per cent egg viability in a lifetime was predicted at the 95% and 50% levels.

Results

The fecundity of ladybirds *C. sexmaculata* ($F_{5,54} = 25.68$, $P < 0.001$) and *P. dissecta* ($F_{5,54} = 19.14$, $P < 0.001$) increased significantly with the number of matings (Fig. 1). The coefficient of determination for the Gompertz model which defined asymptote of fecundity in *C. sexmaculata* and *P. dissecta* was 0.98 ($P < 0.001$) and 0.99 ($P < 0.001$) respectively. In *C. sexmaculata*, the number of matings needed for 50% and 95% of maximum theoretical fecundity were 2.25 and 13.25 matings in a lifetime respectively. *Propylea dissecta* required 12.95 and 3.50 matings for 95% and 50% respectively of maximum theoretical fecundity.

Per cent egg viability did not increase significantly in *C. sexmaculata* with an increase in matings ($F_{5,54} = 4.14$, $P > 0.05$) (Fig. 2). *Propylea dissecta* did, however, show a significant increase in egg viability with increased matings ($F_{5,54} = 56.71$, $P < 0.001$). The asymptote defining the Gompertz model had a significant coefficient of determination for *C. sexmaculata* ($R^2 = 0.97$; $P < 0.001$) and *P. dissecta* ($R^2 = 0.98$; $P < 0.001$). In *C. sexmaculata*, the number of matings required for 95% of maximum theoretical per cent egg viability was 8.95 matings in a lifetime. Fifty per cent of maximum theoretical per cent egg viability could not be plotted in *C. sexmaculata* as at no point was 50% or less egg viability attained. In *P. dissecta*, the
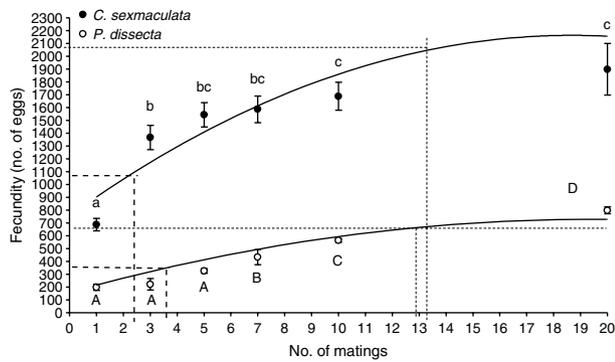
Fig. 1. Gompertz curves depicting the effect of number of matings on fecundity of two ladybirds, and the identification of optimal number of matings required for 95% and 50% maximum theoretical fecundity in a lifetime. Values are means ± SD. Thin and thick dashed lines indicate 95% and 50% maximum theoretical fecundity respectively. Values followed by different letters indicate significant differences at $P < 0.001$ within a species.

Fig. 2. Gompertz curves depicting the effect of number of matings on egg viability of two ladybirds, and the identification of optimal number of matings required for 95% and 50% maximum theoretical egg viability in a lifetime. Values are means ± SD. Thin and thick dashed lines indicate 95% and 50% maximum theoretical viability respectively. Values followed by different letters indicate significant differences at $P < 0.001$ within a species.
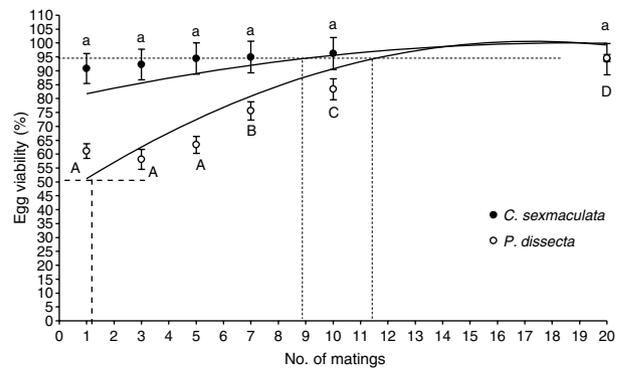
number of matings required for 50% and 95% egg viability were 1.0 and 11.25 matings respectively.

Discussion

The results reveal that the fecundity of both ladybirds increased non-linearly with an increase in the number of matings. The lack of a linear increase in fecundity with an increase in the number of matings is indicative of the existence of optimal mating rates, which were identified through the use of the Gompertz models. About 13.25 matings in a lifetime in *C. sexmaculata* and 12.95 matings in *P. dissecta* were identified as the requisite optimal number of matings for attainment of the 95% of maximum theoretical fecundity. The optima may change within species subject to numerous abiotic and biotic factors.

Increased fecundity and per cent egg viability with an increase in matings has been observed previously in a few ladybirds (Omkar & Srivastava, 2002; Omkar, 2004; Omkar & Pervez, 2005) but in all such studies the adults were subjected to a maximum of five matings and hence conclusions on the optimal number of matings could not be drawn.

The observed plateau (asymptote) in fecundity may be as a result of the fixing of an upper limit of oviposition through selection. The limit on the number of ovarioles in ladybirds (Dixon & Guo, 1993) also supports an upper limit of oviposition beyond which it cannot increase. This limitation of female fitness by fecundity is the reason behind the very concept of an optimal mating rate (Bateman, 1948). Such a relation between fecundity and matings has been established for a number of insect orders by the meta-analysis of Arnqvist and Nilsson (2000). This relation was not affected by the insect order or the food availability or nutritional access to the mating pairs,

although insects involved in nuptial feeding showed stronger positive correlations than those bereft of this benefit. In ladybirds, nuptial benefits are not known. It is, however, possible that there might be nutritional gains from the ejaculate, thereby affecting both fecundity and per cent egg viability and allowing the high number of matings required for attaining 95% of maximum theoretical per cent egg viability. The high number of matings required for 95% maximum theoretical per cent egg viability is otherwise surprising, although it is worth observing that this number is consistently lower than the number of matings required for the same level of theoretical fecundity.

A single optima is, however, not always obtained, with a double optima being reported in some studies. Double optima with a trough in the middle has been reported in the bruchid beetle, *Callosobruchus maculatus* (Fabricius) (Nilsson, 2004). In the bruchid beetle, either a single mating or more than three matings resulted in high reproductive performance, with two matings showing a negative effect. It has been suggested that females mating at low rates may efficiently minimise the costs of mating, while those mating at high rates may instead maximise the benefits of mating (Wedell *et al.*, 2002). The analysis by Arnqvist and Nilsson (2000) advocates the existence of intermediate optimal mating rates.

A significant increase in per cent egg viability with increased number of matings was observed only in *P. dissecta* and not in *C. sexmaculata*. This ambiguous relation between per cent egg viability and number of matings obtained here is indicative of the general trend obtained in insects as such. The detailed compilation of such data in insects and their analysis revealed the absence of any such clear correlation between the two. Although general trends have revealed positive correlations, this relation differed strongly between orders, being the strongest in Diptera and weakest in Heteroptera (Arnqvist & Nilsson, 2000).

A single mating is clearly not sufficient for female fitness because it does not provide a sufficient amount of sperm or gonadotropins to maintain a high production of viable eggs through a lifetime. On the other hand, high mating rates also tend to decrease the net reproductive fitness of females, primarily because of the reduction in lifespan. Between these two extremes lies the intermediate optimal mating rate at which females maximise their reproductive fitness (Arnold & Duvall, 1994; Arnqvist & Nilsson, 2000).

Thus, the present study clearly reveals the presence of optimal mating rates in the two ladybirds studied. However, there is still a need for rigorous studies on this subject. Identification of optimal mating rates in ladybirds can help improve the mass multiplication of these ladybirds by the maximisation of reproductive fitness.

Acknowledgements

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