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VIRGIN MALES ARE BETTER MATING PARTNERS THAN THE EXPERIENCED ONES: A CASE STUDY OF *MENOCHILUS SEXMACULATUS* (COLEOPTERA: COCCINELLIDAE)

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ABSTRACT

We studied the effect of male-mating history on mating performance and reproductive output of a predaceous of ladybird beetle, *Menochilus sexmaculatus* by manipulating copulation between virgin females and males of varying mating experience, *viz*. (i) unmated, (ii) once, (iii) twice, and (iv) thrice. Virgin males were more agile and longing to copulate than the once, twice and thrice mated ones. Previously virgin males initiated the mating much earlier (2.82±0.22 min) than the thrice mated ones (4.21±0.28 min). The mating duration, fecundity and egg viability (%) were maximum when virgins copulated and decreased with male-mating history. Both fecundity and egg viability exhibited a declining trend with an increase in the copulation with males having greater mating experience. The offspring of previously unmated male developed fastest with a high larval survival (%), adult emergence (%) and growth index than those of previously mated ones and slowed down as their mating experiences increased. Thus, we conclude that the virgin males of *M. sexmaculatus* are better mating partners than the experienced ones.

Keywords: Coccinellidae, Mating, Development, Fecundity, Fitness, Survival.

INTRODUCTION

In insects, high incidences of promiscuity and polygyny are attributed to the male mating strategies, as they are seemingly deriving evolutionary benefits due to this approach (Ronkainen et al. 2010; Yadav and Pervez 2022; Pervez and Jahan, 2024). According to studies, the spermatophores of virgin males grow larger and produce greater number sperm which fertilize greater number of eggs and enhance female fecundity and fertility (King and Fischer 2010; Xiaoguo et al. 2011; Michaud et al. 2013). Virgin males offer additional substances to females other than sperm in the form of seminal fluid than the copulated ones (Fox et al. 1995). Multiple mating and polygyny decrease male longevity (Oliver and Cordero 2009) and the potency of spermatophore causing sperm depletion, as ejaculate size, sperm production and replenishment are limited (Hughes et al. 2000; McNamara et al. 2009). Seemingly, males can limit ejaculate amount to maximize their future reproductive success (Wedell et al. 2002; Lewis and Wedell 2009), and can detect the mating history of their female partners and strategically allocate sperm to maximize their probability of paternity (Loose and Koene 2008). For females, however, mating with a sexually active male can be harmful because it results in fewer sperm, fewer nutrients from the nuptial gifts, a longer copulation time, a higher risk of infertility, or a shorter lifespan (Hughes et al. 2000; Wedell and Ritchie 2004; Monalisa et al. 2020). The females even reject or show refusals to mating with the unsuitable males with inferior attributes (Pervez et al. 2022).

Numerous studies show that the females who mated with multiple mated males have lower lifetime fecundity than those mated with virgin ones (Jimenez-Perez and Wang 2004; Pervez et al. 2004; Jiao et al. 2011; Michaud et al. 2013). Female reproduction may be hampered by the size and composition of ejaculate. As a result, females exhibit assortative mating in regard to polygyny, preferring do not mate with males who have recently ejaculated and have insufficient sperm resources to provide appropriate fertilization (Dewsbury 1982).

Adult male and female ladybirds, *Epilachna paenulata* Germar contribute defense alkaloids to the eggs, which males transfer to the females during mating. Predaceous ladybirds (Coleoptera: Coccinellidae) indulge in promiscuity, as they can copulate for a long period and switch their mating partners (Nedved and Honek 2012). Sperm transfer in lepidopterans may cost heavily to the males as it accompanies with loads of nutrients (Arnqvist and Nilsson 2000). The male insects can also influence female reproductive physiology, which can negatively affect progeny quality (Ridley 1988; Gerofotis et al. 2015). Given the importance of parental impacts, we examine how male-mating history of *M. sexmaculatus* affects reproduction and offspring development, survival and growth. We hypothesized that (1) virgin males are better partner in terms of quickly approaching females and mating for longer durations than experienced males, (2) male-mating history has an inverse relationship with fecundity and egg viability, and (3) male-mating history can decline the progeny quality in terms of survival, growth and development.

MATERIALS AND METHODS

Collection and rearing of insects

Adults of *M. sexmaculatus* were collected from cowpea plants (*Lablab purpureus* (L.) Sweet) near the suburbs of Kashipur, India (29.2104°N, 78.9619°E) preying on aphid, *Aphis craccivora* (Koch). These adults were brought to the laboratory and paired in Petri dishes (2.0 cm height × 9.0 cm diameter) containing sufficient quantity of above aphids. Thereafter, the Petri dishes were kept in the Environmental Test Chamber (*REMI*, Remi Instruments) at controlled environmental conditions (27 ± 1°C; 65 ± 5% RH; 14L: 10D. The adults mated, and females after copulation were isolated in different Petri dishes (size and prey, as above). These females laid eggs, which were isolated in separate Petri dishes (size and prey, as above) and were reared from egg-hatch till adult emergence. Newly emerged adults were isolated individually in Petri dishes for further experimentation.

Experimental design

(i) Impact of male-mating history on mate choice

Three mating combinations were made by pairing 10-day-old virgin male and female ladybirds, *M. sexmaculatus* in a Petri dish (size and food, as above) and allowing (i) single, (ii) twice, and (iii) thrice mating between them. We used a new female for each mating treatment. Thereafter, the male ladybird of each Petri dish was isolated in a separate Petri dish (size and food, as above). Thereafter, a 10-day-old virgin female was kept together with 13-day-old (i) virgin, (ii) once, (iii) twice and (iv) thrice mated males in a Petri dish (size and food, as above). The observation was taken 30 min using each mating pair.

(ii) Impact of male-mating history on female reproductive attributes

Three mating combinations were made by pairing 10-day-old virgin male and female ladybirds, *M. sexmaculatus* in a Petri dish (size and food, as above) and allowing (i) single, (ii) twice, and (iii) thrice mating between them. A new female was used in each mating treatment. Thereafter, the male ladybird of each Petri dish was isolated in a separate Petri dish (size and food, as above). Thereafter, a 10-day-old virgin female was paired with 13-day-old (i) virgin, (ii) once, (iii) twice and (iv) thrice mated males in Petri dishes (size and food, as above). Each pair was recorded for mating till 30 min. For each experimental method, initiating time of mating and mating duration were recorded. The experiment was conducted in 10 replicates. Each ladybird was used only once in the experiment. After mating, the females from each treatment were isolated in Petri dishes (size and food, as above). Their daily oviposition and egg-hatch were observed for next 20 days. The fecundity and egg viability were recorded in ten replicates. The adults were used only once during the experiment.

(iii) Impact of male-mating history on pre-adult development, growth and survival

We collected one hundred eggs per mating treatment in Petri dishes (size as above). The number of eggs hatched and their incubation period were recorded. Randomly selected 100 neonates were transferred into plastic beakers (15.0 × 12.0 cm; 10 instars per beaker) covered with muslin and fastened with rubber band. The larvae were fed and reared on *ad libitum A. craccivora* till adult emergence. The number of larvae and pupae survived and reached pupation and emergence, respectively, along with each instar duration, larval period, pupal period and total developmental period were recorded. Larval survival (%) (Total pupae × 100 / total number of first instars), Adult emergence (%) (total number of adults × 100 / total number of pupae), developmental rate (1 / total developmental period), and growth index (adult

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emergence (%) / mean developmental period) were calculated. We analyzed mean values per replicate.

Statistical analysis

Chi-square (χ^2) Goodness-of-fit test was used to examine the null hypothesis of random mating for adults with different mating histories. Arcsine square root transformation were subjected to all percent data prior ANOVA. The data on growth, developmental, survival and adult reproductive parameters were checked for normality using Bartlett's test prior to subjecting One-way ANOVA followed by Tukey's post hoc honest significance test at 5% level. Percent larval survival, percent adult emergence, developmental rate and growth index were also subjected to regression analysis against different mating histories. All analyses were conducted on PC using statistical software MINITAB 2.

RESULTS

(i) Impact of male-mating history on mate choice

The unmated males were more agile and longing to copulate and they approached the female earlier than the once, twice and thrice mated males (χ^2=10.64; P<0.01; N=20; Table 1; Plate-1).

Plate-1: Mating behaviour of *M. sexmaculatus*.

(ii) Impact of male-mating history on mating probability and female reproductive attributes

The initiating time of mating was significantly reduced when virgin male approached and copulated than the thrice mated ones (5.19; P < 0.05). The mating duration (F = 9.83; P < 0.0001), fecundity (F = 10.77; P < 0.0001) and egg viability (F = 8.83; P < 0.0001) were maximum when virgins copulated and decreased as the number of male-mating increased (Table-2). Both fecundity and egg viability exhibited a declining trend with an increase in the copulation with males having greater mating experience (Fig. 1).

(iii) Impact of male-mating history on pre-adult development, growth and survival

Incubation period of eggs of *M. sexmaculatus* in different mating treatment was significantly (F=3.18; P=0.035; df=3,36; Table 3) influenced by the male mating status. Total development period of offspring was (F=7.63; P=0.00; d.f. = 3,36; Table 3) tended to decrease as the male mated more times. Offspring of females mated with virgin male developed fastest followed by once, twice and thrice-mated males (Table 3). The percent larval survival (F = 16.34; P = 0.000; d.f. = 3,36; Fig. 2), percent adult emergence (F = 28.18; P = 0.000; d.f. = 3,36; Fig. 3), developmental rate of immature stages of *M. sexmaculatus* (F = 2.87; P = 0.000; d.f. = 3,36; Fig. 4) and growth index (F = 16.02; P = 0.000; d.f. = 3,36; Fig. 5) declined with increase in paternal matings.

Table 1. Impact of male-mating history on mating preference of female *M. sexmaculatus* (n = 20).

Male mating histories	**Female mating preference**
Unmated	9
Once mated	7
Twice mated	3
Thrice mated	1
χ^2- value	**10.64; P<0.01**

Table 2. Influence of male-mating history on reproductive attributes of *M. sexmaculatus*.

Male mating histories	**Initiating time of mating (in min)**	**Mating Duration (in min)**	**Fecundity (for 20 days)**	**Egg viability (in %)**
Unmated♂ × virgin ♀	2.82±0.22a	134.20±3.52a	303.0±17.57a	91.02±0.53a
Once mated♂ × virgin ♀	3.48±0.21ab	127.40±5.31a	259.0±13.00ab	88.09±0.97ab
Twice mated♂ × virgin ♀	3.53±0.25ab	123.60±6.92a	196.0±22.29bc	85.62±1.47bc
Thrice mated♂ × virgin ♀	4.21±0.28b	98.80±2.89b	179.0±15.64c	81.90±1.83c
F- value	**5.19; P < 0.05**	**9.83; P < 0.0001**	**10.77; P< 0.0001**	**8.83 ; P < 0.0001**

Values are Mean ± SE; d.f. = 3,36

Values followed by different alphabets show significant differences in the data within the column.

Table 3. Influence of male-mating history on developmental period (in days) of immature stages of *M. sexmaculatus*.

Male mating histories	Incubation period	From hatching to pupation (in days)					Pupal period	Total developmental period
		1st instar	2nd instar	3rd instar	4th instar	Total larval period		
Unmated♂×virgin ♀	3.15±0.27a	2.30±0.30a	2.50±0.16a	2.30±0.21a	2.10±0.17a	9.20±0.29a	2.80±0.32a	24.35±0.64a
Once mated♂ × virgin ♀	3.41±0.24ab	3.10±0.27ab	2.60±0.26a	2.40±0.22a	2.20±0.24a	10.30±0.51ab	3.25±0.22ab	27.26±1.11ab
Twice mated♂ × virgin ♀	3.74±0.07ab	3.20±0.29ab	2.70±0.21a	2.80±0.29a	2.40±0.22a	11.10±0.56ab	3.45±0.21ab	29.39±1.13b
Thrice mated♂ × virgin ♀	3.94±0.10b	3.40±0.26b	2.70±0.30a	2.90±0.27a	2.50±0.16a	11.50±0.60b	4.01±0.10c	30.95±1.15b
F- value	3.18	2.89	0.16	1.36	0.779	3.98	4.65	7.63
P- value	0.035	0.048	0.925	0.272	0.513	0.015	0.08	0.000

Values are Mean ± SE; d.f. = 3,36

Values followed by different alphabet show significant differences.

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Fig. 1. Impact of male-mating history on fecundity and percent egg viability in *M. sexmaculatus*.

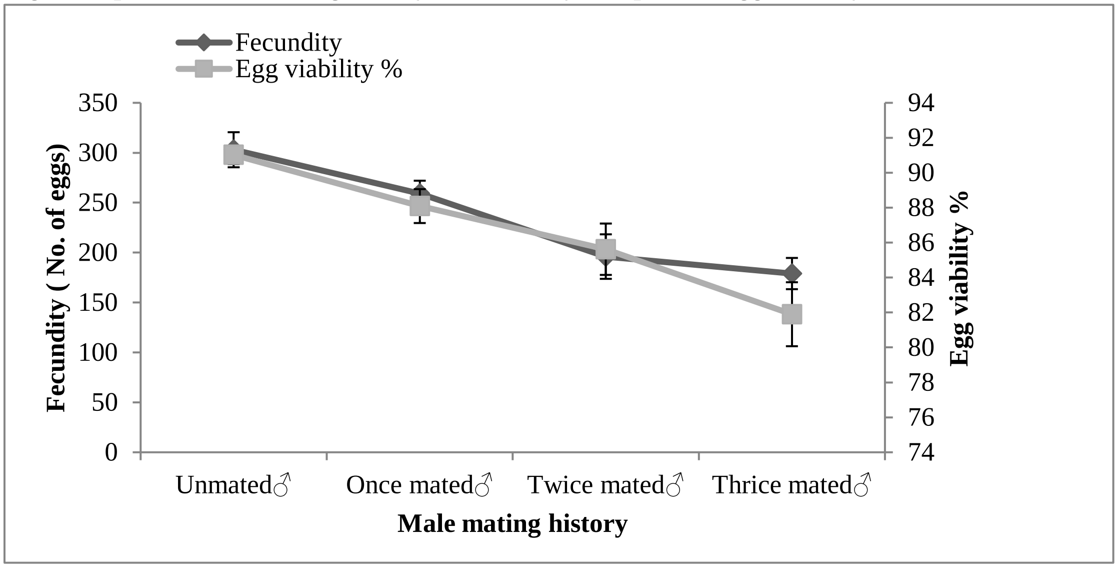


Fig. 2. Impact of male-mating history on percent larval survival of *M. sexmaculatus*.

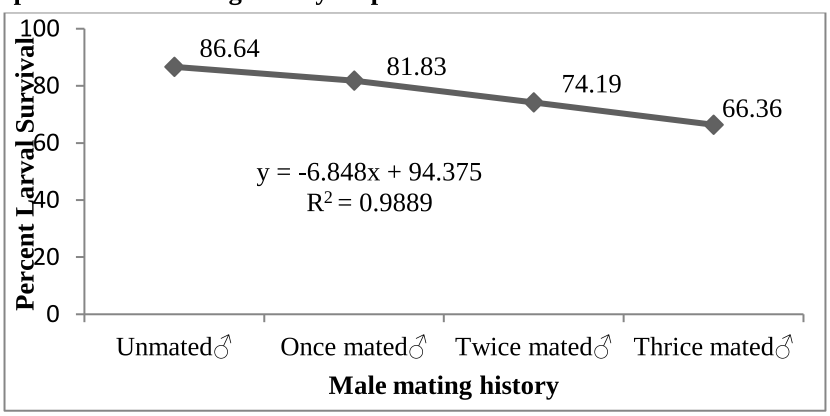


Fig. 3. Impact of male-mating history on percent adult emergence of *M. sexmaculatus*.

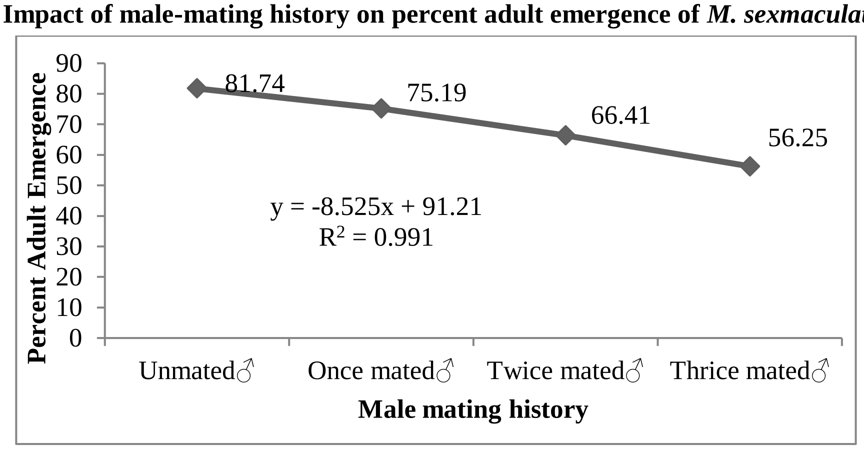


Fig. 4. Impact of male-mating history on developmental rate of *M. sexmaculatus*.

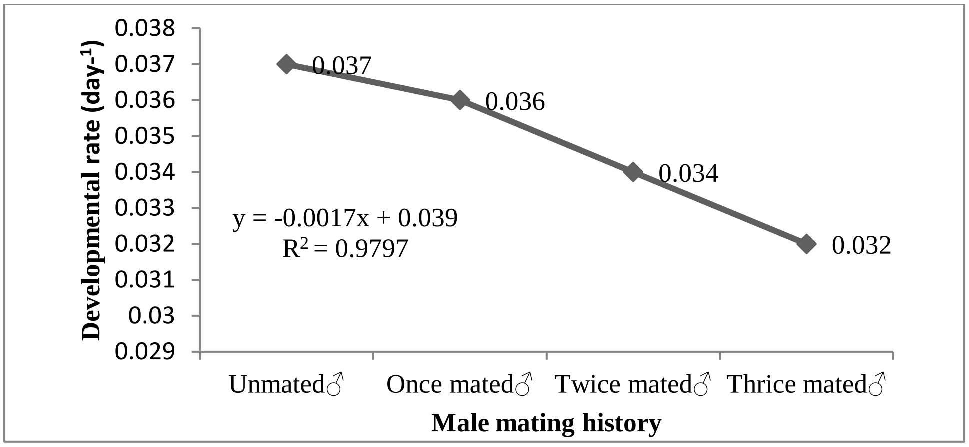
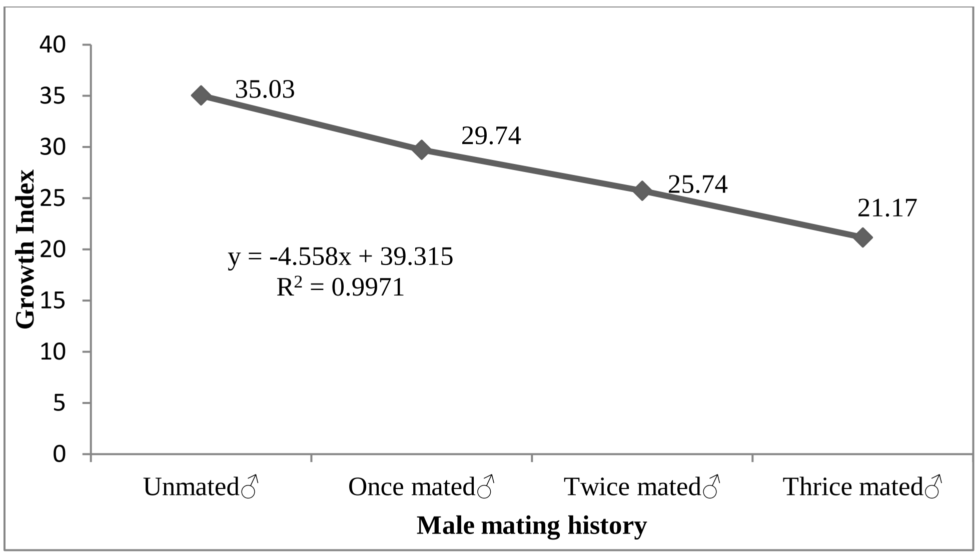


Fig. 5. Impact of male-mating history on Growth Index of *M. sexmaculatus*.



DISCUSSION

The results indicate that mating experience is an important determinant for the mating probability in *M. sexmaculatus* with a declining trend as mating experience is inversely proportionate to mating probability. Female ladybirds were more approached by unmated male than once, twice and thrice mated male. The skewness of mating probability towards virgin male indicates male-fitness in terms of larger ejaculate size, more sperm supply or nutrients and their viability as compared to more spent and mated males (Wedell et al. 2002; Lewis et al. 2011) and associated increased chances of paternity (Jiao et al. 2011; Gerofotis et al. 2015). Experienced males took greater time to initiate mating than the virgin male. This was also reflected in the mating duration, fecundity and egg viability which declined with increase in mating experience. Decline in mating duration of experienced males may be attributed to male exhaustion. Mating with a virgin male win the chance of fertilization of egg (De Jong et al. 1993) and thus, longer mating duration can assume the paternity share to the male.

Male-mating history has a negative effect on both fecundity and percent egg viability. An increase in male mating experience decreases female fecundity, which probably indicate that males' capacity for stimulating ovulation in females is decreasing (Poiani 2006). Seemingly, the females mated with experienced males had smaller ejaculates that are insufficient to effectively induce complete oviposition or fertilization (Torres-Vila and Jennions 2005; Lauwers and van Dyck 2006; Marcotte et al. 2007). Male ejaculates include components that promote female egg formation in a way

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that is similar to a dose-dependent response (Spencer et al. 1995; Eberhard 1996). As a result, females that receive larger ejaculates from unmated males during initial mating are stimulated more to increase the rate of egg maturation (Bonduriansky 2001; Marcotte et al. 2005; Gershman 2010). Studies also revealed that sperm donors carefully distribute their sperm based on the mating histories of their mates to increase their chances of paternity (Loose and Koene 2008). We agree with studies on other insects that discuss ecological advantages when virgins copulated (Vahed 2007; Morse 2010; Helinski and Harrington 2011). However, some studies show no effect of male mating experience on female reproductive performance, as in the almond moth *Cadra cautella* Walker, the fruit fly *Drosophila simulans* Sturtevant (Taylor *et al.*, 2008) and the mosquito *Aedes aegypti* (Linnaeus) (Helinski and Harrington 2011). An enhanced egg-viability when virgins mated may also indicate an increased number of spermatophores resulting in fertilization of most eggs (Rincon and Garcia 2007; King and Fischer 2010; Xiaoguo et al. 2011). Unmated males provide more nutrients and have a high probability of paternity (Bonduriansky 2001; King et al. 2005).

Long-term impacts of male-mating history were evident in their offspring. The male-mating history has an impact on the overall developing duration, percentage of pupal survival, percentage of adult emergence, developmental rate, and growth index of the progeny. The total developmental period was shortest for the offspring of virgin females mated with unmated males. However, offspring of virgin females mated with thrice times mated males showed slowest development. Progeny fitness may change based on differential investment in eggs by females in response to male quality (Cunningham and Russell 2000; Sheldon 2000; Prokop et al. 2007) or nutrients provided by the male during mating (Torres-Vila and Jennions 2005; Marcotte et al. 2005; Rincon and Garcia 2007). It is also suggested that with an increase in male-mating history, the seminal fluids become depleted of hormonal factors that serve to speed offspring development, causing slower development (Michaud et al. 2013). Increased percent pupation, percent adult emergence, developmental rate and growth index of offspring developed by unmated males, and subsequent decline in these parameters with increase in male mating history further suggest that in *Coccinella* spp. seminal components influence sperm viability in the female reproductive tract, possibly *via* sperm activation, nutrition or transport to the spermatheca, and that these resources are prone to depletion after multiple mating (Michaud *et al*., 2013). Thus, this study concludes that: (i) virgin males have high mating probability to mate than mated ones, (ii) Male-mating experience reduces fecundity and egg viability, and (iii) offspring from virgin female mated with unmated male grow the fastest and had highest survival rate.

DECLARATIONS

Availability of data and materials

Not applicable. The authors can't submit the raw data as they will further be used to developing suitable rearing techniques.

Conflict of Interests

The authors declare that they have no conflict of interests.

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