

Pre-copulatory Mate Choice in Relation to Age, Virginity, and Body Weight in *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract

The common cutworm moth, *Spodoptera litura* (Lepidoptera: Noctuidae) is a major agro-forestry pest worldwide. This pest has strong insecticide resistance and thus environmental friendly management methods are imperative to control this pest. In the present study, the pre-copulatory mate choice in relation to age at mating, virginity and body size were investigated and discussed in *S. litura*. Results show males significantly preferred young females to middle aged or old females for copulation but did not appear to demonstrate any preference between middle aged and old females. However, females significantly preferred middle aged males to young or old males for mating but did not appear to have any preference between young and old males. When offered virgin and mated potential partners, the male selector significantly preferred virgin to mated females while female selectors did not have any preference over selectees' mating history. Both males and females significantly preferred heavy mates for mating. These data provided useful information for the control of *S. litura* by using environmental friendly management methods, such as the sterile insect technique.

Keywords: *Spodoptera litura*, tobacco cutworm, mate choice, pest control.

INTRODUCTION

Sexual selection (Darwin, 1871) is one of the most active areas in behavioural and evolutionary ecology because it directly influences the reproductive fitness of animals. Individuals select their partners because potential mates vary in quality, quantity and availability. Sexual selection may occur through intrasexual selection where males or females compete for mates or through intersexual selection where females or males choose their mates with certain characteristics (McDonald & Pizzari, 2018; Xu & Wang, 2009; Jennions & Petrie, 1997; Thornhill & Alcock, 1983). Sexual selection may occur pre- or post-copulation or even during copulation (McDonald &

Pizzari, 2018; Xu & Wang, 2009; Simmons, 2001; Parker, 1970).

The understanding of mate preference and discovering of traits selected by males and females could provide information necessary for the implementation of behaviour-based control tactics. Sterile insect technique (SIT) has been successfully used in pest control worldwide (Lo *et al.*, 2000; Caprio & Suckling, 1995; Suckling *et al.*, 1990) and has been suggested for the control of *Spodoptera litura* (Seth *et al.*, 2016). The SIT consists of mass rearing a pest species, irradiating it to cause sterilization, and releasing millions of sterilized insects to mate with the pest in the wild either before an outbreak becomes significant or once an outbreak is reduced with other methods (Knipling, 1955). One SIT issue recognized since the beginning (Knipling, 1955) is

whether sterilized males can successfully attract and mate with wild females. Thus, whether or not this technique is successful in the control of an insect pest largely depends on our understanding of the mating behaviour of the pest (Cardé & Minks, 1995; Knippling, 1955). The difficulties with SIT may be reduced in some cases if the factors that contribute to male mating success and female preference in mate choice are better known. For example, many empirical studies have showed that male mating success is correlated with his age at mating and body size (see review in Brooks & Kemp, 2001) and thus it is expected that releasing sterilized males of optimal body size and at their optimal age is more likely to achieve higher control efficacy. Indeed, Shelly *et al.*, (2007) showed that releasing mid-aged sterilized males had significant higher control efficacy than releasing younger ones in the Mediterranean fruit fly, *Ceratitis capitata*.

The common cutworm moth, *Spodoptera litura* (Lepidoptera: Noctuidae) is a serious agro forestry pest worldwide (Zhou & Huang, 2002; Armes *et al.*, 1997). This pest showed high insecticide resistance (Rehan *et al.*, 2011; Zhou & Huang, 2002) and thus environmental friendly control methods such as pheromone traps (e.g. Vernon *et al.*, 2014) and SIT (Seth *et al.*, 2016) is imperative to control this pest. In the present study, therefore, the pre-copulatory mate choice in relation to age at mating, virginity and body size were investigated and discussed in *S. litura*. These data provided useful information for the control of *S. litura* by using SIT (Seth *et al.*, 2016).

MATERIALS AND METHODS

Insects

Insect rearing, body weight weighing and categorizing followed the methods described in (Li *et al.*, 2014).

Mate Choice in Relation to Age at Mating

To test whether males and females performed mate choice based on their partners' age, we allowed a 1-day-old moth (selector) to choose from three potential partners (selectees) of different ages (1-, 4- and 7-day-old) for copulation. For each replicate, we released a selector and three selectees into a plastic box (25 cm long, 15 cm wide, 8 cm high) and observed mating events during the entire scotophase until copulation occurred. Virgin insects of average weight were used in this experiment. We performed 44 replicates for male selectors and 49 replicates for female selectors. Selectees were randomly marked with different trace color powder (Magruder Color Company, USA). The mark did not influence mate choice (Binominal test, $P > 0.05$).

Mate Choice in Relation to Virginity

Our previous studies (Li *et al.*, 2014; Li *et al.*, 2012) have indicated that all males and about 40% females accepted a second mating in the subsequent scotophase after the first mating. To test whether males and females performed mate choice based on their partners' mating history, we leased a 1-day-old virgin moth (selector) and two potential partners (one 2-day-old virgin moth and one 2-day-old once-mated moth (mated at 1-day-old)) into a plastic box and observed mating events during the entire scotophase until copulation occurred. Insects of average weight were used in this experiment. We performed 20 replicates for male selectors and 31 replicates for female selectors. Selectees were marked as above.

Mate Choice in Relation to Body Weight

To test whether *S. litura* males and females discriminated between partners based on their body weight, we caged a virgin selector (average weight) in a plastic box with three potential virgin mates (light-, average-, and heavy-weight) and recorded mating events during the entire scotophase until copulation occurred. All insects used in this experiment were 1-d-old virgin moths. A total of 50 males and 42 females were used as selectors. Selectees were marked as above.

Statistics

The Marascuilo procedure of the nonparametric analysis (Daniel, 1990) was used to assess the effect of age, body weight and virginity on mate choice. All analyses were made using SAS 9.1 (SAS Institute, Cary, NC, U.S.A.) (SAS, 2006), rejection level was set at $\alpha < 0.05$.

RESULTS

Mate Choice in Relation to Age at Mating

When offered females of three different ages, males significantly preferred 1-day-old females to 4- or 7-day-old females for copulation but did not appear to demonstrate any preference between 4- and 7-day-old females (Table 1). However, female *S. litura* significantly preferred 4-d-old males to 1- or 7-d-old males for mating but did not appear to have any preference between 1- and 7-day-old males (Table 1).

Table 1 Mate choice in relation to age in *S. litura**

| Selector | Selectee | | | | DF | U_0 | P |
|------------------|----------|---------|---------|----|----|-------|---------|
| | 1-d-old | 4-d-old | 7-d-old | n | | | |
| Male (1-d-old) | 27 A | 10 B | 7 B | 44 | 2 | 15.86 | < 0.001 |
| Female (1-d-old) | 12 a | 26 b | 11 a | 49 | 2 | 8.61 | < 0.05 |

*numbers with different letters in rows are significantly different ($P < 0.05$).

Mate Choice in Relation to Virginity

When offered virgin and mated potential partners (selectees), the male selector significantly preferred virgin to mated females while female selectors did not have any preference over selectees' mating history (Table 2).

Table 2 Mate choice in relation to virginity in *S. litura**

| Selector | Selectee | | | DF | U_0 | P |
|----------|----------|-------|----|----|-------|--------|
| | Virgin | Mated | n | | | |
| Male | 16 A | 4 B | 20 | 1 | 7.20 | < 0.01 |
| Female | 14 a | 17 a | 31 | 1 | 0.29 | > 0.05 |

*numbers with different letters in rows are significantly different ($P < 0.05$).

Mate Choice in Relation to Body Weight

Both *S. litura* males and females selected their mates based on their mates' body weight and showed similar selection pattern (Table 3), where both male and female selectors significantly preferred heavy selectees.

Table 3 Mate choice in relation to body weight in *S. litura**

| Selector | Selectee | | | | DF | U_0 | P |
|------------------|----------|---------|-------|----|----|-------|--------|
| | Heavy | Average | Light | n | | | |
| Male (Average) | 26 A | 13B | 11 B | 50 | 2 | 7.95 | < 0.05 |
| Female (Average) | 23a | 7 b | 12 b | 42 | 2 | 9.57 | < 0.01 |

*numbers with different letters in rows are significantly different ($P < 0.05$).

DISCUSSION

Our results show that *S. litura* males preferred younger females for copulation when given a choice. This strategy allows males to gain the maximum reproductive return as younger females have higher reproductive potential in this insect (Yu *et al.*, 2014). However, females of this species significantly preferred 4-d-old males to 1- or 7-d-old males for mating.

This preference is unlikely for direct benefit (higher fecundity and fertility) or indirect (genetic) benefit (higher offspring fitness) because age of males has negative effect on female fecundity (Yu *et al.*, 2014). Kawasaki (1985) show that male responsiveness to female sex pheromone peaked four days after emergence and declined afterward in *S. litura*. This could be the reason why 4-d-old males have higher mating success than 1- or 7-d-old ones in this species.

Mating history in males and females has different consequences. Male's preference for virgin to mated females for mating may be his strategy to reduce sperm competition. Unlike some other species, such as *C. Jactatana* (Jimenez-Perez & Wang, 2004b) where females prefer virgin to non-virgin males for mating, our study shows that *S. litura* females do not discriminate their partners in terms of virgin or not. This difference may be due to the fact that male mating history has negative effect on female reproductive fitness in *C. Jactatana* (Jimenez-Perez & Wang, 2004a) while this does not have significant effect on female fecundity and fertility in *S. litura* (Li *et al.*, 2014).

Similar to other species (Rhainds, 2017; MacLaren & Rowland, 2006; Schafer & Uhl, 2005), both sexes of *S. litura* prefer larger mates to mate (Table 3). Body size is generally considered a cue of the female reproductive potential with large females tending to offer greater

reproductive returns to males than small ones (Bonduriansky & Brassil, 2005). In *S. Litura*, male preference to mate with larger females is likely to gain direct benefit because female fecundity and fertility increased with her weight (J. Xu, unpubl. Data). Large size may also be a cue of good quality in males, such as having better genes and more sperm supply over smaller ones (Bissoondath & Wiklund, 1996; Phelan & Barker, 1986). In many species of Lepidoptera, the size of ejaculate transferred during first mating is positively correlated with male body size (reviewed in Bissoondath & Wiklund, 1996), suggesting that females mated to large males may gain direct benefit in terms of more sperm and nutrition. For example, Wiklund & Kaitala (1995) show that in *Pieris napi* larger males inseminated larger ejaculates to females and that females receiving more male invested nutrients had higher fecundity.

Fisher's (1958) genetic model explains why females prefer ornamented males: a female choosing a male with an attractive trait will have sons and daughters that can both carry alleles for the attractive trait. Body size is heritable (e.g. Xu & Wang, 2013; Davis & Landolt, 2012; Iyengar & Eisner, 1999). Therefore, a female choosing a large mate will have large offspring and thus she will gain indirect genetic benefit because her large sons and daughters possess higher fitness in pre-copulatory mate choice (Rhainds, 2017; MacLaren & Rowland, 2006; Schafer & Uhl, 2005), and probably also in post-copulatory mate choice (Harano *et al.*, 2012; MacLaren & Rowland, 2006; Keller & Reeve, 1995).

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