



## Male Lifetime Multiple Mating Potential and Reproductive Success in the Tobacco Cutworm *Spodoptera litura*

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### Abstract

A male's reproductive success primarily depends on the number of females he can inseminate. Multiple mating are common in males but the reproductive potential of a male is much less well understood, probably because measurements of the reproductive success of males in their entire lifespan are extremely uncommon compared to females. In the present study, therefore, we tested male lifetime multiple mating potential and reproductive output in *Spodoptera litura*. Results showed that males that had access to a new virgin female daily for their lifespan mated up to 8 times successfully (produced viable offspring) with the mean number of matings being 6.25 ( $\pm 0.23$ ). Mating duration increased significantly with the increase of male mating experience. Analysis showed that male copulation history had significant effect on female fecundity and fertility. However, this effect was found only in the last mating. Therefore, the effect of male mating history on female fecundity and fertility is limited or minor in this insect. On average, a male in his lifetime fertilized 8380 ( $\pm 571$ ) eggs.

**Keywords:** *Spodoptera litura*; Mating Duration; Multiple Mating; Lifetime Reproductive Success

### Introduction

It is widely believed that a male's reproductive success depends mainly on the number of mates he can inseminate [1]. Compared to females, however, the reproductive potential of a male is much less well understood, probably because measurements of the reproductive success of males in their entire lifespan are extremely uncommon [2,3].

In a landmark paper, Dewsbury [4] shows that the cost of producing ejaculate, often considered to be very low, is nontrivial. Later studies also revealed that sperm production is costly and limited in many other species [4-7]. Males may also invest nutrients in reproduction besides sperm. In some insect species, males provide females with a nuptial gift during courtship or copulation [8]. Nutrients contained in the spermatophore have been found in the eggs and soma of females [9,10]. However, most non-pollen feeding Lepidoptera do not feed on a protein source as adults, instead, sequester most of the protein needed for egg production and basal

maintenance during their larval feeding stage [11]. Even in those nectar-feeding Lepidoptera, only small amounts of protein may be obtained in some nectars [12]. Male Lepidoptera therefore have a limited protein supply and are likely to incur substantial costs such as sperm depletion or reduced survival through mating [13].

Male investment of gametes and seminal products can have important consequences for female fitness [14]. Therefore, male copulation experience may have a profound impact on female reproductive success if male reproductive investment declines over consecutive copulations. Some studies show that females that mate with nonvirgin males have lower lifetime reproductive success than those that mate with virgin males while other studies do not find this relationship [14]. This inconclusive scenario may occur because male mating experience is affected by a number of factors. The size, quality and number of spermatophores delivered by males have been shown to be highly sensitive to such factors as male body size, age at mating, larval and adult feeding regime, mating order and the duration between consecutive matings [15].

## Aim of the Study

In the present study, therefore, we tested male lifetime multiple mating potential and reproductive output in the common cutworm moth, *Spodoptera litura*.

## Materials and Methods

### Insects

Insect rearing, body weight weighing and categorizing followed the methods described in Li, *et al* [16]. Unless stated otherwise, all insects used in the present study were 1-d-old virgin moths with average body weight.

### Male multiple mating potential and reproductive output

In the first experiment, a 1-d-old virgin male was caged with a 1-d-old virgin female at the beginning of the scotophase. If mating occurs, the mated female was removed and then a newly 1-d-old virgin female was induced to the male. Such treatment was repeated until the end of the scotophase. This experiment allows us to test whether *S. litura* males can copulate more than once during one night. Twenty males were used ( $n = 20$ ). The copulated females were caged individually for their lifespan in the plastic boxes (25 cm long, 15 cm wide, 8 cm high). Female fecundity and fertility were recorded as described in Li, *et al* [16].

In the second experiment, a 1-d-old virgin male was allowed to copulate with a 1-d-old virgin female, and then offered a 1-d-old virgin female every 24h until he died. Twenty males were tested ( $n = 20$ ). Mating duration was recorded. The copulated females were caged individually for their lifespan in the plastic box. Female fecundity and fertility were recorded as above.

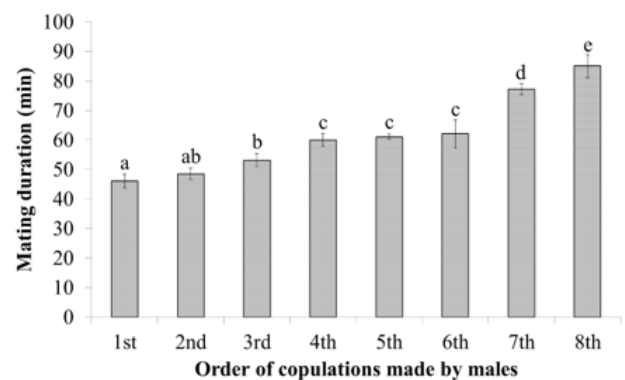
### Statistics

The difference between treatments in female fecundity and fertility, mating duration were analyzed using an analysis of variance (ANOVA) followed by Tukey's studentized range test. All analyses were made using SAS 9.1 (SAS Institute, Cary, NC, U.S.A.) [17]. Rejection level was set at  $\alpha < 0.05$ . All values reported were Mean  $\pm$  SE.

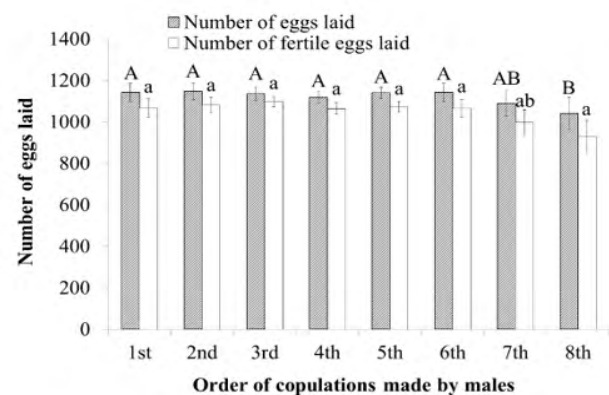
## Results

Results of the first experiment showed that almost all males ( $19/20 = 95\%$ ) copulated once, while only a few males ( $5/20 = 25\%$ ) copulated twice within the 10 h scotophase and no males copulated more than two times. Moreover, female fecundity and fertility test showed that the first copulation can normally fertilize eggs while the second copulation is infertile (could not fertilize any eggs).

In the second experiment, males that had access to a new virgin female daily for their lifespan mated up to 8 times successfully (produced viable offspring) with the mean number of matings being  $6.25 (\pm 0.23)$ . Mating duration (Figure 1) increased significantly with the increase of male mating experience ( $DF = 7, 181; F = 22.49; P < 0.0001$ ). Male copulation history had significant effect on female fecundity ( $DF = 7, 118; F = 2.22; P < 0.05$ ) and fertility ( $DF = 7, 118; F = 5.26; P < 0.0001$ ) (Figure 2). On average, a male in his lifetime fertilized 8380 ( $\pm 571$ ) eggs ( $n = 20$ ).



**Figure 1:** Mating duration under different insemination status of male *S. litura*. Bars with different letters are significantly different ( $P < 0.05$ ).



**Figure 2:** Number of eggs and number of fertile eggs laid by female *S. litura* under different insemination status.

## Discussion

A male's reproductive success primarily depends on the number of females he can inseminate [1]. Multiple matings are common in lepidopteran males but male remating rate varies between species. For example, *Cnephasia jactatana* males can mate 6 times [18] while *Cydia pomonella* males can mate 18 times [19].

*S. litura* adults are nocturnally active [20]. A few males (25%) can mate twice within the same scotophase but these second matings cannot fertilize any eggs. However, if the second mating occurs in the subsequent scotophase after the first mating, it can fertilize eggs normally (Figure 2). Therefore, males of this species need a recovery period for at least 24h between successive matings. Similar results have been found in *C. jactatana* [18], *Lobesia botrana* [15] and *Ephestia kuehniella* [21]. Furthermore, like many other studies in the Lepidoptera [19,22-24], the current study demonstrates that male mating duration significantly increased with the increase of male mating experience. These facts generally support the hypothesis that sperm production is costly and limited [4-7,25] and follows a circadian rhythm [21,26].

In *Zeiraphera canadensis* [27] and *C. jactatana* [18], male mating history showed significant effect on female fecundity and fertility. In the present study, analysis also showed that male mating history had significant effect on female fecundity and fertility (Figure 2). However, Tukey's studentized range test showed that this effect only found in the last mating. Therefore, the effect of male mating history on female fecundity and fertility is limited and can be neglected. From a meta-analysis Torres-Vila., *et al.* [28] shows that the reproductive fitness of females in species with low female remating rates is less negatively impacted by smaller male reproductive investments than that in highly polyandrous species. Female reproductive output is affected by sperm number, oviposition duration and male derived nutrition [18,29,30]. *S. litura* females do not obtain male nutritional investment for fecundity [16]. They lay all their eggs within a short period after mating (about 5 days) [20], reducing the chance of sperm loss during storage due to sperm ageing or female hostile condition [29]. This may explain why male copulation history only has minor effect on female fecundity and fertility in this species. In contrast, the obvious reduction of female reproductive output due to male mating history in *C. jactatana* may be because females have a long oviposition period (15d) and male derived nutrition can enhance female fecundity [18].

## Conclusion

This inconclusive scenario may occur because male mating experience is affected by a number of factors. The size, quality and number of spermatophores delivered by males have been shown to be highly sensitive to such factors as male body size, age at mating, larval and adult feeding regime, mating order and the duration between consecutive matings.

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