Bodyweight-Related Polyandry in the Tobacco Cutworm Moth *Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract

Recent studies on polyandry evolution have revolutionized our understanding of sexual selection. Therefore, study on traits in females, such as sensory perception and remating strategies, is expected to disclose a greater role of sexual selection in female evolution. Here, we tested how bodyweights of both sexes affected female and male remating behavior in *Spodoptera litura* (Fabricius, 1775) (Lepidoptera: Noctuidae). Results show most (74.4%; unmated virgin females may be sexually immature) females and males mated the first time in the second scotophase after eclosion. In the subsequent scotophase, 41.9% once-mated females and 94.1% once-mated males mated the second time with a novel virgin mate. Analyses indicate that female remating probability significantly increased with the increase of her own and the second male’s bodyweight but decreased with the increase of the first male’s bodyweight, while bodyweights of both sexes have no significant effect on male remating probability. Considering the nature of the low level of polyandry (two times on average) and last male precedence in *S. litura*, we propose that 1) females are more choosy when remating, from which females will benefit indirectly by sequential mate comparison and remate to replace sperm from the previous mates with those from higher quality mates, and 2) males will generally like to remate with any possible females, from which males will gain direct benefit by siring more offspring. The outcome of polyandry rate is likely to be the result of male investment × female choice interactions.

Key words: *Spodoptera litura*, body weight, mate choice, polyandry, sexual selection

The Darwin–Bateman paradigm predicts that males’ battle for access to multiple mates is the main force of sexual selection, which drives the evolution of male traits carrying advantages for intrasexual competition (reviewed in Kvarnemo and Simmons 2013, McDonald and Pizzari 2018). However, recent studies on the evolution of polyandry have revolutionized our understanding of sexual selection; increasing studies find that females also benefit directly or indirectly from multiple matings, which can generate sexual selection on female traits that influence their mating success (reviewed in Kvarnemo & Simmons 2013, Taylor et al. 2014, McDonald and Pizzari 2018). Therefore, study on traits in females, such as sensory perception and remating strategies, is expected to reveal a greater role of sexual selection in female evolution (Kvarnemo and Simmons 2013). Moreover, interests of males and females may be in conflict and benefits/costs of multiple mating are unlikely to be equal between sexes (Parker and Birkhead 2013). Therefore, it strongly suggested to study the evolutionary significance of multiple mating by considering the benefits and costs of both sexes (Kvarnemo and Simmons 2013, Parker and Birkhead 2013, McDonald and Pizzari 2018).

Studies have suggested that polyandry allows females to gain direct benefits by replenishing sperm and nutrition from the ejaculate (Meade et al. 2017), and indirect benefits in terms of offspring genetic diversity and ‘good genes’ (Taylor et al. 2014, Rafter et al. 2018). However, there are many costs of mating in general, particularly in multiple matings, such as energy costs and disease transmission (Okada et al. 2017). To maximize the benefits under the costs of mating, females should have evolved various strategies for mate choice during the process of multiple matings (Taylor et al. 2014). For example, studies have found that females from six invertebrate species and two vertebrate species discriminate against previous mates and favor novel mates in their subsequent matings, which enhanced offspring’s genetic diversity (reviewed in Li et al. 2014).

To obtain genetic benefits in terms of ‘good genes’, females should seek rematings with males of higher genetic quality, such as larger ones, than their previous mates to achieve high-quality
offspring (Taylor et al. 2014, Culina et al. 2015). Body size or weight is a key determinant of an organism’s ecological and physiological properties and one of the most important traits involved in mating success (e.g., Schafer and Uhl 2005, Xu and Wang 2009, Rhainds 2017). Therefore, the study of body-size-related multiple matings in different taxa will provide evolutionarily deep insights into reproductive strategies and sexual selection in females.

Here, therefore, we tested how bodyweights of both sexes affected female and male remating behavior in the tobacco cutworm Spodoptera litura. The data of this study and previous studies in this species allowed us to report the body-weight-related polyandry pattern in S. litura, and to discuss the consequences and evolutionary significance of such pattern.

Spodoptera litura adults close at dusk and no mating occurs in the eclosion scotophase (0-d-old). Maximum mating (approximately 70%) occurs during the subsequent scotophase after eclosion (1-d-old) (Bloch et al. 2013). The remaining unmated moths mate during the third scotophase (2-d-old). Both males and females mate multiply (Li et al. 2014). About 35% of females mate again 24 h after the first mating (Li et al. 2014).

Materials and Methods

Insects

Spodoptera litura larvae were reared on an artificial diet (Li et al. 1998), and adult moths were fed a 10% honey solution under a 14:10 (L:D) h photoperiod, at 26°C and at 60–80% relative humidity. The male and female pupae were collected from the colony and housed separately in plastic boxes. Newly eclosed moths (<12 h old) were weighed to an accuracy of 0.0001 g using an electronic balance. The mean bodyweights (mean ± SD) of the male and female moths were 152.6 ± 19.5 mg and 200.4 ± 25.3 mg, respectively. We categorized the bodyweights as light (less than 1 SD below the mean), average (within 1 SD of the mean) and heavy (more than 1 SD above the mean) (Lu et al. 2017). In the present study, all used insects were reared using the same food and conditions as above, which allow the body size variation in adult moths is more likely due to genetic variation (e.g., Sharp and Agrawal 2009).

Effect of Bodyweights of Both Sexes on Female and Male Remating

At the beginning of the second scotophase after eclosion, we selected 50 light, 150 average, and 50 heavy 1-d-old virgin females and 50 light, 150 average, and 50 heavy 1-d-old virgin males from the colony. These moths were mixed thoroughly and then were randomly selected and paired in plastic boxes (one pair one box). Once-mated males and females in this scotophase were separated and held individually in the box. In the subsequent scotophase, 186 once-mated females (64 pairs did not mate in the previous scotophase and thus were discarded) were individually paired with novel 1-d-old virgin males (include 38 light, 110 average, and 38 heavy males) randomly in the boxes (one pair one box) for the entire scotophase. Similarly, those 186 once-mated females were individually paired with 1-d-old virgin females (include 38 light, 110 average, and 38 heavy females) randomly. The bodyweight and mating events of all used insects were recorded.

Statistical Analysis

The Logistic procedure was used to analyze the effect of bodyweight on female and male remating. The relationship between female remating probability and body weight of their own and their mates was given by the equation: \[ y/(1-y) = \exp (\beta_0 + \beta_1 x_{m1} + \beta_2 x_{f1} + \beta_3 x_{m2} + \beta_4 x_{f2}), \]

where \( \beta_0, \beta_1, \beta_2, \beta_3, \) and \( \beta_4 \) are model parameters; \( x_{m1} \) is the male bodyweight; \( x_{f1} \) and \( x_{m2} \) are the first and second males’ bodyweight, respectively. Similarly, the relationship between male remating probability and bodyweight of their own and their mates was given by the equation: \[ y/(1-y) = \exp (\beta_0 + \beta_1 x_{f1} + \beta_2 x_{m1} + \beta_3 x_{f2}), \]

where \( x_{f1} \) is the male bodyweight, \( x_{f2} \) and \( x_{m2} \) are the first and second females’ bodyweight. Only significant terms, after running the full regression models, were kept in the final models. Rejection level was set at \( \alpha < 0.05 \). All analyses were made using SAS 9.1 (SAS 2006).

Results

Most (186/250 = 74.4%) males and females mated the first time in the second scotophase after eclosion. In the subsequent scotophase, 41.9% (78/186) once-mated females and 94.1% (175/186) once-mated males mated the second time with a novel virgin mate.

The Logistic procedure analysis indicates that the bodyweights of females and males have a significant effect on female remating probability (Global likelihood ratio test for \( \beta = 0 \): df = 3,183; \( \chi^2 = 52.34; P < 0.0001 \)). Analysis of maximum likelihood estimates indicates that female remating probability significantly increased with the increase of her own and the second male’s bodyweights but decreased with the increase of the first male’s bodyweight (\( y/(1-y) = \exp ((-7.8343 + 0.0177x_1 + (-0.0243)x_{f1} + 0.0487x_{m2}) \) (Fig. 1).

Analyses indicate that the bodyweights of both sexes have no significant effect on male remating probability (Global likelihood ratio test for \( \beta = 0 \): df = 3,183; \( \chi^2 = 1.36; P > 0.1 \)).

Discussion

The phenomenon that heavier (Fig. 1) or larger females are more likely to remate has also been reported in other insect species (e.g., Schafer and Uhl 2005, Xu and Wang 2009, Engqvist et al. 2014). In insects, including S. litura (Xu et al. 2018), heavy females usually have an inherently greater egg-laying capacity and thus need to obtain more sperm and male-derived factors from remating to achieve maximum reproductive gains (Shapiro et al. 1994).

Schafer and Uhl (2005) report that in the cellar spider, the chance of a female remating is significantly predicted by female body size but not by the body size of the second or first male. Using male dummies of different sizes in a fish species, Poecilia latipinna, MacLaren and Rowland (2006) demonstrated that females prefer larger male size in the sequential settings. In the adzuki bean beetle, the remating probability of large females was not affected by first male size, whereas small females that mated first with smaller males were more likely to remate, which may be due to small females suffering greater harm from copulating with larger males (Harano et al. 2012). More interestingly, the present study demonstrated that the probability of female remating was predicted by the bodyweights of her own and the first and second males: the heavier the female and the second male were, the lighter the first male was, the more likely the female was to remate (Fig. 1).

In the present study, most (74.4%); those unmated ones may be sexually immature; Bloch et al. 2013) S. litura females mated the first time in the pairing scotophase and only 41.9% females mated the second time in the subsequent scotophase. Therefore, S. litura females may mate with any males they encounter for their first mating to ensure fertilization (Pitcher et al. 2003) and then accept further mating only if subsequent males are of higher quality (e.g., larger) than previous mates. Our previous study (Li et al. 2014) indicates that S. litura females favor novel mates in their second matings and
strategically save eggs to facilitate fertilization by the second mates. This evidence suggests that *S. litura* females have evolved strategies for mate choice during the process of multiple matings, which allow them to seek rematings with novel males (Li et al. 2014) and males of higher quality (such as heavier; Fig. 1) than their previous mate to achieve direct and/or indirect benefits.

However, direct benefits hypothesis cannot explain this female preference (i.e., females prefer heavier males in multiple matings; Fig. 1) because neither male body weight (Xu et al. 2018) nor female remating (with the same or different males) (Li et al. 2014) has significant effect on female fecundity, fertility, and longevity in *S. litura*.

When direct benefits are absent, indirect benefits may be important to maintain such female preference (Kirkpatrick 1996). When male traits reflect male attractiveness and/or viability (i.e., good genes) and are heritable, females mating with such males can indirectly increase their fitness by increasing the reproductive success of their sons (Fisher 1958) and/or the viability of their offspring (Andersson 2006). In insects, phenotypic and genotypic quality often is positively correlated with body size. For example, larger males have higher probability of obtaining mates (Sokolovska et al. 2000), seducing nonvirgin females (Lewis et al. 2013) and mating more often (Alcock 1990). Similar to other insect species (e.g., Davis and Landolt 2012), body size is heritable in *S. litura* (Xu et al. 2018).

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**Fig. 1.** Predicted female remating probability in response to bodyweight of her own and the first and second males in *S. litura* \(|y/(1−y) = \exp((-7.8343) + 0.0177x_f + (-0.0243)x_m1 + 0.0487x_m2)|\). (a) light females, make \(x_f = 149.8\) mg (mean of female bodyweight − 2SD) in the equation, (b) average females, make \(x_f = 200.4\) mg (mean of female bodyweight) in the equation, and (c) heavy females, make \(x_f = 251\) mg (mean of female bodyweight + 2 SD) in the equation.
Therefore, *S. littura* females that mate with larger males are most likely to gain genetic benefits as their larger sons and daughters will carry alleles for those beneficial characters.

In addition, besides female choice as mentioned above, male investments, including 1) energy, such as the second larger males may have better energy supply for courtship behavior, and 2) ejaculate, such as the first larger males may transfer more sperm to females than smaller ones (Bissoondath and Wiklund 1996), may also have some effects on bodyweight-related female remating probability (Fig. 1). Therefore, the outcome of female remating rate is the result of male investment $\times$ female choice interactions.

Considering the nature of the low level of polyandry (two times on average) (Li et al. 2014) and last male precedence in *S. littura* (Seth et al. 2002), we propose that 1) females are more choosy when remating (Fig. 1), from which females will benefit indirectly by sequential mate comparison and remate to replace sperm from the previous mates with those from higher quality mates, and 2) males will generally like to remate with any possible mates (this study), from which males will probably gain direct benefit by siring more offspring.

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