



Female Remating Behavior in a Lekking Moth

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Abstract

In polyandrous species, male reproductive success will at least partly be determined by males' success in sperm competition. To understand the potential for post-mating sexual selection, it is therefore important to assess the extent of female remating. In the lekking moth *Achroia grisella*, male mating success is strongly determined by female choice based on the attractiveness of male ultrasonic songs. Although observations have indicated that some females will remate, only little is known about the level of sperm competition. In many species, females are more likely to remate if their first mating involved an already mated male than if the first male was virgin. Potentially, this is because mated males are less well able to provide an adequate sperm supply, nutrients, or substances inhibiting female remating. This phenomenon will effectively reduce the strength of pre-copulatory sexual selection because attractive males with high mating success will be more susceptible to sperm competition. We therefore performed an experiment designed both to provide a more precise estimate of female remating probability and simultaneously to test the hypothesis that female remating is influenced by male mating history. Overall, approximately one of five females remated with a second male. Yet, although females mated to non-virgin males were somewhat more prone to remate, the effect of male mating history was not significant. The results revealed, however, that heavier females were more likely to remate. Furthermore, we found that females' second copulations were longer, suggesting that, in accordance with theory, males may invest more sperm in situations with an elevated risk of sperm competition.

Introduction

In many animal species, males compete for access to receptive females generating sexual selection on traits such as weaponry or ornaments which increase male mating success (see Andersson 1994; Andersson & Simmons 2006). However, if females mate with more than one male (henceforth remating), mating success will not be the only determinant of male reproductive success; instead, this will at least partly be affected by males' success in sperm competition (Parker 1970; Birkhead & Møller 1998; Simmons 2001). The probability of female remating is therefore an important characteristic of a species' mating system, because polyandry will set the stage for post-mating sexual selection. Accordingly, males from polyandrous

species or populations are often characterized by a series of adaptations, such as larger testes (e.g. Harcourt et al. 1981; Hosken & Ward 2001) or more competitive sperm (Martin-Coello et al. 2009; Firman & Simmons 2011).

Nonetheless, future female remating is naturally not in the interest of current male partners, and this may in turn lead to male adaptations aiming to prevent its occurrence (Sillén-Tullberg 1981; Polak et al. 2001; Andersson et al. 2004; Engqvist 2007). In this respect, male ejaculate size often plays a prominent role in the reduction of female receptivity (Wedell 1993, 2005; Vahed 2006). Females may be sperm-constrained and remate to renew their sperm supply (e.g. Proshold 1995). In addition, the ejaculate may contain nutrients or male manipulative substances,

and receiving smaller amounts of these may affect female receptivity and female incentive to seek additional matings (Thornhill 1983; Simmons & Gwynne 1991; Wedell 1993; Sakaluk et al. 2006; Vahed 2006). In terms of male reproductive success, provisioning of a large ejaculate may therefore have manifold benefits: In addition to potentially increasing females' and thus directly the males' own reproductive output (Arnqvist & Nilsson 2000), more sperm will apparently be advantageous in sperm competition, increasing the probability of fertilization (e.g. Gage & Morrow 2003; Engqvist et al. 2007), and a larger ejaculate may also decrease the probability that females actually do remate at all in the first place (Oberhauser 1989; He & Tsubaki 1991; Wedell 1993; Wiklund & Kaitala 1995).

This effect of ejaculate size on female receptivity and future remating propensity has some important consequences for sexual selection. In many insects, in particular in the Lepidoptera, male ejaculate size usually decreases in successive male matings (Svärd & Wiklund 1986; Bissoondath & Wiklund 1996; Torres-Vila & Jennions 2005; Cordes et al. 2013). Virgin males therefore often transfer considerably larger ejaculates than already mated males, and females in many species consequently remate sooner after initial matings with non-virgin males (Oberhauser 1992; Cook & Gage 1995; Kaitala & Wiklund 1995; Wedell & Cook 1999; Marcotte et al. 2006; McNamara et al. 2009). High male mating success may thus come at a cost of reduced ejaculate size (Preston et al. 2001; Tazzyman et al. 2009; Engqvist 2011; Vahed & Parker 2012) and higher incidence of sperm competition. This virgin male advantage has the potential to reduce the strength of pre-copulatory sexual selection (e.g. Snedden 1996), because of the resulting covariance between mating success and paternity lost to other males (see Shuster et al. 2013). Understanding the features of this link between pre- and post-copulatory sexual selection remains a challenge for future research in this area (Kvarnemo & Simmons 2013).

The lesser wax moth, *Achroia grisella* (Lepidoptera: Pyralidae), represents a well-known model system for studying the evolutionary ecology of pre-copulatory female mate choice and the evolution of male secondary sexual characters (e.g. Jang & Greenfield 1996, 1998; Reinhold et al. 1998; Brandt & Greenfield 2004; Danielson-François et al. 2006; Zhou et al. 2011). Males aggregate in small leks (Greenfield & Coffelt 1983; Spangler et al. 1984), and male mating success is strongly determined by the attractiveness of male ultrasonic songs (Jang & Greenfield 1998). In

strong contrast, much less is known about the potential of post-copulatory mechanisms (but see Cordes et al. 2013). However, observations have indicated that at least some females remate (Greenfield & Coffelt 1983), implying that sperm competition might influence male reproductive success, too. Furthermore, ejaculate size is decreased by ca. 40% in once mated compared with virgin male matings (Cordes et al. 2013). If ejaculate size has an effect on female receptivity in *A. grisella*, we would therefore expect remating to be more likely following matings with non-virgin males. In this case, attractive males which may conceivably encounter sperm limitation would suffer from an increased risk of sperm competition, and this in turn would alter the magnitude of the benefit from attractive advertisement. *A. grisella* is also notable for lacking any manner of adult nutrition in both sexes (mouthparts are atrophied). Thus, this species is exceptional in its complete reliance of resources acquired by the larva. This will make any adult resource allocation trade-offs not only likely but of central importance.

To further explore the potential for post-mating sexual selection in the mating system of the lesser wax moth, our aim here is to provide a detailed estimate of female remating probability and simultaneously test the hypothesis that female remating propensity is influenced by male mating history and thus male mating success.

Materials & Methods

Animal Breeding

Experimental animals were obtained from a laboratory population of *A. grisella*; this population was derived from approx. 100 individuals that were collected from a wild population in bee hives near Bielefeld, Germany, in October 2009. Larvae were given food *ad libitum* (see Cordes et al. 2013 for details) and reared in 30-ml plastic cups under 12:12 h light/dark photoperiod, at 26°C, 40% relative humidity, using a breeding design which minimizes inbreeding. Males and females used in the experiment were isolated into individual cups during second to last instar to ensure virginity. Eclosion was monitored on a daily basis; the age of newly emerged adults was therefore 0–24 h, and we define the age of such individuals to be zero days. Thus, for instance, 1-day-old males and females are between 24 and 48 h old. The experiment was performed in two replicates – one during October/November 2012 and one during February 2013.

Experimental Mating Trials

On the day of emergence, females were either mated to a virgin male or to an already mated (non-virgin) male. Virgin and non-virgin males were of matching age. In replicate 1, non-virgin males had mated with one female on the preceding day, whereas in replicate 2 they had mated with two females on the two preceding days. Hence in replicate 1, the mating with the focal female was the mated males' second lifetime mating, whereas in replicate 2, it was their third one. On their first mating, the mated males were 2 d old. Thus in replicate 1, males were 3 d old when mating with the focal female, whereas in replicate 2, they were 4 d old. All mating trials were performed in the first hours of the scotophase using the same small plastic containers that females emerged in (height: 2.5 cm; \varnothing ca. 3.5 cm). Males were carefully placed in the container, and subsequent courtship interaction was immediately observed intensively, and mating latency and copulation duration were recorded.

Experimental Remating Trials

On the day following females' initial mating, the experimental females' remating propensity was tested. Before the mating trial, we recorded whether females had laid eggs overnight or not. Similar to the experimental design in Greenfield and Coffelt (1983), females were presented with an experimental 'lek' to mimic natural conditions which consisted of three displaying males (age in replicate 1: 2–3 d; in replicate 2: 3–4 d h). Mating trials were this time performed in larger containers (height: 13 cm; \varnothing ca. 11 cm) to ensure that females were unconstrained in their ability to counteract mating. Here, the three males were first placed in the container and the actual trial started when the female was placed inside. We monitored whether females remated or not, and if they did, we recorded mating latency and copulation duration. In replicate 1, trials were observed for 2 h, and females that had not started mating by then were classified as *not remating*. The vast majority of females that remated did so relatively quickly, that is, >50% within the first 2 min. We therefore restricted remating trials in replicate 2 to 1 h. In replicate 1, we also recorded the occurrence of female mate rejection behavior (running away from or fighting the male) to assess female potential to resist male mating attempts.

All remating trials took place during the first 4 h of the scotophase when individuals are reproductively most active (Greenfield & Coffelt 1983; Jang et al.

1997), and they were performed blindly with respect to male and female mating history.

Body Mass Measurements

To analyze whether their own body weight or the weight of their first mate affected females' remating propensity, we measured individuals' body weight to the nearest 0.01 mg using a Kern 770 electronic balance (Kern & Sohn GmbH, Balingen, Germany). However, due to logistic constraints, it was only feasible to measure animals after the experiment. Males and females were immediately frozen after they had been used in their last experimental mating and weighed at a later time point. The potential shortcoming of this procedure is that body weight might change during mating. Virgin male mates may thus appear heavier because they will have performed fewer matings and transferred fewer spermatophores at the time of weighing. Similarly, remated (i.e., twice-mated) females may seem heavier because they will have received two spermatophores. We therefore investigated this effect by using unpublished data that we had collected during a previous experiment (Cordes et al. 2013). Here, 49 virgin pairs were weighed immediately before a mating trial and again immediately thereafter, using the same procedure and equipment as described above. There was a close to perfect correlation of pre- and post-mating weight both in males ($r = 0.961$, $p < 0.001$) and females ($r = 0.996$, $p < 0.001$). The expected weight decrease in males was indistinguishable from measurement error ($t_{48} = 0.006$, $p = 0.99$; mean weight loss: $0.4 \pm 66 \mu\text{g}$), whereas females' weight gain was statistically significant (mean weight gain: $0.109 \pm 0.048 \text{ mg}$, $t_{48} = 2.26$, $p = 0.03$). Nevertheless, this relatively small effect size should be viewed in the light of the large interindividual variation in body weight (estimated standard deviation females: 3.95 mg, males: 1.59 mg). Thus, in our main experiment, the major part of the variation in post-experimental body weight clearly is due to pre-experimental variation between individuals rather than variation caused by differential mating history.

Statistical Analysis

In all analyses, family IDs (both females' and males') were included as random effects to account for possible dependence of data points due to common ancestry. Dependent variables with binary responses (occurrence of female remating and oviposition) were analyzed with generalized linear mixed models

using a binomial error distribution and a logit link function. Copulation duration and mating latency were analyzed using linear mixed-effects models (LME) assuming normal distribution of the data (appropriate after log-transformation of mating latency). Significance testing was based on deviance when removing respective terms from the model. The change in log-likelihood was compared to a chi-square distribution. For reproductive traits where we obtained several measurements from each individual, we used the intraclass correlation coefficient as a measure of the between-individual variance relative to total variance (i.e., repeatability). We employed a LME-based repeatability approach as described by Nakagawa and Schielzeth (2010), fitting a mixed-effects model to estimate variance components using restricted maximum likelihood. Subsequently, we used parametric bootstrapping with 1000 iterations, based on a method described by Faraway (2006), to estimate confidence intervals. All statistical analyses were carried out using R version 2.13.2 (R Development Core Team 2011); all tests were two-tailed, and null hypotheses rejected at $p < 0.05$. Unless otherwise stated, point estimates are given as mean \pm SE.

Results

Remating Rate

In total, across both replicates, 35 of 185 females remated, resulting in an overall remating rate of 0.189. In both replicates, females mated to virgin males had slightly lower remating rates than females mated to non-virgin males (Fig. 1). However, this difference was not statistically significant – neither in the separate replicates (first replicate: virgin males: 11/48, non-virgin males: 16/48; GLMM, log odds ratio: 0.65 ± 0.49 , $\chi_1^2 = 1.52$, $p = 0.22$, second replicate: virgin males: 2/44, non-virgin males: 6/45; log odds ratio: 1.32 ± 1.03 , $\chi_1^2 = 2.30$, $p = 0.13$) nor in an

overall test (log odds ratio: 0.75 ± 0.42 , $\chi_1^2 = 3.3$, $p = 0.07$). While the effect of male mating status was similar across replicates (treatment \times replicate interaction: $\chi_1^2 = 0.55$, $p = 0.46$), female remating rate was considerably higher in replicate 1 than in replicate 2 (effect of replicate: $\chi_1^2 = 11.2$, $p < 0.001$, Fig. 1). In these analyses, we used male and female body weight as covariates. The weight of females' first mate had no influence on their subsequent remating propensity (first replicate: $\chi_1^2 = 0.14$, $p = 0.71$, second replicate: $\chi_1^2 = 0.83$, $p = 0.36$, overall: $\chi_1^2 = 0.85$, $p = 0.36$), yet heavier females were more likely to remate (replicate 1: 0.19 ± 0.068 log odds/mg, $\chi_1^2 = 8.56$, $p = 0.003$; replicate 2: 0.024 ± 0.13 log odds/mg, $\chi_1^2 = 0.04$, $p = 0.82$; overall: 0.136 ± 0.052 log odds/mg, $\chi_1^2 = 6.85$, $p = 0.009$). Although the effect of female weight seems conspicuously larger in the first replicate than in the second, there was no statistically significant difference (female weight \times replicate interaction: $\chi_1^2 = 2.1$, $p = 0.15$).

There was no effect of male mating status on the occurrence of female oviposition in-between matings (replicate 1: $\chi_1^2 = 0.24$, $p = 0.62$; replicate 2: $\chi_1^2 = 0.03$, $p = 0.87$; overall: $\chi_1^2 = 0.22$, $p = 0.64$), nor was there any significant association between female egg laying and remating behavior (replicate 1: $\chi_1^2 = 1.23$, $p = 0.27$; replicate 2: $\chi_1^2 = 0.49$, $p = 0.49$; overall: $\chi_1^2 = 0.24$, $p = 0.62$).

Male Mating Behavior

To establish males of different mating status, males were recurrently mated to virgin females (see methods). This repeated use of the same males allowed us to compare aspects of male mating behavior (copulation duration, mating latency) between mating situations and individual males (replicate 1: $n = 48$; replicate 2: $n = 44$ males). Male mating status had a significant effect on copulation duration (first replicate: $\chi_1^2 = 7.90$, $p = 0.005$, second replicate: $\chi_2^2 = 29.8$, $p < 0.001$; see Fig. 2a). In replicate 1, copulation

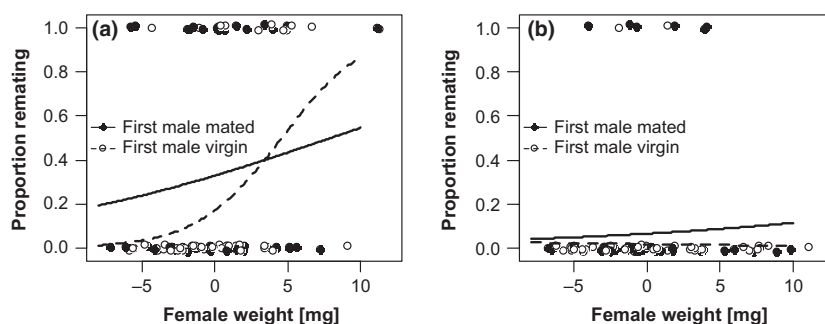


Fig. 1: The occurrence of remating and estimated remating probability of non-virgin females in (a) replicate 1 and (b) replicate 2 of the remating trials. Lines show GLMM predictions (1st male mated: solid lines and marks; 1st male virgin: dashed lines and open marks).

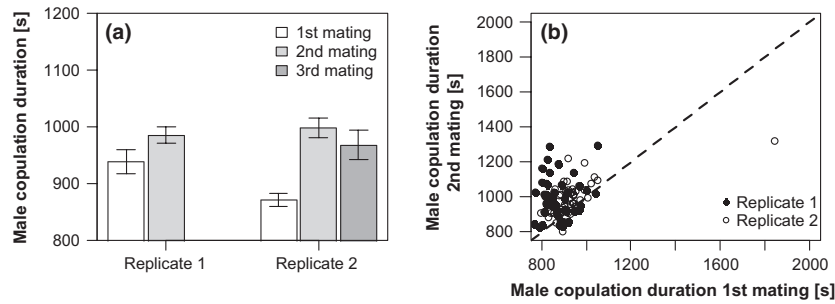


Fig. 2: Copulation duration in consecutive male matings. In (a), the estimated means (\pm SE) are shown illustrating the effects of male mating history over two (replicate 1) and three (replicate 2) consecutive male matings. In (b), the additional effect of male individual is illustrated in the first two matings for both replicates. Each data point represents one male that mated twice (replicate 1: solid marks; replicate 2: open marks). Dashed line depicts expected values assuming no systematic difference between matings.

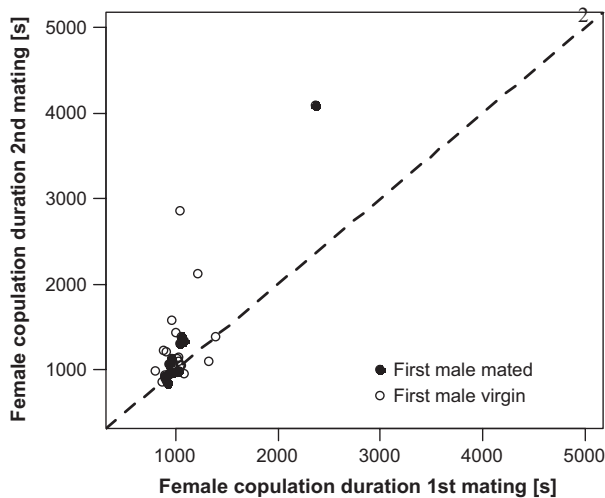


Fig. 3: Copulation duration in consecutive female matings. Each data point represents one female that mated twice (solid marks: females' first mated to mated males; open marks: females' first mated to virgin males). Dashed line depicts expected values assuming no difference between copulations.

duration was longer in males' second mating and in replicate 2 a post hoc comparison revealed that males' first copulation was shorter than the following, but there was no difference between males' second and third copulation (Tukey's test: first vs. second: $z = 5.72$, $p < 0.001$; first vs. third: $z = 4.36$, $p < 0.001$; second vs. third: $z = -1.38$, $p = 0.35$). Furthermore, males also differed consistently and highly significantly in the duration of their copulations (replicate 1: repeatability, controlling for male mating history: 0.582 ± 0.097 , $\chi^2_1 = 19.4$, $p < 0.001$; replicate 2: repeatability: 0.333 ± 0.103 , $\chi^2_1 = 10.5$, $p = 0.0012$; overall (controlling for male mating history and replicate): repeatability: 0.398 ± 0.075 , $\chi^2_1 = 16.5$, $p < 0.001$; see Fig. 2b). In contrast, mating latency did not

differ across matings (mean [95% CI] replicate 1: first: 33.0 s [25.8–45.9 s], second: 36.4 s [24.2–52.4 s], $\chi^2_1 = 0.25$, $p = 0.62$; replicate 2: first: 33.3 s [24.2–45.9 s], second: 40.2 s [28.5–56.7 s], third: 35.6 s [24.1–52.4 s], $\chi^2_2 = 0.62$, $p = 0.73$) or between males (replicate 1: repeatability: 0.305 ± 0.130 , $\chi^2_1 = 3.97$, $p = 0.046$; replicate 2: repeatability: 0 ± 0.048 , $\chi^2_1 = 0$, $p = 1$; overall (controlling for male mating history and replicate): repeatability: 0.093 ± 0.070 , $\chi^2_1 = 0.034$, $p = 0.85$).

Female Mating Behavior

For the $n = 35$ females that remated ($n = 27$ from replicate 1 and $n = 8$ from replicate 2), we were also able to compare differences in copulation duration between mating situations (virgin vs. already mated females) and individual females. Copulations involving already mated females were significantly longer than copulations with virgin females (virgin: 1035 ± 44 s, non-virgin: 1245 ± 107 s, estimated difference: 210 ± 75 s, $\chi^2_1 = 7.18$, $p = 0.007$; Fig. 3). This effect was statistically indistinguishable in the two replicates ($\chi^2_1 = 2.44$, $p = 0.12$) and did also not depend on whether the first male was virgin or not ($\chi^2_1 = 0.011$, $p = 0.92$). Copulation duration also differed strongly between individual females, where some females consistently copulated longer than others (repeatability, controlling for female mating history: 0.570 ± 0.122 , $\chi^2_1 = 13.4$, $p < 0.001$; Fig. 3).

In replicate 1, we recorded the occurrence of female mate rejection behavior during the remating trials. In 42 of 96 cases (44%), females were observed to resist male mating attempts. The likelihood of remating within 2 h was considerably higher in those trials where no mate rejection behavior was observed ($\chi^2_1 = 23.4$, $p < 0.001$, Fig. 4).

Discussion

Our results demonstrate a potential for post-mating sexual selection in the mating system of the lesser wax moth. We observed a female remating probability of $q = 18.9\%$. This would correspond to an overall sperm competition risk of $2q/(1 + q) = 31.9\%$ (cf. Parker et al. 1997). Thus, our data indicate that approximately one of three male mating opportunities may result in sperm competition with other males over fertilization, which suggests that sperm competition success may be a non-trivial source of variation in male reproductive success. Moreover, female mate rejection behavior was mostly successful (see Fig. 4), suggesting that female remating is to a large degree under female control and not a manifestation of male harassment and sexual conflict, in which case exposing females to many males would potentially overestimate female remating propensity. Indeed, our observations support the conclusion in Greenfield and Coffelt (1983) that remating females appear to solicit mating. The majority of remating females almost immediately approached males and more than 50% mated within 2 min and 75% within 10 min.

The hypothesis that the previous mating history of males has an effect on the remating probability of their female mating partners could not be confirmed in our study. On the other hand, the lack of a statistically significant difference does not necessarily mean that there is no such effect. In fact, our data revealed a strong tendency in the expected direction. In our balanced design, 23 of 35 females that did remate had previously mated with a non-virgin male, revealing that within our data set the odds were twice as high that a female previously mated to a non-virgin male would remate compared with the odds for a female mated to a virgin male. Furthermore, the 95% confidence interval of the odds ratio spans the values

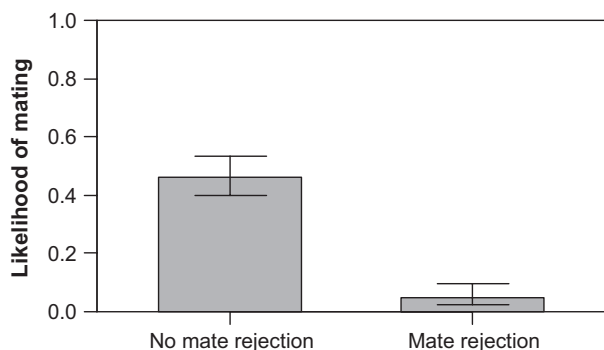


Fig. 4: Likelihood of mating in remating trials dependent on whether female mate rejection behavior was observed or not. Bars represent GLMM estimates and error bars standard errors.

0.93–4.8, indicating that there is possibly only a minor effect of male mating history, but also that the odds for a female previously mated to a non-virgin male to remate could even be four to five times larger than the remating odds for a female previously mated to a virgin male. Thus, even though the estimates are based on almost 200 female remating trials, our data unfortunately remain inconclusive on this matter due to statistical power issues. Males' previous mating success may reduce their ability to induce female receptivity and consequently lead to higher sperm competition risk for such males, resulting in lesser opportunity for sexual selection (see Shuster et al. 2013), but the evidence for this is weak at present.

Nevertheless, we did find evidence that male mating history affects some other reproductive parameters. Copulation duration was significantly longer for non-virgin males. Previous studies have demonstrated that ejaculate size is reduced by ca. 40% in non-virgin matings (Cordes et al. 2013), and possibly it becomes more demanding to package and transfer a spermatophore of adequate size resulting in prolonged copulations (see e.g. Bissoondath & Wiklund 1996; Lauwers & van Dyck 2006). Copulation duration was also repeatable across male matings, and this may correspond to the established intermale variation in ejaculate size (Cordes et al. 2013).

We also were able to explain some variation in female remating behavior: remating propensity increased with female weight. However, as female weight was measured post-experimentally, we need to be careful with the interpretation. From these data alone, it would be difficult to conclude whether heavier females remate more frequently or rather whether females become heavier by mating (e.g., by receiving a spermatophore). However, data from an earlier control experiment (see Materials and methods) showed that female weight gain during mating (ca. 0.11 mg) is at least an order of magnitude smaller than the difference in weight between mated and twice-mated females (1.78 mg). Thus, mating itself cannot account for this effect. Similar size effects on remating propensity have been reported repeatedly for female Lepidoptera (Torres-Vila et al. 1997; Bergström & Wiklund 2002; McNamara et al. 2008). Although the ultimate causes for this phenomenon are unresolved, potential proximate explanations include an allometric relationship between body weight and the size of the reproductive tract. The mechanical pressure on the stretch receptors in the bursa, which have been shown to be involved in the induction of female non-receptivity (Sugawara 1979; Drummond 1984), may be weaker if the female and

her reproductive tract is large (see e.g. Torres-Vila et al. 1997).

We also found large differences in overall female remating probability between the two separate replicates. However, a convincing scenario explaining these differences is difficult to find. Replicates were performed at different times of the year, and this uncontrolled effect of climatic variation outside the laboratory may have affected moths' reproductive behavior; although this explanation seems far-fetched. Remating trials were also shorter in the second replicate (one instead of 2 h), but even if we disregard the rematings that took place during the second hour in replicate 1 (merely three), the difference is still obvious. The only additional difference between replicates was that males used in the remating trials were on average 1 day older in replicate 2. Instead of 2–3 d, they were 3–4 d old. Males age fast and have a typical life expectancy (in the lab) of 10–20 d (Brandt & Greenfield 2004). In this time, they also show reproductive senescence and the quality of important song properties decline with age (Jang et al. 1997; Brandt et al. 2005). This is however not particularly evident before the age of 100 h (Jang et al. 1997), and even then the decline in attractiveness should not be as abrupt as suggested by the difference in remating rate between replicates. Nevertheless, age effects on male reproductive parameters have only been evaluated in relation to the preference functions of virgin females, which are usually eager to mate. Subtle male age effects on song parameters may thus be amplified in mating trials with (hypothetically more choosy) non-virgin females, potentially explaining the differences in remating frequency between replicates.

Our data also revealed that copulation duration was significantly longer in females' second matings compared with females' first matings. This observation can either be a true difference or caused by an experimental bias. As the focus of the experiment was on other issues, the experimental setup differed between the two situations. In virgin matings, females had no option which male to mate with, whereas in the remating trials females could choose between three males. This potential non-random sampling of males could generate the observed pattern, given that the successful males in the remating trials are the ones that copulate the longest. A strong argument against this simple explanation is that the observed difference in copulation duration between female matings (cf. Fig. 3) is roughly three times the standard deviation of the estimated intermale variance in copulation duration (cf. Fig. 2b). An additional effect of the

experimental setup was that males also differed in mating status between females' first and second matings. In females' second matings, all males were virgin, whereas some females' first mates were non-virgin. However, this would have generated a difference in the other direction, as male virgin matings are usually shorter (see Fig. 2a). Thus, the true effect of female mating status on copulation duration must even be larger than estimated here to have overridden this effect.

If we assume that the observed difference is due to true differences between female virgin and non-virgin matings, one straightforward explanation could be that it is more difficult for males to transfer a spermatozoon to an already mated female. This may seem a compelling hypothesis in cases where spermatophores are large and mating plug-like. Although it is difficult at present to completely rule out this possibility, it does seem unlikely. Spermatophores in the lesser wax moth are not at all conspicuously large, in fact they seem relatively small (cf. Drummond 1984; Cordes et al. 2013) and the data on male weight loss and female weight gain during mating support this impression. The most intriguing explanation would be that males adaptively increase copulation duration in females' second matings. As copulation duration correlates positively with sperm transfer (Cordes et al. 2013; T. Schmoll, M. Ruoff, N. Cordes & L. Engqvist, unpubl. data), the obvious reason for this would be that males may strategically allocate sperm depending on female mating status and risk of sperm competition (Parker et al. 1997; Wedell et al. 2002; Engqvist & Reinhold 2006). In the lesser wax moth, matings with already mated females will certainly result in future sperm competition, whereas matings with virgin females will result in sperm competition with a probability of ca. 20%, that is, the probability that females will remate. Thus, investing a larger amount of sperm in non-virgin females could be advantageous, and possibly the longer copulations in non-virgin females reflect an increase in ejaculate size (see also Cordes et al. 2013). Interestingly, a relative increase in sperm investment in matings with non-virgin females is predicted to be especially pronounced in relatively monandrous species such as the lesser wax moth compared with polyandrous species where the effect should be weaker (Parker et al. 1997; Engqvist & Reinhold 2006) or even reversed (Engqvist & Reinhold 2006; see also Kelly & Jennions 2011). This phenomenon has not been particularly acknowledged, but there is indeed evidence from studies on *Drosophila* that males from monandrous species make more distinct allocation decisions based on female mating status than males in polyandrous species.

drous species (Lize et al. 2012). Nonetheless, the socio-sexual environment also differed between the two matings in our experimental design – to mimic natural situations, remating trials were performed in ‘leks’ with other males present. As this may also increase males’ perceived sperm competition risk (see Gage 1991; Olsson 2001), it is possible that the longer copulation duration observed in female rematings reflects *future* rather than *past* sperm competition risk (sensu Parker et al. 1997). It is of course also premature to say whether the longer copulation duration in females’ first matings observed here truly signifies the transfer of a larger ejaculate, but the observation is conspicuous and calls for future experiments testing male strategic sperm allocation in this interesting mating system. Similarly, the realization here that there is a significant chance in remating and polyandry in female wax moths suggests that this may indeed be a fruitful study system for understanding the association between pre- and post-copulatory sexual selection.

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