Sex-Specific Control and Incomplete Matings: Sperm Removal Behaviour in a Bush Cricket Species

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Acknowledgements

We sincerely thank Sonja Schindler for her assistance in field collection of animals and data acquisition in Greece. We are further grateful to Berfin Deniz Güney Calışkan and Srishti Mittal for conducting a pilot field study on the same *M. ornatus* population in 2023; their dataset was integrated into this study to enhance the total sample size. Finally, we express our gratitude to colleagues in the Department of Evolutionary Biology at Bielefeld University for their constructive feedback during discussions of this experiment and its results.

Abstract

In sexually promiscuous species, sperm removal behaviour (SRB) is a male strategy to increase reproductive success by displacing rival sperm prior to insemination. This behaviour may, however, impose costs on both sexes, generating sexual conflict. We investigated the sex-specific control over SRB in *Metaplastes ornatus*, a bush cricket species exhibiting this behaviour. We used a double mating design experiment and recorded morphometric measurements from a wild population in Greece. We found that lighter females likely had more successful matings, while body mass did not affect SRB duration in either sex. Repeatability analyses suggested a potential, albeit weak, female influence on SRB duration. We also identified high rates of unsuccessful matings after the initiation of SRB, where behaviour was initiated but terminated before spermatophore transfer. These events were associated with shorter SRB durations and fewer behavioural breaks. A pilot analysis revealed that males with narrower subgenital plates may have greater mating success. Our results suggest that female traits and behaviour could play a critical role in shaping the outcome of SRB, indicating potential female counter-adaptations in this sexually antagonistic system.

Keywords

Metaplastes ornatus, sperm removal behaviour, promiscuity, precopulatory sexual selection, sexual conflict

1. Introduction

Sexual conflict is caused by different mating strategies between males and females (Parker 1979). Males benefit from multiple matings due to the low cost of sperm (Bateman, 1948), to increase the likelihood of producing high-quality offspring. In contrast, female eggs are limited and need higher investment (Bateman 1948). This results in female choice for the most attractive male (Balmford 1991, Bateman 1948, Darwin 1871) driving sexual selection (Lyon 2012). To counter female mating control, males have evolved different mechanisms (e.g., Borgia 1981) that can harm females. Examples include toxic peptides present in the seminal fluid in the fruit fly *Drosophila melanogaster* (Chapman et al. 1995), forced copulations in different species of waterfowl (Brennan et al. 2007) or an increased infection risk due to traumatic insemination in bedbugs, *Cimex lectularius* (Otti et al. 2017). As a response, females could have developed counter strategies to get back more control on mating behaviour like multiple sperm storage organs in damselflies (Cordero-Rivera 2017, Nakahara, Tsubaki 2007), cryptic female choice (Jennions, Petrie 2000) or maze-like reproductive organs in hyenas (Glickman et al. 1987) and waterfowl (Brennan et al 2007). These adaptations drive an intersexual arms race, with greater divergence in mating strategies intensifying sexual conflict.

Sexual conflict is predicted to be most intense in polyandrous and promiscuous species, compared to species with other mating systems (Chapman et al. 2003). Males in such species have evolved various pre- and postcopulatory behaviours or mechanisms to increase their chances of paternity (e.g., Breedveld 2019, Clutton-Brock, Parker 1995, Hooper et al. 2024, Koene, Schulenburg 2005, Greenfield, Coffelt 1983). One such behaviour called sperm removal involves males removing sperm from previous female matings before transferring their own ejaculate (e.g. Waage 1979). Sperm removal behaviour (SRB) is reported among various taxa like the crayfish *Austropotamobius italicus* (Galeotti et al. 2008), the pholcid spider, *Holocnemus pluchei*, (Calbacho-Rosa et al. 2013), the yellow spotted longicorn beetle, *Psacothea hilaris*, (Yokoi 1990), the earwig *Euborellia plebeja* (Kamimura 2005), the damselflies ebony jewelwing, *Calopteryx maculata*, (Waage 1979), *Calopteryx cornelia* (Tsuchiya, Hayashi 2014) and the ancient greenling, *Hemiphlebia mirabilis*, which shows that SRB is already existent since the Permian period (Cordero-Rivera 2016). Considering all known species that perform SRB, three types have been described. First, in external fertilizing species, males remove sperm of competitive males outside the females' body, for example in the brown frillfin, *Bathygobius fuscus*, through tail-fanning above the eggs (Takegaki et al. 2020). Second, in internal fertilizing species, males actively remove sperm stored within females, like males of the cuttlefish *Sepia esculenta* which scrape out sperm with their third arm (Wada et al. 2005). Thirdly, males can trigger females to release sperm (example in *Metaplastes ornatus* (von Helversen, von Helversen 1991)).

Sperm removal behaviour (SRB) is an intersexual shared mating trait, that drives sexual conflict (Cordero-Rivera 2017). In *Metaplastes ornatus*, males benefit by reducing sperm competition through SRB (Winkler et al., 2019), while females incur costs such as the loss of previously stored sperm and physical damage to genitalia (von Helversen & von Helversen, 1991; Matsumura et al., 2020). However, participating in SRB is also the only way for females to remate and gain new sperm, including a nutritious nuptial gift. Acquiring the spermatophore could also be quite beneficial for females because it could increase egg production, as shown in, for example, the bush cricket *Kawanaphila nartee* (Pärssinen et al. 2024). This results in sexual antagonism in the trait where the fitness optima diverge for males and females. Different traits—such as body size or mass—are critical determinants of quality and fitness in animals (Herdman et al. 2004, Honěk 1993, Shuster, Wade 2003), influencing SRB outcomes: heavier males may exert greater control over sperm displacement, while females with greater body mass could be better at resisting manipulation or allocate resources to repair damage. Despite advances in understanding SRB's role in mating dynamics, critical gaps remain in elucidating the extent of sex-specific control over the duration of SRB, and the role of body condition in mediating trade-offs between SRB costs and benefits.

To investigate which sex dominates the duration of SRB, we carried out a double mating experiment using wild caught *M. ornatus*. We hypothesized that female weight would have an effect on the duration of SRB with heavier females participating in longer durations of SRB and having higher copulation success as males would perceive them to be of higher quality and invest longer in removing sperm. Therefore, female weight can be a driver of the variation in this trait. Additionally, we also tested for the effect of body weight on the mating latency, our prediction being that lighter females will mount males faster to gain the nutritious nuptial gift. To further quantify sex-specific control over the traits, we conducted a double mating experiment to collect repeated measures of SRB duration from both sexes and quantify inter-individual differences. We hypothesized that males would be more consistent in this behavioural trait, since we assume that they are having a higher influence over the duration of SRB. Additionally, we performed a pilot-study on a possible influence of the subgenital plate morphology on mating success and SRB duration. We hypothesise that the duration of SRB is longer and the copulation success higher when the spurs on that plate are longer as the females cannot separate from the males easily. We further predict that males with a width:length ratio of the subgenital plate bigger than 1 have a longer SRB duration, as they should take longer to trigger sufficient sperm release, but this ratio should not influence mating success.

2. Materials and methods

2.1 Study system

Metaplastes ornatus

In the promiscuous bush cricket *M. ornatus* Ramme 1931 (Orthoptera, Tettigonoidea, Phaneropteridae), after a male and a female pair for mating, the male starts SRB by inserting his subgenital plate into the females' genital chamber. The male thrusts it back and forth repeatedly to trigger sperm release from the spermatheca of female, using microscopic spines on his plate. During this process, the pair separates for a short period of time, so that the female can clean her genital area with her mouthparts and the male his subgenital plate before resuming SRB (von Helversen, von Helversen 1991). Finally, the male attaches a protein-rich spermatophore to the female's genital opening which consists of an ampulla, containing sperm, and a spermatophylax, the nuptial gift (Heller et al. 1998). The female starts feeding on the spermatophylax (von Helversen, von Helversen 1991) because of its nutritional quality (Voigt et al. 2008, Lehmann, Lehmann 2016). In this species, the occurrence of SRB is independent of female mating history (including virgins) (Foraita et al. 2017). During SRB, females can get damages in their genital area due to the spines and hook-like spurs of the subgenital plate (Matsumura et al. 2020). These hooks are important to hold the subgenital plate inside the genital chamber. As an adaptation, females have resilin, a semi-fluidlike substance, in their genital area to reduce damage (Matsumura et al. 2020).

M. ornatus occurs in the southern Balkan Peninsula (Pavićević et al. 2014). We collected individuals in two consecutive years in Paleokastro, central Greece. In 2023, we caught a total of 20 male and 20 female subadults between 20th May and 06th June at one field site (38°56'6.0"N 22°2'2.4"E). It was not possible to collect adults due to a cold spring in that region which is why we waited to conduct the mating trials until the animals were adults. In 2024, we captured 147 female and 146 male adults between 16th June and 5th July at three field sites in a total of nine batches. In the experiment, we used 112 males and 113 females. Each field site was approximately ± 300 m along a road (centres of the tracks: 38°59'14.8"N 21°53'59.4"E for the first, 38°58'55.1"N 21°53'40.7"E for the second and 38°59'16.9"N 21°53'19.9"E for the third collection site (**Supplements 1**)).

Animal keeping

In both years, we first weighed the animals (Kompakte Analysenwaagen HR-100A, measurement error ± 0.0001 g) and kept them individually in cylindrical cups (8 x 13 cm). Each cup contained a test tube with leaves from the pedunculate oak (*Quercus pannonica*) which was sealed with moist cotton wool. The cups of the females contained smaller cups with moistened sand for laying eggs. We numbered the cups with the animals and stored them in large trays separated by sex. Daily, we checked food and water supply as well as their health. Unhealthy looking or parasitized animals were excluded. After the animals had acclimatized for two days, we used them for the mating trials. After the trials, we let the animals free again at the locations where we collected them from. Since all collected animals in 2024 were adults, we assumed that most of the animals were non-virgins. This assumption was supported by the fact that most of the females laid eggs before we tested them in the trials.

2.2 Experimental overview

To investigate SRB, we prepared a cage (14 x 7 x 9 cm) for every trial with an oak leaf, a moistened cotton ball and a twig (**Supplements 2**). First, we put the females in the mating arena and waited 5 minutes before adding the corresponding male (SRB 1). As soon as the male began to sing, we started a stopwatch and noted the start time of the sperm removal behaviour. This is the moment when the female mounts the male and the male inserts the subgenital plate in the females' genital chamber (measurement error ± 5 sec) (exemplary picture **Supplements 3**). The time between when the male started singing and the beginning of SRB was defined as the mating latency. We also noted the number and duration of breaks which occurred when the pair separated in between to clean their genitalia as well as whether the mating was successful in terms of spermatophore transfer (exemplary picture **Supplements 4**). If that was the case, two days later the animals could be used again in another trial with another mating partner (SRB 2) (time window based on von Helversen, von Helversen 1991 and personal observation by K. Reinhold). In case a male did not start to sing within an hour, we exchanged it with a different male. This also applies to pairs which did not start to show sperm removal behaviour after two hours. In those cases, we exchanged either the male or the female with a new one and started a new trial.

For a pilot study, we measured the median keel length and the width of the subgenital plates with an electronic digital calliper (*Imatec*, Type I, CR2032, measurement error ± 0.05 mm) two times and calculated the mean. We used the subgenital plate ratio as an estimate for the morphology of the subgenital plates:

$$Subgenital\ Plate\ Ratio = \frac{Median\ Keel\ Length}{Width\ between\ lobes} \tag{1}$$

To get an estimate for the size of the spurs on each subgenital plate, we took pictures with a camera (camera model: *Panasonic* DMC-G70, lens model: Panasonic Lumix G Macro 1:2.8/30) and analysed them in *ImageJ* (Schneider et al. 2012). Firstly, we measured a picture of the scale ten times and secondly, we measured the tip of the forceps ten times (measurement error ± 0.005 mm). The forceps were the reference point on each picture to, thirdly, measure a straight line from the origin of the spur on the median keel ten times each. We used the mean for the following analysis (**Figure 1**). During this, we took measurements from the median keel length and width, too, to compare these results with our results from the measurements with the calliper.

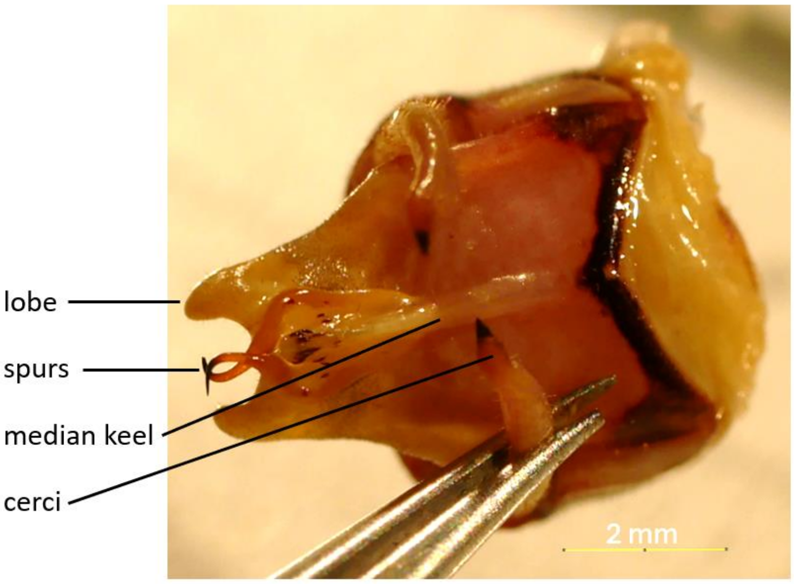

Figure 1: Dorsal side of a male subgenital plate (sample). Visible are the lobes, the cerci, the median keel and the spurs which are crossed. The tip of one spur is broken.

2.3 Data analysis

In total, we carried out around 27 trials in 2023 and exactly 203 trials in 2024, out of which we excluded 4 pairs because the males never started to sing. The first mating (SRB 1) was successfully completed in the form of females receiving a spermatophore by 13 pairs (2023) and 41 pairs (2024). 4 pairs (2023) and 10 pairs (2024) completed SRB 2. In our analysis, we included all successfully completed trials, independent of whether they have been SRB 1 or SRB 2. Then, we analysed the influence of weight on copulation success and SRB duration (the duration includes only the time during which SRB was exhibited and excludes all break times). This also applies to the analysis of mating latency and incomplete matings after the initiation of SRB, for which we only used data from 2024 because this data was only collected in that year. Incomplete matings after the initiation of SRB are defined by a pair exhibiting SRB initially, but the process is indefinitely interrupted and does not start again plus the female does not receive a spermatophore. In contrast, in completed matings, the sperm removal terminates and male transfers a spermatophore to the female. For the pilot-study on possible effects of the subgenital plate on mating behaviour, we collected samples from 19 males in 2024.

For the analysis, we applied Generalized Linear Mixed Models (GLMMs) to our data to examine effects of female and male weight on successful and unsuccessful matings. Successful matings are defined as pairs in which males transferred a spermatophore to the female and the pair separates. In case the spermatophore fell, it still counted as successful. In unsuccessful matings, females did not receive any spermatophore. Next, we looked for the influence of mating latency on mating success and SRB duration. In these models, we used ID as random effect. We used a bootstrap repeatabilities test (n = 1000) for female and male ID to determine which sex predominantly influences the duration of SRB.

As a pilot study, we examined the influence of morphological characteristics of the subgenital plate of males (formula 1) on the SRB duration and mating success, for the latter using Cohen's d. We also conducted a post-hoc analysis on complete and incomplete matings after the initiation of SRB.

We documented the raw data in a *Microsoft Excel* (Microsoft Corporation 2018) file and analysed the data in *R* (v4.2.2, in *RStudio* v2024.09.0+375 (R Core Team 2017)). During this analysis, we used the packages \`readr´ v.2.1.5 (Wickham et al. 2024) to import the raw data and manipulated it with \`dplyr´ v.1.1.4 (Wickham et al. 2023), \`tidyr´ v.1.3.0 (Wickham et al. 2023) and \`Matrix´ v.1.6-5 (Bates et al. 2024). For the statistical analysis, we needed the packages \`lme4´ v.1.1-35.3 (Bates et al. 2015) and \`lmerTest´ v.3.1-3 (Kuznetsova et al. 2012). To carry out the bootstrap test, we used \`rptR´ v.0.9.22 (Stoffel et al. 2017) and for calculating Cohen´s d, we used the package \`effectsize´ v.1.0.0 (Ben-Shachar et al. 2020). Finally, for the plots, we used \`ggplot2´ v.3.5.1 (Wickham 2016) and \`ggpubr´ v.0.6.0 (Kassambara 2023).

3. Results

3.1 Influence of body mass on successful mating and SRB Duration

To explain differences in mating success and SRB duration, we used GLMMs to assess the effect of weight (g) depending on the sex. We found a trend for a negative correlation between female body weight and the completion of the mating, suggesting that female weight influences successful mating ($p < 0.1$, effect size = -4.35, Std. error = ± 2.44, odds ratio (OR) = 0.01) (Table in **Supplements 5**) which means that lighter females likely complete matings with a higher rate. There is no significant effect of female weight on the duration of SRB ($p > 0.5$, effect size = -4.45, Std. error = ± 7.59, OR = 0.01) (Table in **Supplements 6**). Male weight did not show an effect either on copulation success ($p > 0.1$, effect size = 2.80, Std. error = ± 2.01, OR = 16.41) nor on the duration of SRB (min) ($p > 0.1$, effect size = -4.97, Std. error = ± 6.61, OR < 0.01).

3.2 Influence of mating latency on copulation success and SRB duration

Mating latency is a key trait that can influence mating success. However, our results show that mating latency did not influence whether a mating is successful ($p > 0.1$, effect size = -0.01, Std. error = 0.01, OR = 0.99) (Table in **Supplements 7**) or not. Similarly, no correlation was observed between mating latency and sperm removing duration ($p > 0.05$, effect size = -0.02, Std. error = 0.01, OR = 0.98) (Table in **Supplements 8**). This suggests that the motivation of a pair to copulate in terms of how quickly they start exhibiting SRB after pairing does not influence the outcome and the duration of it.

3.3 Sex-specific repeatability of sperm removal duration

We investigated individual variation within each sex to determine which sex shows greater consistency in sperm removal duration between the first and second mating trials. We expected males to exhibit more consistent durations than females. To test this, we calculated bootstrap repeatabilities for female and male IDs. Female ID had a stronger and more consistent effect on removing duration ($R = 0.409 \pm 0.22$, CIs = 0 to 0.77) in contrast to male ID ($R = 0.134 \pm 0.22$, CIs = 0 to 0.69) which suggests that females could have a greater control over sperm removal duration than males. But, likely due to a small sample size with repeated measures from only 12 females and 9 males, large and overlapping confidence intervals between the sexes resulted, indicated a high level of uncertainty in the estimates. Thus, it was not possible to predict the sex-specific domination of sperm removal duration based on our data and this model.

3.4 Post-hoc analysis on incomplete matings after the initiation of SRB

During the trials, we observed that 41 out of 83 pairs which started to exhibit SRB did not finish copulations by transferring a spermatophore. Therefore, we performed a post-hoc analysis on incomplete matings after the initiation of SRB and its duration, the number of breaks during sperm removal and the mating latency of these pairs. For unsuccessful matings, both the removing duration ($p < 0.001$, effect size = 5.72, Std. error = 0.65, OR = 305.82) (**Figure 2a**, Table in **Supplements 9**) and the number of breaks ($p < 0.001$, effect size = 3.17, Std. error = 0.74, OR = 23.84) (**Figure 2b**, Table in **Supplements 10**) were significantly shorter and fewer than in cases in which the male finally transferred a spermatophore to the female. Furthermore, there was the trend that pairs mated more often successfully when they showed a shorter mating latency ($p < 0.1$, effect size = -20.82, Std. error = 11.80, OR = < 0.01) (**Figure 2c**, Table in **Supplements 11**). This suggests that mating either occurs or is interrupted after only a few executions of SRB, resulting in an incomplete mating attempt, and that the probability of unsuccessful matings increases with a longer mating latency.

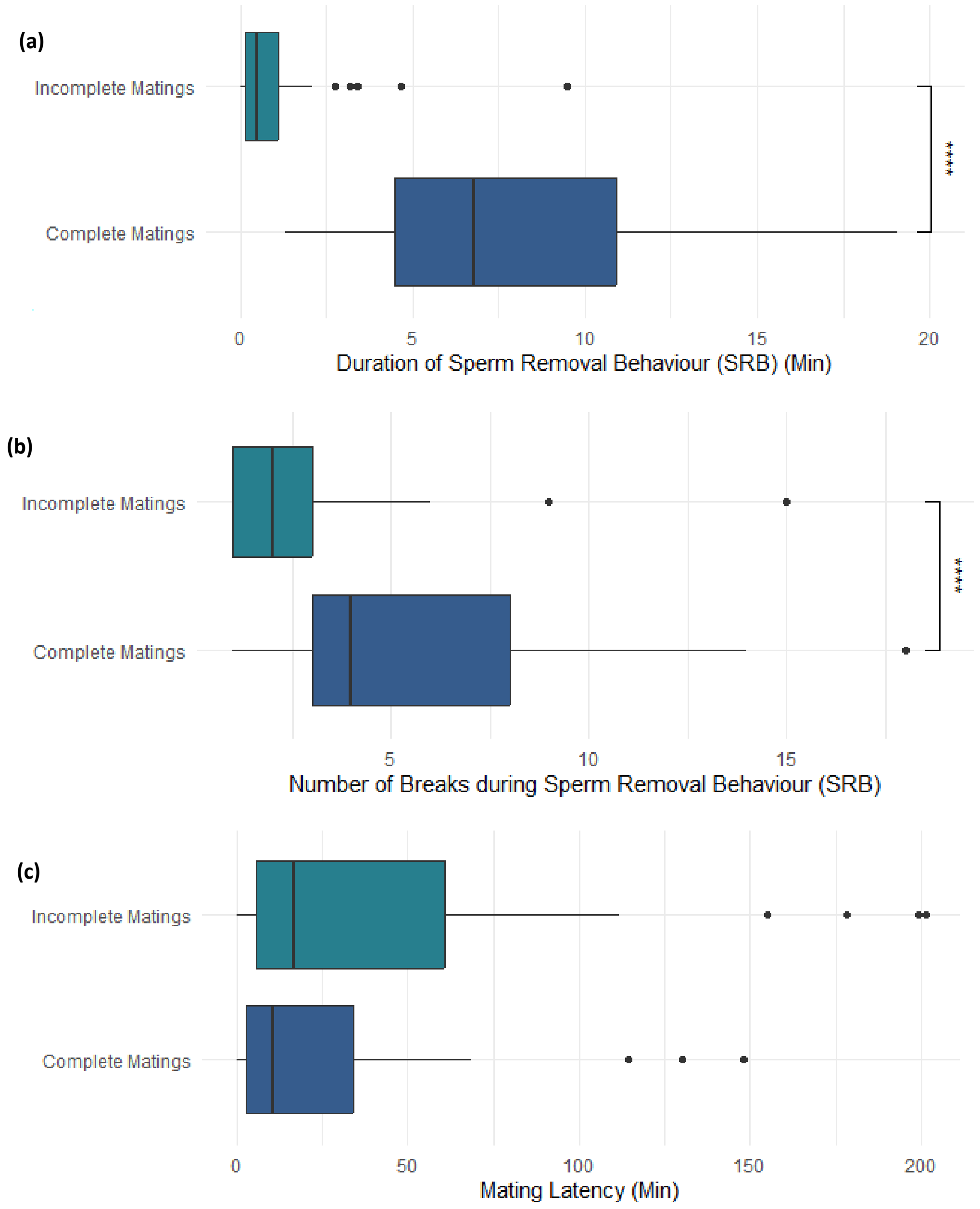

Figure 2: (a) Duration of sperm removal behaviour (SRB) (min) depending on whether matings included spermatophore transfer (completed matings) or not. The total removing duration was significantly longer in

complete mating attempts than in incomplete ones. The mean value for the SRB duration for incomplete mating attempts was 1.33 min (95 % CI: 0.55 to 1.70 min) and for complete ones 7.77 min (95 % CI: 6.38 to 9.15 min). **(b)** Differences in the number of breaks during SRB depending on complete and incomplete attempts. The number of breaks was significantly higher in cases of complete matings than in incomplete ones. The mean value for the number of breaks during SRB for incomplete matings after the initiation of SRB was 2.59 (95 % CI: 1.77 to 3.40) and for complete ones 5.84 (95 % CI: 4.64 to 7.04). **(c)** Differences in the mating latency (min) between complete and incomplete matings after the initiation of SRB. Pairs showing complete matings after the initiation of SRB were not more likely to have a shorter mating latency than pairs showing incomplete matings. The mean value for the mating latency for incomplete mating attempts was 51.5 min (95 % CI: 28.2 to 74.7 min) and for complete ones 24.7 min (95 % CI: 13.4 to 36.0 min).

3.5 Pilot study on mating behaviour and subgenital plate morphology

We hypothesised that the duration of SRB is longer and the mating success higher when the spurs on the male's subgenital plate are longer as the females likely cannot separate from the males during SRB that easily. We observed no tendency for narrower subgenital plates to be associated with a longer SRB duration (p = 0.14, effect size = 21.43, Std. error = ± 6.49, OR = $2.03*10^9$) (**Supplements 12a**). Next, we hypothesised that males with a width:length ratio of the subgenital plate bigger than 1 have a longer SRB duration, but that ratio should affect mating success. The mean of the subgenital plate ratio (Formula 1) was 0.95 (95 % CI: 0.88 – 1.00) for successful copulations and 0.92 (95 % CI: 0.89 – 0.97) for unsuccessful copulations. A Cohen's d of 0.81 (95 % CI: -0.59 to 2.16) hints that subgenital plate ratio had a large influence on mating success, albeit it is not significant due to the confidence interval. This indicates that individuals with narrower subgenital plate are more likely to transfer a spermatophore (**Supplements 12b**). Initial results indicated that a smaller mean spur length is not associated with shorter SRB duration (p = 0.53, effect size = 3.03, Std. error = ± 3.50, OR = 20.67) (**Supplements 12c**). Due to a Cohen's d of -0.48 (95 % CI: -1.49 to 0.54), there seemed to be a small effect of spur length on the probability of successful copulation, as the completed matings of males with shorter spurs had a mean of 0.95 (95 % CI: 0.84 – 1.07) and with longer spurs with a mean of 1.02 (95 % CI: 0.94 – 1.10). So, individuals with shorter spurs could be more likely to participate in a successful mating (**Supplements 12d**). However, both confidence intervals of the Cohen´s ds included 0 which could suggest that maybe there was no effect of subgenital plate ratio or of spur length on copulation success at all. We also identified broken spurs in 5 of 19 samples (26.3%), with no subgenital plate exhibiting bilateral spur damage. Additionally, 8 samples displayed crossed spur orientation (exemplified in **Figure 1** for broken spurs and **Supplements 13** for intact spurs).

3.6 Remarks

During the experiment, a notable issue regarding the health of the bush crickets was that 47 individuals (16.2 % of the total) were visibly infected with parasites, either because these were ectoparasites or because they left the host during the course of the experiment. The latter parasites were identified as horsehair worms ($n_{\text{visibly infected bush crickets}}$ = 26) and the former as mites ($n_{\text{visibly infected bush crickets}}$ = 23). The mortality rate was 97 out of 292 individuals. This included all animals with visible worm infection that perished on the same day as the worms were discovered outside their bodies. To estimate the number of animals infected with worms that had not yet been released out of the body, 32 animals from the third location were dissected. The dissection revealed that 12.5 % of these animals had worms in their bodies. This finding suggests the presence of a slightly greater number of unobserved cases within the population which in turn could have contributed to the overall low mating rate.

4. Discussion

We conducted an experimental study using a wild population to test sex-specific effects on sperm removal behaviour in the bush cricket, *Metaplastes ornatus*. We found an increasing rate of non-completed matings, i.e. matings that contained sperm removal behaviour but no spermatophore transfer, with increasing female body mass. Additionally, our results also indicate the possibility of a female effect on the duration of SRB and greater inter individual consistency for this trait among females. Our study is one of the first to presents data on the frequency of incomplete matings that are interrupted before spermatophore transfer. These incidences can play an important role in the fitness of the involved individuals and may impose additional selection pressures on males and females.

We found that lighter females tend to have more matings that included spermatophore transfer than heavier ones, while male weight showed no effect on mating completion. Successful copulation was lower for heavier females as they could be choosier and hence reject males more often than lighter females. On the other hand, lighter females may be more receptive or cooperative during mating, facilitating both higher copulation rates. Body mass of either sex however did not have an effect on the duration of SRB. This result challenges the assumption that physical condition or size-based advantages would influence the intensity of SRB. In other species, larger males are often able to exert more control during copulation (Dong et al. 2023), either through physical dominance or prolonged mating durations, while heavier females may resist or endure male manipulations more effectively due to superior body condition (Oviedo-Diego et al. 2025). However, our findings suggest that, in *M. ornatus*, the mechanics governing SRB duration may be more tightly linked to behavioural or morphological traits rather than overall mass.

While testing which sex exhibits more consistent individual differences in sperm removal behaviour (SRB), female identity had a marginally stronger and more consistent effect than male identity. However, the small sample size resulting from low mating rates (25.1%) and even lower remating frequencies led to overlapping confidence intervals in bootstrap analyses, limiting statistical power. The reduced mating rate may be attributed to poor population health, as 16.2% of individuals exhibited visible parasitic infections-a factor known to suppress reproductive activity under the Hamilton-Zuk hypothesis (Hamilton & Zuk, 1982). Therefore, our results are weak evidence of female control over the duration of SRB and can be further investigated in the future with a larger sample size for more reliable results. Nevertheless, female agency in mating systems is well-documented, including genital coevolution to counter male manipulation (e.g., beetles: Genevcius et al., 2020), cryptic sperm selection to avoid inbreeding (e.g., *Teleogryllus oceanicus*: Tuni et al., 2013), and behavioural resistance to minimize mating costs (Birkhead, 1998). These mechanisms align with our observations, suggesting that female *M. ornatus* may similarly modulate SRB outcomes through physiological or behavioural adaptations, albeit masked here by population-wide stressors.

To the best of our knowledge our study presents one of the first descriptions of incomplete matings after the initiation of SRB in *M. ornatus* and this can have significant implications for sexual conflict in this species. Males experience time and energy loss by attempting to mate with a female but not transferring any sperm in the end and likely also an increased risk for predation during unsuccessful matings. Such interrupted matings likely intensify sperm competition by allowing residual rival sperm to persist in the female reproductive tract, potentially driving the evolution of male adaptations such as more efficient sperm removal mechanisms. For females, these incomplete sperm removal attempts may represent an opportunity for cryptic choice, allowing them to bias fertilization towards preferred males. However, they also face trade-offs between retaining sperm from previous mates and the physical costs associated with repeated mating attempts. This high rate of mating interruption suggests an ongoing evolutionary arms race between the sexes, where males strive for complete sperm removal and spermatophore transfer, while females may be evolving mechanisms to terminate costly or undesirable mating prematurely. Yet, incomplete matings after the initiation of SRB in *M. ornatus* is only mentioned briefly in another study by Matsumura et al. (2020). In a post-hoc analysis, we found that SRB gets interrupted already after a short duration and its probability rises with increasing mating latency. Moreover, the higher mating latency could reflect a lower motivation of females to mate with the male they encountered.

Our findings support that the morphology, especially the width of subgenital plates could play a role for mating behaviour (suggested by Winkler et al. 2019). Unlike Lehmann et al. in their study on titillator length in the middle European bush cricket, *Roeseliana roeselii* (2021), we observed that females could be more willing to participate in SRB when spurs are shorter. The difference could be explained by the fact that, in contrast to that study, we did not experimentally change the titillators or, in our case, the spurs, but used natural variance. Such a manipulation could have an influence on female choice as females showed more resistance when mating with males with experimentally shortened titillators (Lehmann et al. 2021). In addition, we detected that five tips of spurs have been broken which is similar to observations by Matsumura et al. (2020). Hence, a further study on the effect of naturally broken or experimentally manipulated spur(s) on male mating success is needed. If SRB duration could be longer when spurs are longer too, it could explain why male *M. ornatus* with longer spurs had longer SRB durations. Also, titillators are important to reduce female resistance (Wulff, Lehmann, 2016) which could hint that longer spurs are better for controlling female resistance and thus allowing longer SRB duration. Apart from this, it is unknown whether males can actively move their spurs, whether spur-crossing has a biologically relevant reason and whether the amount of haemolymph a male can pump in his subgenital plate effects mating behaviour. Overall, the subgenital plate could be crucial in explaining inter-individual differences in the duration and success of mating behaviour in *M. ornatus*.

Overall, our study contributes to a deeper understanding of sexual selection and the complex coevolutionary dynamics underlying male-female counter-adaptations. The apparent female influence over sperm removal behaviour (SRB) may reflect a division of control, with each sex exerting dominance during different phases of the mating process. Alternatively, it is plausible that females exercise cryptic choice based on male quality or health that becomes apparent only after mating initiation, thereby strongly shaping SRB outcomes. This ongoing sexual conflict likely drives females to evolve nuanced resistance strategies at multiple stages of mating, counterbalancing male control exerted through the act of sperm removal. These findings highlight the intricate interplay of conflict shaping reproductive strategies and underscore the need for further research into the mechanisms of female choice and control during shared copulatory sexual interactions.

Ethics approval

All animals were handled in accordance with the ASAB guidelines for the treatment of animals in behavioural research and teaching.

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Supplements

Supplements 1: Collection sites of our experiment to study which sex dominates SRB in M. ornatus. We collected the individuals 2024 around the village Paleokastro, Central Greece (@ Google Maps).

Supplements 2: This was one of the experimental arenas we used to study which sex dominates SRB. Each contained oak leaves as food, a wet cotton ball for drinking and a climbing branch. A female M. ornatus is visible in the foreground on the cotton ball, while a male is climbing on the branch in the top left corner (@ C. Flaskamp).

Supplements 3: Bottom view of SRB in M. ornatus. Previously, the female mounted the male, and, on that picture, the male inserted his subgenital plate into the genital chamber of the female to thrust it back and forth, while he held her with his cerci. The female flexed the posterior part of her body towards the male to allow the male to exhibit SRB (@ C. Flaskamp).

Supplements 4: Top view on a female (left) and male (right) directly after the transfer of a spermatophore, following an observed SRB in one of our trials. The female started to tilt her abdomen in the direction of her mouthparts to start feeding on the obtained spermatophore (@ C. Flaskamp).

Supplements 5: GLMM of mating success depending on female and male weight. Individual IDs were random factors. The effect of female weight on successful copulation hints towards that lighter females could have been more likely to mate successfully. Male weight did not show an effect.

	Estimate	Std. Error	Z value	Pr (>|z|)
(Intercept)	0.102	1.80	0.057	0.955
Female weight	-4.348	2.44	-1.780	0.075
Male weight	2.798	2.00	1.395	0.163

Supplements 6: GLMM to analyse a possible effect of female and male weight on the SRB duration. Both were not significant and did not show any effect on SRB duration at all.

	Estimate	Std. Error	df	t value	Pr (>|t|)

(Intercept)	11.875	6.01	36.636	1.977	0.056
Female Weight	-4.445	7.59	26.106	-0.586	0.563
Male Weight	-4.972	6.61	28.621	-0.752	0.458

Supplements 7: GLMM of the analysis of the effect from mating latency on mating success, which was not significant.

	Estimate	Std. Error	Z value	Pr (>|z|)
(Intercept)	0.148	0.31	0.467	0.640
Mating Latency	-0.009	0.01	-1.569	0.117

Supplements 8: GLMM of the analysis of the effect from mating latency on the duration of SRB, which was not significant.

	Estimate	Std. Error	df	t value	Pr (>|t|)
(Intercept)	4.943	0.68	68.274	7.234	< 0.001 *
Mating Latency	-0.019	0.01	59.629	-1.934	0.058

Supplements 9: GLMM of the effect of SRB duration on mating success, which was highly significant, indicating that the total accumulated SRB duration was much shorter in unsuccessful pairings than in successfully copulating ones.

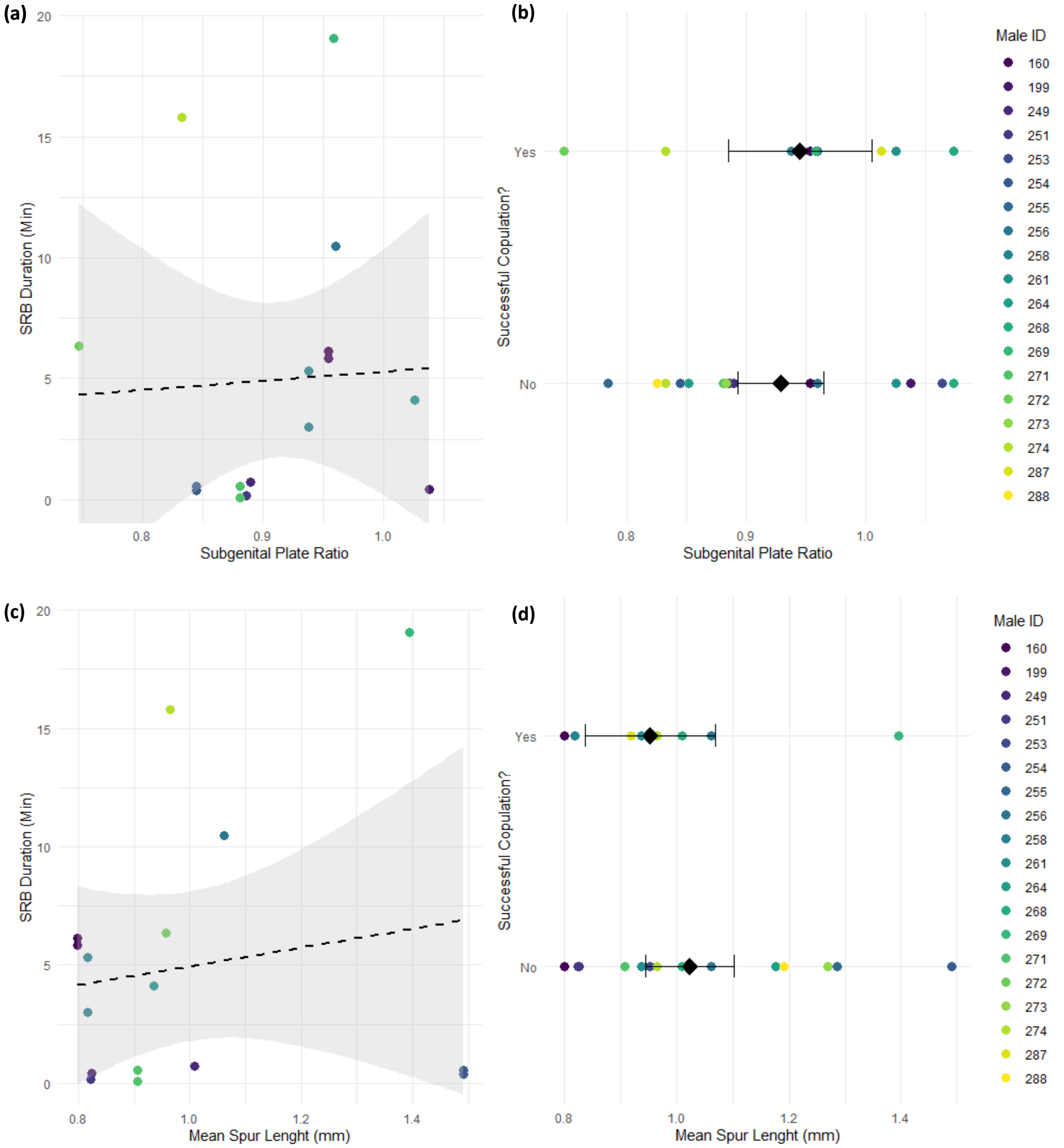
	Estimate	Std. Error	df	t value	Pr (>|t|)
(Intercept)	1.619	0.53	74.744	3.065	0.003 *
Successful Copulation	5.722	0.65	24.537	8.743	< 0.001 *

Supplements 10: GLMM of the number of breaks during SRB on mating success, which was highly significant, indicating that the number of breaks was much lower in unsuccessful pairings than in successfully copulating ones.

	Estimate	Std. Error	df	t value	Pr (>|t|)
(Intercept)	2.617	0.51	72.067	5.106	< 0.001 *
Successful Copulation	3.170	0.74	75.324	4.306	< 0.001 *

Supplements 11: GLMM of the mating latency between the male starting to sing and the first execution of SRB on mating success, which was significant, indicating that the mating latency was longer in unsuccessful pairings than in successfully copulating ones.

	Estimate	Std. Error	df	t value	Pr (>|t|)
(Intercept)	45.967	8.40	73.000	5.475	< 0.001 *
Successful Copulation	-20.821	11.80	73.000	-1.765	0.082



Supplements 12: (a) SRB duration (min) and (b) copulation success depending on the median keel ratio which was calculated from the median keel mean length and mean width. In (a), attached are a trend line and its 95 % confidence interval. In (b), marked with black diamonds are the means and the CIs are given. The IDs are coded by colour. (*c*) SRB duration (min) and (*d*) copulation success depending on the mean spur length (mm). In (*c*), attached are a trend line and its 95 % confidence interval. In (*d*), marked with black diamonds are the means and the CIs are given. The IDs are colour coded.

Supplements 13: Dorsal side of a males' subgenital plate. Visible are a top view on the tip of the media keel, the lobes and the uncrossed spurs without broken tips (@ C. Flaskamp).

Reference in Supplements:

Google (n. d.) Map of Paleokastro, Central Greece (2024), maps.google.com