From mating to sperm storage: density-dependent plasticity in pre- and post-copulatory shared mating traits

Tuba Rizvi*[1], Cristina Tuni[2], Klaus Reinhold[1]

1 Evolutionary Biology, Bielefeld University, Bielefeld, Germany

2 Department of Life Science and Systems Biology, University of Turin, Italy

*Correspondence: tuba.rizvi@uni-bielefeld.de

ABSTRACT

Mating interactions depend on traits expressed jointly by males and females, yet the extent to which each sex controls variation in these shared mating traits remains unclear. Because the expression of such traits (like mating latency, copulation duration, and sperm transfer) depends on both partners, their evolution is constrained by intersexual correlations yet facilitated by behavioural plasticity that allows each sex to adjust trait expression across environments. In this study we investigated whether shared mating traits are determined by male or female control or if the observed outcomes result from interactions of the developmental environment of both partners. Drawing from the well-known mating system of the banded cricket, *Gryllodes sigillatus*, we used a fully factorial mating design using combinations of male and female partners reared at high or low density and tested how they shape shared pre- and post-copulatory traits. We found that female developmental density affected mating latency, with low-density females exhibiting longer latencies, suggesting female control and mate choice. In contrast, male developmental density affected sperm transfer and subsequent sperm storage, with males from high-density treatments transferring significantly more sperm consistent with adaptive ejaculate adjustment to sperm competition risk, as well as contributing to higher sperm storage in females. Copulation duration varied with female body mass but not density, indicating plastic responses to partner quality rather than social context. By partitioning environmental effects between the sexes, our study highlights how developmental context can be used to examine sex-specific contributions to shared mating traits.

Keywords: behavioural plasticity, male-female interactions, sexual conflict, social environment, phenotypic plasticity

INTRODUCTION

Mating interactions are a fundamental driver of reproductive success and a key arena for sexual selection and sexual conflict (Andersson 1994; Chapman et al. 2003; Mazzi et al. 2009). These interactions often involve traits inherently expressed by both sexes, such as mating latency, copulation duration, sperm transfer because their expression depends on the interactive behaviour of both males and females during a single mating event (hereon referred to as 'shared mating traits'). These shared mating traits are central to reproductive success because they determine the timing and outcome of fertilization, yet they are rarely fixed. Instead, they frequently exhibit substantial behavioural plasticity which allows individuals to adjust their reproductive strategies in response to fluctuating ecological and social conditions (Lizé et al. 2012; Nwajei et al. 2024; Simmons and Lovegrove 2024). Such plasticity enables males and females to balance the costs and benefits of mating across contexts (Dore et al. 2021), but it also complicates our understanding of how variation in these traits arises and how it is partitioned between the sexes.

Shared mating traits are central to sexual selection. Pre-copulatory mechanisms (e.g., female mate choice reflected in mating latency or copulation timing) mediate access to fertilization opportunities, while copulatory and post-copulatory processes (such as sperm transfer, sperm storage and usage) determine actual mating success and the potential for sperm competition (Simmons 2001). Frequently, shared mating traits benefit one sex but impose costs on the other, and in such cases sexual conflict arises as males and females have different fitness optima (Arnqvist and Rowe 2005). For example, in species such as *Gryllodes sigillatus,* where ejaculate takes the form of a device that adheres to the female genitalia, post-copulatory dynamics are complex, allowing for reduced male control over sperm transfer, cryptic female choice and sexual conflict during mating and over fertilization (Burns-Dunn et al. 2024). Overall, it is clear that the resolution of the conflict will strongly depend upon the degree to which males and females are able to control the processes in question. Yet, much of our understanding of the evolution of these traits derives from studies that manipulate or observe one sex in isolation, preventing us from quantifying estimates for the relative effects males and females have on them. Therefore, receiving such estimates is what we are aiming for with the present study.

From an evolutionary perspective, such shared mating traits are not static but often exhibit behavioural plasticity, allowing individuals to fine-tune their strategies in response to changing ecological and social environments. The social environment, particularly conspecific density, plays a key role in shaping reproductive behaviours because it influences encounter rates, mate

availability, and mating strategies (Jirotkul 1999; Han and Brooks 2015; Morimoto et al. 2016). At the pre-copulatory stage, variation in density can alter how males and females express mating-related behaviours. Males exposed to high densities often experience elevated competition and may increase signalling effort, courtship intensity, or persistence to secure matings (Kokko and Rankin 2006; Callander et al. 2013; Choi and Hebets 2021). Females, in turn, may adjust their selectivity depending on perceived mate availability or competition, leading to changes in mating latency or copulation duration. For example, under low-density conditions or when mate encounters are infrequent, females often exhibit reduced choosiness (Holveck et al. 2015; Scott et al. 2020) and shorter mating latencies, reflecting a trade-off between selectivity and the risk of remaining unmated (Etienne et al. 2014; DuVal and Kapoor 2015). Beyond the initial stages of mate choice and mating, social context also shapes post-copulatory processes such as sperm competition and cryptic female choice. In species with internal fertilization, males often tailor ejaculate allocation — for instance, sperm number or seminal fluid composition — in response to cues of sperm competition (Wigby et al. 2009; Bretman et al. 2011). Females, conversely, can exert post-copulatory choice by differentially storing or utilizing sperm, influencing paternity outcomes after mating (Firman et al. 2017; Wang et al. 2024; Kustra et al. 2025). Manipulating developmental or social density can therefore provide a powerful approach to disentangle sex-specific effects on both pre- and post-copulatory traits.

Much of the empirical work investigating sexual selection and reproductive trait plasticity has focused on manipulating the environment or phenotype of either males or females in isolation (Bretman et al. 2009; Bailey et al. 2010; Rebar et al. 2011; Churchill et al. 2021). While these studies provide valuable insights into sex-specific strategies, they often overlook interactive effects between the sexes that occur within natural mating systems. Manipulating only one sex can obscure the extent to which shared mating traits are the product of coordinated, antagonistic, or context-dependent influences from both partners. As a result, it is difficult to attribute control of these traits to males or females, and partition the observed variance, especially when behavioral responses or plasticity in one sex trigger compensatory changes in the other. Moreover, behavioural observations alone cannot reveal whether apparent sex-specific effects arise from differential control or context-dependent feedback between partners. Experimental manipulations, for example, that independently vary the social environments of both sexes can provide a powerful framework for determining which sex exerts greater control over shared copulatory traits, as has been discussed in more detail in a recent review by the authors (under review, Behavioural Ecology and Sociobiology). Such approaches can reveal whether males and females adjust their

mating behaviour towards different optima when the socio-sexual context changes and whether these adjustments are cooperative, conflicting, or compensatory. Ultimately, changes in the social environment can alter the optimal expression of mating traits for each sex, driving dynamic patterns of behavioural plasticity and potentially fueling ongoing sexual coevolution.

In this study, we employed a fully factorial mating design and variance-partitioning approach, using banded crickets, *Gryllodes sigillatus*, to investigate whether shared pre- and post-copulatory mating traits (mating latency, copulation duration, sperm investment of males and sperm storage in females) are controlled by males, females, or both sexes. The banded cricket is a cosmopolitan cricket that has become a well-established model in studies of sexual selection and sexual conflict (Sakaluk et al. 2019). Males attract females through long-distance calling songs and switch to softer courtship songs upon proximity to a female (**Figure 1**). During copulation, males produce a spermatophore complex (a two part ejaculate package) consisting of a gelatinous spermatophylax that is consumed by the female (i.e., nuptial gift) while sperm transfer proceeds via the ampulla (Sakaluk 1984). Unlike direct insemination, this mating system allows females to have the ability to terminate sperm transfer prematurely by removing the ampulla, directly impacting the number of sperm received and subsequent paternity (Sakaluk and Eggert 1996). This dynamic sets the stage for sexually antagonistic coevolution, where males evolve strategies to maximize sperm transfer and nuptial gift effectiveness, while females evolve resistance mechanisms to retain control over fertilization (Vahed et al. 2014; Kamimura et al. 2021). We raised male and female crickets in two treatments: same-sex low and high density from nymph to adult stage. Through variance partitioning, we estimated the proportion of variance in each trait attributable to male and female developmental social environment as well as their interaction. We hypothesized that male density treatment would explain greater variance in traits related to duration of copulation and sperm investment, reflecting adaptive adjustments to sperm competition, whereas female density treatment might contribute more strongly to variance in traits linked to mating latency and sperm storage, consistent with the potential for female choice. Furthermore, we also predicted that male and female treatments might interact to shape some of the shared mating traits, reflecting the potential for coordinated, antagonistic, or context-dependent contributions from both partners during mating. By integrating both pre- and post-copulatory traits within a single experimental framework, our study provides novel insights into how male and female social environments jointly shape shared reproductive behaviours and outcomes.

METHODS

Animal rearing

Animals used in the study originated from a stock of approximately 200 individuals (adults and subadults) that were sourced from an insect breeder firm in April 2022 (ReptilienKosmos). For maintaining the laboratory population, in each generation, when adults were detected, moistened cotton wool was provided in a plastic cup as an oviposition substrate. Hatching nymphs were collected en masse and approximately 200 nymphs were allocated at random to each container (28cm x 17.5cm x 17cm) to establish the next generation. This process minimises inbreeding in generation. Crickets were kept inside a laboratory room at 27±1 °C on a 12hr:12hr light:dark cycle. They were provided with *ad libitum* food (Nekton cricket breeding concentrate), water in a glass vial (22 ml) plugged with cotton wool and egg cartons for shelter. Individuals for the experiment were sourced from the 8th, 9th and 10th generations of the lab population at the nymphal stage.

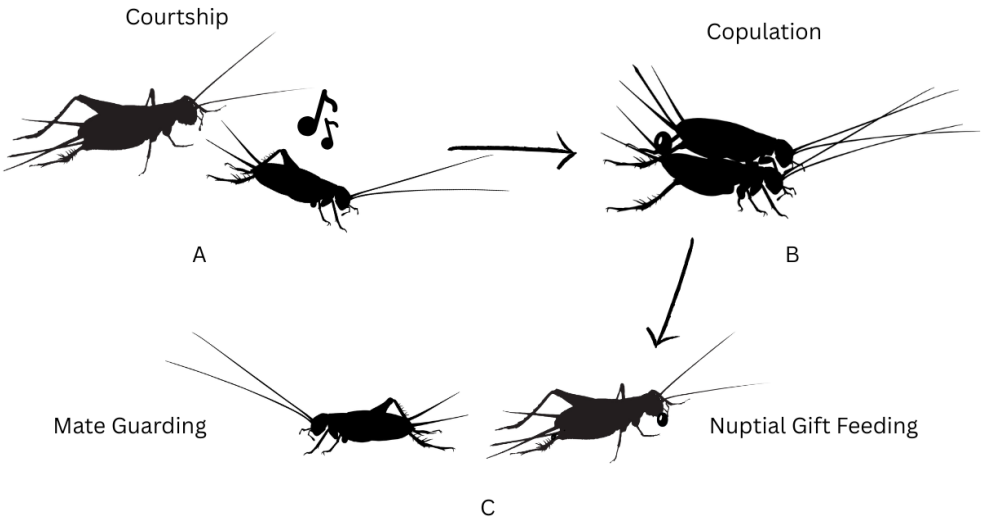

Figure 1. Simplified illustration of the mating behaviour of *Gryllodes sigillatus.*

A) Male courts the female by singing B) Female mounts the male dorsally and copulation starts, male produces a spermatophore C) After transfer of spermatophore (spermatophylax+ampulla) to the female, the pair separates and female starts feeding on the spermatophylax while the male performs post-copulatory mate guarding.

Density treatments

To investigate the effects of rearing density on shared mating traits in *Gryllodes sigillatus*, we reared males and females under two same-sex density treatments – high and low (**Figure 2**). At 3-4 weeks of age, nymphs were individually sexed using a stereo microscope (Leica MS5, 1.6x magnification) based on the morphology of the posterior abdomen: females were identified by the presence of a developing ovipositor, while males lacked this structure. After sexing, individuals were assigned to either the low-density (1 individual) or high-density (8 individuals) treatment and placed in plastic containers (l:16.9 cm w:10.5 cm h:7.4 cm). Crickets remained in their assigned density and sex-specific groups throughout the remainder of development until sexual maturity (8 weeks). To prevent the transmission of adult male auditory cues to the females (therefore not giving a perception of male treatments to the females before the mating trials), the male and female containers were maintained in different rooms with the same temperature and photoperiod conditions. Males in high- and low-density treatments were further separated into two different rooms to prevent exposing low-density males to the auditory cues of high-density males. The density treatments were designed to simulate varying levels of social environment during development, allowing assessment of density-dependent effects on adult mating traits.

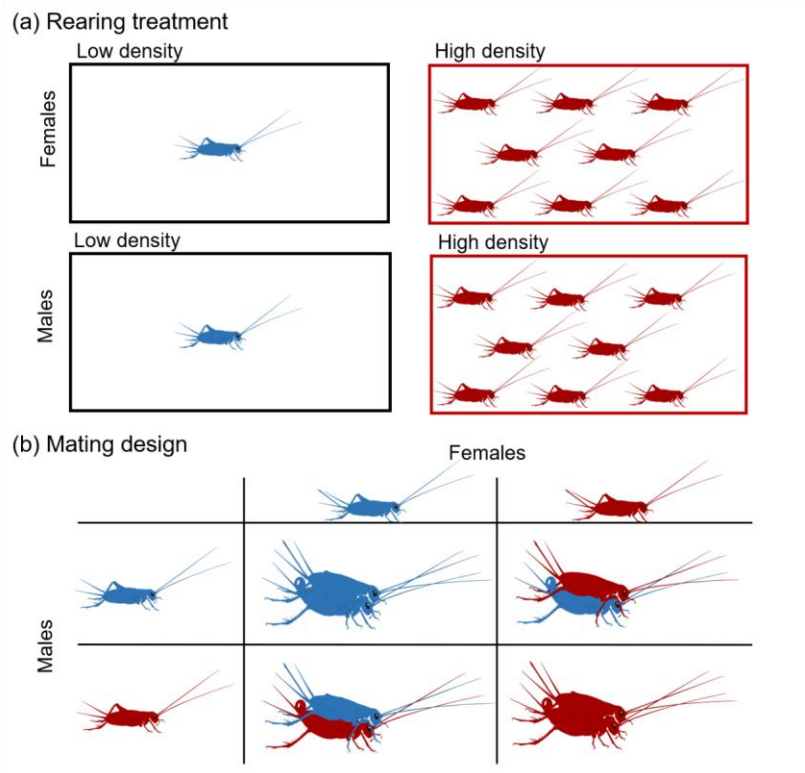

Figure 2. Overview of the experimental design. (a) Nymph males and females were reared independently under either low-density (blue) or high-density (red) conditions until adulthood. These treatments manipulated each sex's developmental social environment. (b) Adults were then randomly paired in a full factorial crossing design, allowing us to test how male and female developmental density—independently and interactively—affected shared mating traits.

Mating trials and trait measurement

Mating trials followed a full factorial design, in which males and females independently raised at one of two density treatments (low or high) were crossed, generating four possible male–female treatment combinations (Figure 2, for sample sizes refer to S1). During each no-choice trial, one female and subsequently one male, were introduced into a standardized mating arena (dimensions) under controlled laboratory conditions (temperature: 27 °C; photoperiod: 12:12 h light:dark). Only one individual at random from each high density replicate of both sexes was used for the mating trials to avoid pseudo replication (Forstmeier et al. 2016). If a pair did not mate in the first 30 minutes, we exchanged the male or female partner, thereby giving such individuals two chances to mate. Pairs were observed in red light continuously until copulation was completed and measures of mating latency and duration were taken. We defined mating latency as the time from male starting to sing to the onset of copulation (female mounting the male dorsally), and mating duration as the elapsed time from the initiation to the termination of copulation (i.e. transfer of spermatophore to the female and separation of the pair as a result of dismounting). These

timings were recorded by one observer using a stopwatch (ATP digital stopwatch TIM1166). The data for mating latency and mating duration was collected in 3 batches (batch 1 in February 2024, batch 2 in April 2024, batch 3 in October 2024). Following copulation, the attached ampulla was immediately extracted from the female's posterior using fine-tipped forceps to count the number of sperm transferred by the male. This data was collected from the first batch of the experiment.

To calculate the number of sperm stored by females we ran a different set of mating trials over 2 batches of animals. Here, females were separated from the male partner after a completed copulation and sperm transfer from the ampulla into the female was allowed. After 16±1 hours post-mating, females were dissected under a stereo microscope (Leica MS5, 1.6x magnification) and the spermatheca was extracted for the sperm count assay (time window based on pilot testing by TR). Both males and females were weighed the morning after the mating trials (Kern 770-60 electronic analytical balance).

Sperm count assay

For quantifying sperm contained in the ampulla of the spermatophore and the female spermatheca, samples of an individual ampulla or spermatheca were suspended in 2 ml PBS solution. Each sample was first sheared with microscissors, and then by pushing the suspension multiple times through a 25 G needle attached to a 1-ml syringe until the sample was 'cloudy' (Schaus and Sakaluk 2001). Sperm were systematically quantified on a *Leja* standard count 2 chamber slide under light microscopy (Olympus BX50, 40x magnification). The slide consisted of two chambers of 6µl, so the sperm from each sample was counted twice (2 aliquots) and recorded. The mean sperm count per sample was used as the measure of sperm number in statistical analyses. Repeatability of sperm counts within males was high (ICC = 0.83, 95% CI: 0.70–0.90).

Statistical Analyses

All statistical analyses were performed in R version 4.4.2 (R Core Team 2024), with packages loaded via *pacman* (Rinker and Kurkiewicz 2018) to ensure reproducibility. We performed generalized mixed effects models and linear mixed models implemented in lme4 (Bates et al. 2015) and glmmTMB (Brooks et al. 2017) depending on the data and error structure.

For all models (unless stated otherwise), male density (high and low), female density (high and low) and their two-way interaction were included as fixed effects while Batch ID (number of batches) was added as a random effect. If the interaction term yielded non-significant effects, we also ran an additive model and compared the two using anova. Male and female body weights were summarized using means and standard deviations, and group differences were evaluated

with linear models to confirm that density treatments did not affect body mass (p=0.72 for males, p=0.22 for females). Male and female body weights were subsequently included as covariates in all relevant analyses. Because mating latency data (in seconds) were right skewed, we applied a log transformation to improve normality of residuals and stabilize variances. Specifically, we used log(x+1) to accommodate zero values. We tested post hoc whether mating latency and mating duration were correlated across pairs using Pearson's correlation (Pearson 1895). Data visualization was performed using ggplot2 (Wickham 2016). Post hoc comparisons of main level effects and interactions were performed using the function emmeans and emtrends in the 'emmeans' package *v 1.8.8* (Lenth 2023).

RESULTS

Effect of Density Treatment on Mating Latency

The analysis was based on 108 mating trials conducted across two experimental batches. Mating latency was significantly affected by female density treatment (β = 0.593 ± 0.26, t = 2.30, ***p* = 0.024; Figure 3**), with females in the low-density treatment exhibiting longer mating latencies compared to those in the high-density treatment. The mean mating latency was 106 s for low-density females and 90.3 s for high-density females. Male density treatment and the interaction between male and female treatments did not significantly influence this trait (S2). Similarly, neither male weight nor female weight had a detectable effect. The variance explained by batch was relatively high (proportion of variance = 0.33), indicating substantial differences among the experimental batches.

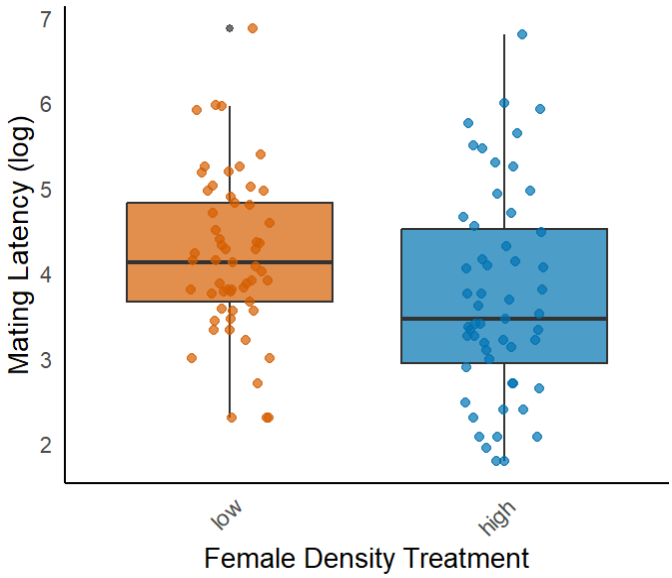

Figure 3. Effect of female density treatment on log-transformed mating latency (measured in seconds). Boxplots show that females from the low-density treatment exhibited longer mating latencies compared to females from the high-density treatment, consistent with model results.

Effect of Density Treatment on Mating Duration

The density treatment of either sex had no detectable effects on mating duration (S3). However, mating duration showed a positive association with female weight (β = 1.843 ± 0.95, t = 1.93, ***p* = 0.04; Figure 4**), with heavier females experiencing slightly longer copulations, but not with male weight (β = 0.39± 0.54, p = 0.49). The random effect of batch accounted for a small variation (variance = 0.008), indicating minimal batch-to-batch differences.

We also tested post-hoc whether mating latency and duration were correlated across pairs using Pearson's correlation. Trials that had zero recorded mating duration (no copulation) despite showing a mating attempt, were excluded from the correlation analysis. There was a weak negative correlation between mating latency and mating duration (r = –0.18, 95% CI: –0.36 to 0.01, p = 0.057). This suggests a tendency for pairs with shorter mating latencies to mate for longer, although the relationship was not statistically significant according to the threshold.

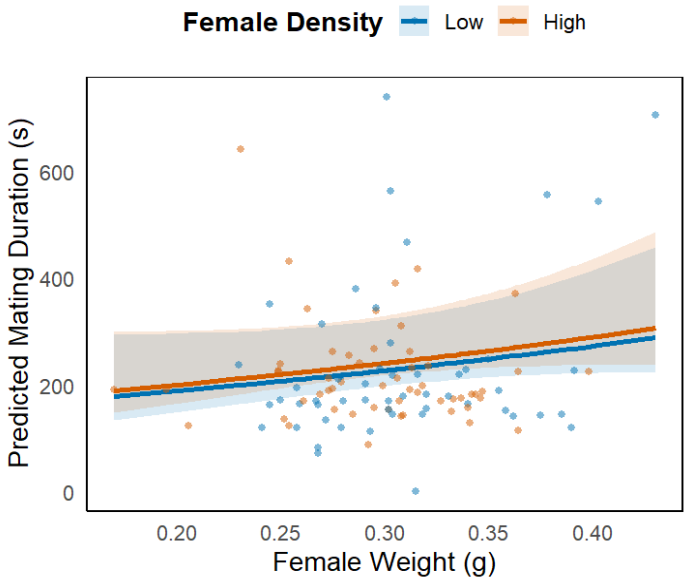

Figure 4. Predicted mating duration for *Gryllodes sigillatus* females as a function of female weight and female density treatment. Model predictions (lines) and 95% confidence intervals (shaded ribbons) are shown for low and high-density female groups, estimated using a generalized linear mixed model. Individual data points are overlaid. Mating duration increased with female weight across both treatments, and was highest for heavier females.

Effect of Density Treatment on number of sperm transferred

Sperm transfer was significantly influenced by male density treatment (β = −0.260 ± 0.075, t = −3.45, ***p* = 0.001, Figure 5**), with males from the low-density treatment transferring fewer sperm than males from the high-density treatment. Female density (p = 0.32) and the weights of either sex did not significantly affect sperm transfer (S4). This pattern is consistent with strategic male adjustment of sperm investment in response to perceived sperm competition risk, a phenomenon previously demonstrated in other species.

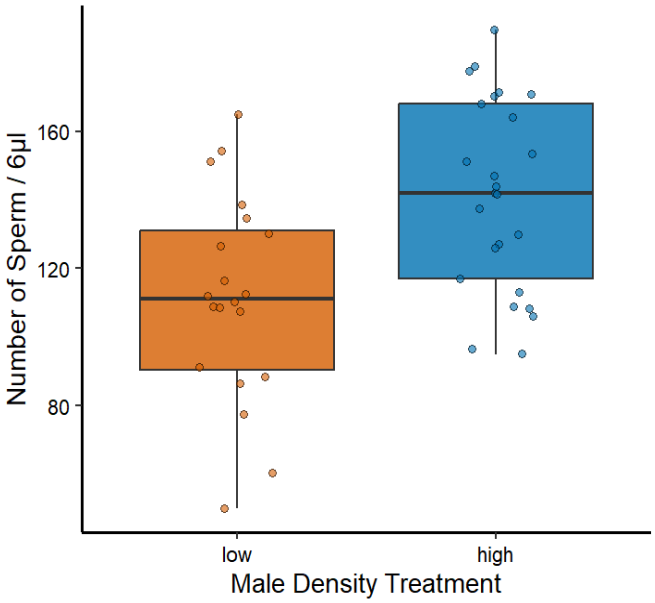

Figure 5. Number of sperm from the male ampulla (counted in 6 µl volume) in *Gryllodes sigillatus,* plotted by male density treatment. Boxplots show that males reared at high density transferred significantly more sperm upon copulation completion than males from low-density treatments. The number of sperm in the ampulla ranged from ~17000-70000.

Effect of Density Treatment on the Number of Sperm Stored by the Female

Using a zero-inflated negative binomial mixed model, we found that female sperm storage was significantly lower when females mated with males from the low-density treatment (estimate= -0.288 ± 0.125, z = -2.31, **p = 0.02; Figure 6**). Female density, the interaction of male and female treatment and both male and female body weights did not significantly affect sperm storage (S5). The random effect of batch once again accounted for very little variation (variance = 0.003), indicating minimal batch-to-batch differences.

We post-hoc quantified sperm transfer and storage across male and female treatments (S6). The proportion of sperm stored relative to that transferred varied with both male and female treatments, ranging from 0.678 in low-density male × high-density female pairings to 0.893 in low-density male × low-density female pairings.

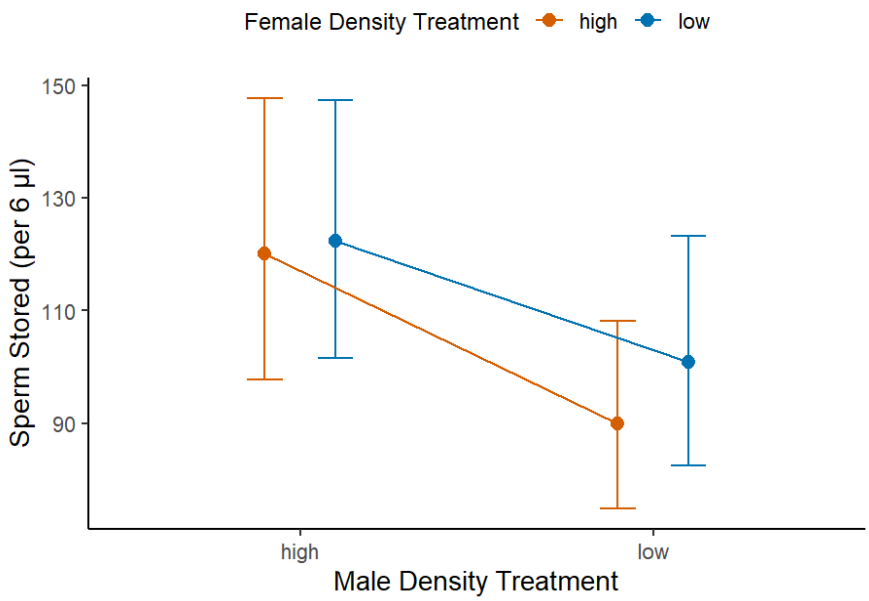

Figure 6. Estimated marginal means of sperm stored (counted in 6 µl) after mating for each combination of male and female density treatment in *Gryllodes sigillatus*. Points and error bars show model-predicted means ± 95% confidence intervals from a negative binomial mixed model. Sperm storage was higher when males developed under high-density conditions and in females developed under low-density conditions, with the greatest sperm storage observed for pairs of high-density males and low-density females.

DISCUSSION

In this study, we tested sex-specific control over key shared mating traits by varying the developmental social environment of males and females independently and then conducting fully factorial, no-choice mating trials. We predicted that each shared mating trait (mating latency, copulation duration, number of sperm transferred, and number of sperm stored) could be primarily under the control of one sex, but that for some traits both sexes might exert influence, potentially leading to interactive effects between male and female developmental environments. Our main finding was that each shared mating trait, except mating duration, was affected by sex-specific density treatment of either male or female without any clear interactive responses, in contrast to a recent review (Daupagne et al. 2025).

Unlike most previous studies that typically manipulate the environment or condition of either males or females to assess sex-specific effects on mating behavior, our experimental design manipulated both male and female developmental densities independently and combined them factorially in mating trials. This approach enabled us to disentangle the contribution of each sex's

environment on mating outcomes and to directly test for male-by-female interaction effects. Such designs are increasingly recognized as necessary to capture the complexity of sexual interactions and to reveal how plastic responses in both sexes jointly shape reproductive outcomes (Simmons and Lovegrove, 2024). Because these plastic responses can alter the expression of key mating traits in context-dependent ways, they may also influence the strength and direction of sexual selection, thereby affecting the potential for adaptive evolution.

Female Control over Mating Latency

Our findings revealed a significant effect of female developmental density on mating latency, with females from low-density treatments exhibiting longer latencies compared to those from high-density environments. This pattern suggests that early-life social conditions shape female behavioral plasticity, potentially by altering perception of mate availability or competitive risk (Díaz-Fleischer et al. 2009; Santana et al. 2020). Mating latency is frequently interpreted as a proxy for female mate choice, where increased latency can reflect greater female selectivity or reluctance to mate, possibly due to assessment of male quality or other mating-context cues (Sharma et al. 2010; Bailey and Zuk 2012; Judge et al. 2014; Kuriwada 2022). In line with a study on *Drosophila* manipulating female density and receiving longer female mating latencies for higher housing density (Churchill et al. 2021) we predicted longer mating latencies when females were reared at high density. However, our result is opposite to our prediction as females from high density social environments take shorter to initiate mating. However, it is plausible that virgin female crickets raised at high same-sex density and therefore experiencing higher female encounter rate without encountering males may have led to an expectation of low male presence and higher competition for mates. As male treatment did not influence mating latencies in our experiment, it is unlikely that males altered the attractiveness of their calling signals (Kelly et al. 2023).

Notably, we did not detect a significant interaction between male and female developmental environments on mating latency. This outcome contrasts with our expectation and results from other studies, eg. Fowler et al. (2022), who demonstrated in *Drosophila melanogaster* that mating latency was determined by an interaction between the plastic responses of both sexes to their social environments. However, mating latency as a mating trait is often affected by differences in experimental protocols, such as type of design, arena size, introduction timing, or observation methods which further complicates standardized measurement and comparability across studies (Dougherty and Shuker 2015). Additionally, behavioral plasticity allows individuals to modulate

behaviours like mating latency adaptively in response to fluctuating ecological and social contexts, demonstrating considerable within- and between-species variability (Karlsson et al. 2010).

High batch variation accounted for 33% of the total variance in mating latency, indicating that a sizable portion of behavioral variability was attributable to differences between experimental batches rather than the modeled fixed effects. Such batch-driven heterogeneity is a common challenge in behavioral ecology which can result from slight but cumulatively impactful variations in environmental conditions (e.g., temperature, humidity, lighting), subtle differences in handling or timing by experimenters, or microhabitat structure within the experimental apparatus across batches. While all the environmental conditions in the lab during breeding and the experimental trials were kept constant in our study across all batches, the crickets used in each batch belonged to a different generation. This also meant that the experiment was performed in different times of year (see Methods) which could have added some unexplained genetic or environmental variation. While batch variation complicates data interpretation, it likely reflects genuine biological and environmental heterogeneity and underscores the need for further replication and variance partitioning to isolate true treatment effects.

Effect of Female Body weight on Copulation Duration

Our findings on prolonged copulation with heavier females may result from male assessment of female reproductive quality, greater sperm storage capacity, or female willingness to prolong mating, all of which can influence fertility outcomes. Larger or heavier females often have higher fecundity and greater capacity for egg production and sperm storage, driving males to allocate reproductive effort preferentially during extended copulations to maximize fertilization success (Sturm 2016; Jiron et al. 2025). Alternatively, the mating duration period could also act as a mate assessment phase for the female (Lehmann 2007), with larger or heavier females needing longer stimulation or more nuptial gift investment before accepting the male's spermatophore, consistent with differential allocation theories of mate choice (Wilson and Walker 2019). This is supported by evidence in *Gryllodes sigillatus* and related species where females can modulate spermatophylax feeding duration impacting sperm transfer and subsequent paternity outcomes (Wedell 1991; Reinhold and Heller 1993; Burns-Dunn et al. 2024).

The finding that heavier females engage in longer copulations in *Gryllodes sigillatus* may also reflect mechanistic factors related to mating posture and genital positioning. During copulation, males use specialized cerci to grasp and coordinate female mounting, facilitating precise alignment necessary for successful spermatophore transfer (Ritz and Sakaluk 2002; Wulff et al.

2017). Larger female size could influence these mechanical interactions, potentially requiring an extended time to achieve or maintain optimal copulatory positioning. Such biomechanical and sensory coordination underscores the role of physical compatibility in shaping mating duration and highlights the integration of morphological and behavioral factors in reproductive strategies of crickets (Alexander D and Otte 1967). Beyond mechanical considerations, prolonged copulation may reflect sexual conflict and negotiation, where females evolve resistance mechanisms to influence sperm transfer while males manipulate copulation duration to maximize reproductive success.

Sperm transfer as a response to sperm competition risk

Our finding that males from high-density treatments transferred significantly more sperm aligns with the well-documented phenomenon of strategic sperm allocation in response to perceived sperm competition risk (Wedell and Cook 1999; Schaus and Sakaluk 2001; delBarco-Trillo 2011; Schaus and Sakaluk 2011; Hopkins et al. 2019; Manas et al. 2025). Such plastic shifts in ejaculate investment are not merely behavioural responses but are widely interpreted as adaptive strategies shaped by selection in fluctuating environments. In this context, the increased sperm transfer by males reared at high density likely reflects an evolved capacity for plastically responding to cues that reliably predict future sperm competition (Hopkins et al. 2019, Koppik et al. 2023). The lack of female treatment or body weight effects also suggests that male investment responses are more directly attuned to intra-sexual competitive environments rather than female social cues. In fact, female size has consistently been shown to have no effect on the amount of sperm transferred by male *G. sigillatus* (Gage and Barnard 1996; Farmer and Barnard 2000). Furthermore, since the males we used were virgins, it is possible that they already possessed fully formed spermatophores when they entered the mating arena and hence would have already been committed to a particular ejaculate expenditure. While the observed differences in sperm number contained in the ampulla can be attributed to variation in sperm competition risk resulting from male social environment, it is possible that females have an indirect effect on the composition of the spermatophylax or the seminal fluid proteins that males are known to adjust based on, not only their social environment, but also female quality (Perry et al. 2013). Female cues, therefore, could shape ejaculate investment in ways that were not captured in our study.

Sperm storage and the possibly uncaptured cryptic female control

Contrary to our predictions, our results show that females stored significantly fewer sperm when mating with males reared under low-density conditions, suggesting that the male social

environment during development or adulthood influences ejaculate traits that affect post-copulatory outcomes. The absence of significant effects of female density treatment or male–female interaction indicates that first mating sperm storage in *Gryllodes sigillatus* is primarily influenced by male-driven variation in ejaculate investment rather than female-mediated storage biases under the tested conditions.

However, this significant male treatment effect does not rule out female influences, as suggested by the differences in proportion of sperm stored in the different mating combinations. It is possible that females respond plastically to male ejaculate cues rather than to the social density they themselves experienced. It also remains possible that females exert subtle, context-dependent influences on sperm storage through mechanisms such as cryptic female choice which were not captured through our experimental design. Females of *G. sigillatus* are well known to exert strong post-copulatory mate choice by adjusting their feeding time of the spermatophylax and can terminate sperm transfer prematurely by removing the ampulla (Sakaluk and Eggert 1996; Ivy and Sakaluk 2007; Gershman and Sakaluk 2010; Burns-Dunn et al. 2024). These hidden female-mediated processes may thus contribute to the observed mean-level trends in sperm storage, even in the absence of statistically detectable treatment effects, as the number of sperm stored mainly seems to be dominated by higher male sperm investment when they were reared under high density. Future studies incorporating repeated mating opportunities, direct assays of spermatophore attachment durations, sperm viability, or transcriptomic responses of the female reproductive tract could help disentangle these potential female-mediated effects from male-driven variation.

Studies using plasticity and heritability to disentangle mating traits

A parallel literature by various authors has explicitly partitioned additive genetic contributions of males and females to shared reproductive traits, demonstrating that both sex-specific additive variance and cross-sex genetic correlations shape the potential for independent evolution of male and female expression (Ratterman et al. 2013; Gaertner et al. 2015; Han et al. 2024). When male and female phenotypes are genetically correlated, evolutionary responses in one sex may inadvertently limit or modify responses in the other. This shows that the potential for sexual selection depends not only on the genetic variance in each sex but also on the extent to which male and female traits are coupled. Our study complements these genetic approaches by experimentally partitioning sex-specific developmental environmental (density) effects and their interactions in a fully factorial design. Rather than estimating additive genetic variance (direct or indirect), we quantify how male and female developmental social environments independently

and jointly shape the expression of shared mating traits via plasticity—information that is orthogonal to, and therefore directly comparable with, additive-genetic decompositions of the same traits.

Conclusion

By independently manipulating both male and female environments and applying a variance partitioning approach, our study provides rigorous support for the existence of independent and plastic sex-specific effects while highlighting that the expression and detection of interaction effects may be more context-specific than previously appreciated. Our results also reveal that shared mating traits are not solely determined by the direct interaction of the sexes but are dynamically shaped by their developmental social environments. By experimentally disentangling male and female density effects, we demonstrate that both sexes exhibit environmentally induced plasticity that jointly determines mating outcomes. This highlights that sex-specific developmental conditions can modulate reproductive interactions before mating occurs, potentially maintaining variation in shared traits even in the absence of strong genetic divergence between the sexes.

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Data and code availability statement

Data and code are publicly available on Zenodo (https://doi.org/10.5281/zenodo.17737514)

Author contributions

TR: Conceptualisation, Data Curation, Formal Analysis, Investigation, Methodology, Funding Acquisition, Project Administration, Software, Visualization, Writing- original draft (lead), review and editing (equal) **CT:** Supervision (supporting), Writing- original draft, review and editing (equal) **KR:** Conceptualisation, Resources, Funding Acquisition, Supervision (lead), Writing- review and editing (equal)

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Appendix

S1: Summary statistics of the four measured shared mating traits (N= sample size, mean and SD= standard deviation, h= high density treatment, l= low density treatment)

Trait	N	Mean±SD
Mating latency	111 ($N_{h♂xh♀}$= 23, $N_{h♂xl♀}$= 33, $N_{l♂xl♀}$= 31, $N_{l♂xh♀}$= 24)	98.4±145 s
Mating duration	111 ($N_{h♂xh♀}$=23, $N_{h♂xl♀}$= 33, $N_{l♂xl♀}$= 31, $N_{l♂xh♀}$= 24)	204±175 s
Number of sperm transferred	46 ($N_{h♂xh♀}$= 10, $N_{h♂xl♀}$= 15, $N_{l♂xl♀}$= 12, $N_{l♂xh♀}$= 9)	128±32.3
Number of sperm stored	66 ($N_{h♂xh♀}$= 13, $N_{h♂xl♀}$= 18, $N_{l♂xl♀}$= 19, $N_{l♂xh♀}$= 16)	97.9±45.7

S2: Mating latency model results

Predictors	Estimate	SE	CI	Statistic	p
(Intercept)	2.81	0.94	0.96 – 4.67	3.01	**0.003**
male density [low]	0.38	0.26	-0.14 – 0.89	1.46	0.148
female density [low]	0.59	0.26	0.08 – 1.11	2.29	**0.024**
male weight	2.50	2.90	-3.26 – 8.26	0.86	0.391
female weight	0.40	2.02	-3.61 – 4.42	0.20	0.842
male density [low] × female density [low]	-0.14	0.36	-0.86 – 0.58	-0.38	0.708
Random Effects					
σ^2	0.83				
Batch	0.33				
ICC	0.28				
N_{Batch}	3				

S3: Mating duration model results

Predictors	Estimates	SE	CI	Statistic	p
(Intercept)	145.01	60.74	63.16 – 332.95	11.88	**<0.001**
male density [low]	1.20	0.15	0.94 – 1.53	1.49	0.139
female density [low]	1.06	0.13	0.83 – 1.36	0.47	0.636

male weight	0.39	0.54	0.02 – 6.20	-0.68	0.500
female weight	6.32	6.01	0.96 – 41.70	1.94	**0.055**
male density [low] × female density [low]	0.89	0.16	0.63 – 1.27	-0.64	0.526

Random Effects

σ^2	0.22
Batch	0.01
ICC	0.04
N_{Batch}	3

S4: Sperm transfer model results

Predictors	Incidence Rate Ratios	SE	CI	Statistic	p
(Intercept)	129.40	55.60	55.35 – 298.35	11.32	**<0.001**
male density [low]	0.77	0.06	0.67 – 0.89	-3.45	**0.001**
female density [low]	0.93	0.07	0.80 – 1.07	-1.01	0.315
male weight	4.55	5.10	0.52 – 41.66	1.35	0.176
female weight	0.51	0.46	0.09 – 2.96	-0.76	0.450

S5: Sperm storage model results

Predictors	Incidence Rate Ratios	SE	CI	Statistic	p
Count Model					
(Intercept)	215.18	86.97	97.44 – 475.17	13.29	**<0.001**
male density [low]	0.75	0.09	0.59 – 0.96	-2.31	**0.021**
female density [low]	1.02	0.14	0.78 – 1.32	0.13	0.893
male weight	0.15	0.23	0.01 – 2.93	-1.25	0.212

female weight	0.57	0.57	0.08 – 4.04	-0.56	0.575
male density [low] × female density [low]	1.10	0.20	0.77 – 1.58	0.52	0.606
(Intercept)	18687.90		720.07 – 2430326.71		
Zero-Inflated Model					
(Intercept)	0.08	0.04	0.03 – 0.21	-5.34	<0.001

S6: Mean level differences in the proportion of sperm stored in different treatment combinations

Male treatment	**Female treatment**	**Sperm transferred (per 6 µl)**	**Sperm stored (per 6 µl)**	**Proportion stored**
high	high	146	119	0.815
high	low	138	108	0.783
low	high	117	79	0.675
low	low	103	92	0.893