Developmental density shapes adult mate guarding strategies in an invertebrate

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Abstract

Post-copulatory mate guarding is a widespread reproductive strategy that reduces sperm competition but can generate sexual conflict when male and female optima diverge. While mate guarding is known to respond plastically to immediate social conditions, the extent to which early-life social environments of both sexes shape adult guarding behaviour remains poorly understood. We experimentally tested how developmental density of males and females influences post-copulatory mate guarding and associated behaviours in the nuptial gift–giving cricket *Gryllodes sigillatus*. Using a full factorial design, we reared males and females under high or low same-sex densities and crossed them in standardized no-choice mating trials. We quantified mate-guarding duration, the intensity of guarding, female feeding time on the nuptial gift and ampulla retention time. Contrary to our prediction, males reared at low density guarded females for significantly longer than males from high density. Females reared at low density were also guarded for longer, with largely additive effects of the two treatments. Further analyses revealed that longer mate guarding prolonged female feeding and ampulla retention. These results demonstrate that developmental social environment can have lasting, sex-specific effects on post-copulatory behaviour and that mate guarding is functionally linked to mechanisms that ensure sperm transfer. Our findings highlight how early social cues can bias adult reproductive tactics and, consequently, the outcomes of sexual selection in polygamous mating systems.

Keywords: sexual conflict, mating tactics, nuptial gift feeding, shared mating traits, density effects, post-copulatory behaviour

Introduction

Post-copulatory sexual selection shapes reproductive strategies across animals, particularly in systems where females mate with multiple males and ejaculates compete for fertilizations (1,2). Under these conditions, selection favours male traits and behaviours that increase the likelihood of paternity, including strategic allocation of time, energy and reproductive effort after mating. Mate guarding represents one such strategy, in which individuals invest time after mating to reduce competition and thereby enhance reproductive success (3–5). Consistent with predictions from sperm competition theory, mate guarding behaviour has evolved repeatedly across the animal kingdom and has been documented in multiple taxa including insects (6–8), crustaceans (9), reptiles (10), birds (11–13) and mammals (14,15).

In post-copulatory mate guarding, both sexes should have an interest to influence the duration towards their own optimum, as each incurs distinct costs and benefits (16). For males, prolonged guarding can reduce sperm competition risk and increase fertilization success, but it also limits opportunities to seek additional mates (17,18). Females, in contrast, may benefit from guarding if it enhances sperm utilization, protection from harassment by other males or access to nuptial gifts, yet extended interactions can also impose costs such as restricted mobility, constrained cryptic choice or reduced genetic benefits (16-21). Studying these divergent interests and how they influence the outcome requires examining both sexes simultaneously, since male and female traits may both have effects on the outcome (22). While sex ratio manipulation studies have revealed plasticity in male mate guarding behaviour, they cannot distinguish sex-specific or developmental effects from immediate contextual responses. By manipulating developmental density in both sexes factorially, we reveal novel sex-specific anticipatory effects on post-copulatory interactions, with implications for how early-life social cues bias sexual selection outcomes in density-variable populations.

Nuptial-gift systems provide an informative context for such research since they integrate pre- and post-copulatory selection pressures: the duration of gift feeding frequently mediates the extent of mate guarding and, consequently, paternity. For example, in species such as *Gryllodes sigillatus*, females continue feeding on the nuptial gift (spermatophylax) while the male guards her, linking male investment and female behaviour. After copulation, the male remains in close proximity to the female, often maintaining physical contact or closely following her movements (23), while the female feeds on the nuptial gift. This strategy is thought to enhance male reproductive success through at least two overlapping, non-mutually exclusive mechanisms. First, mate guarding can prolong the period during which the ampulla of the spermatophore remains attached to the female, thereby increasing the number of sperm transferred (24, 25). Second, as a response to intrasexual competition, guarding can function to exclude rival males by physically deterring them or by making the female less receptive to additional mating attempts (26, 27). Regardless, mate guarding benefits males in ways that can generate sexual conflict if females gain from discarding the gift, earlier ampulla removal or remating (28).

Here we examine the effects of male and female developmental density on post-copulatory mate guarding in *Gryllodes sigillatus*. We used a full factorial design manipulating male and female developmental density to test how early social environment influences mate-guarding behaviour and its underlying components. Specifically, we quantified guarding duration and associated post-copulatory behaviours, including female feeding on the nuptial gift and ampulla retention time, to assess whether density effects operate through changes in male investment, female behaviour, or both. Given the higher benefits associated with males as compared to females, we hypothesized a stronger male effect on the duration of mate guarding and predicted that males raised in higher density would guard females for longer as they have a perception of higher intrasexual competition. We also predicted a causal correlation between intensity of mate guarding and the ampulla retention time by females. By integrating these behavioural measures within a structural equation modelling framework, we aimed to disentangle direct effects of developmental density from indirect effects mediated by post-mating interactions between the mating partners.

Methods

Study organism

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 (15 containers/generation). This process minimizes inbreeding in future generations. 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 9th and 10th generations of the lab population at the nymphal stage.

Experimental design

To investigate the sex-specific effects of density on mate guarding and its associated post-copulatory behaviours in *G. sigillatus*, we reared males and females under two same-sex density treatments – high and low (S1). At 3-4 weeks of age, nymphs from the stock population 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 for 8 weeks throughout the remainder of development until sexual maturity (S1). The density treatments were designed to simulate two levels of social environment during development in the wild, allowing assessment of density-dependent effects on adult post-copulatory mating traits.

Mating trials followed a full factorial design, in which males and females independently raised from one of two density treatments (low or high) were crossed, generating four possible male–female treatment combinations. During each no-choice trial, one female followed by a male were introduced into a standardized mating arena (dimensions) under controlled laboratory conditions (temperature: 27 °C, red light). Only one individual from each high density replicate of both sexes was used at random for the mating trials to avoid pseudo-replication. Pairs were observed by two observers continuously until copulation (transfer of spermatophore from male to the female) and mate guarding was completed. Data was collected for the following traits: mate guarding duration (time for which the male guarded the female by remaining in close proximity or following her around), feeding time (time taken by the female to finish feeding on the spermatophylax), ampulla retention time (time for which the ampulla remained attached to the female) and the number of post copulatory touches (number of times the male made physical contact using antennae or other body parts with the female during mate guarding, particularly when the female attempts to bend down to remove the ampulla). Post-copulatory touches (PCT) were quantified as a measure of mate guarding intensity, since our enclosed mating arenas did not allow the female to escape the male.

Statistical analyses

All analyses were conducted in R (*v*. 4.5.2). To examine the effect of density treatment on mate guarding duration we first evaluated a linear mixed-effects model using *lme4* package (*v*. 1.1.37; 29) including male treatment, female treatment, male and female weight as fixed effects, and batch as the random intercept. As the distribution of mate-guarding time was right-skewed, a natural log transformation was applied to meet assumptions of homoscedasticity and normality. Since batch explained negligible variance, it was subsequently removed, resulting in a final Gaussian linear model with fixed effects.

Residual diagnostics were performed using simulation-based tests (*DHARMa*) to evaluate normality, dispersion, and model fit (30). Estimated marginal means and pairwise contrasts (*emmeans, v*. 1.11.2.8) were used to quantify density-dependent differences in mate-guarding duration (31).

To test hypothesized causal relationships among all the measured behavioural traits – nuptial gift feeding time, ampulla retention time, post-copulatory touches (PCT), and mate-guarding duration – we constructed a piecewise structural equation model using the *piecewiseSEM* framework (*v*. 2.3.1; 32). The model included three component sub-models: (a) nuptial gift feeding time was modeled as a linear function of mate-guarding duration, male and female density treatments, and male and female body mass; (b) ampulla retention time was modeled as a function of mate-guarding duration, feeding time, male and female density, and male and female body mass; and (c) PCT was modeled using a linear mixed-effects model with mate-guarding duration, feeding time, male and female density and male and female body mass as fixed effects and batch as a random effect.

Model fit and directed separation tests were evaluated using the *psem* function. Coefficients from the component models were used to interpret the strength and direction of individual pathways.

To quantify indirect pathways, we performed non-parametric bootstrapping (1000 iterations) by resampling the dataset with replacement. For each bootstrap sample, all relevant component models were refitted and the product of coefficients for each indirect pathway was calculated. Mean indirect effects and 95% percentile confidence intervals were obtained.

Results

By manipulating the developmental density of males and females in a 2x2 factorial design, we found that mate-guarding time was significantly higher for males from low-density rearing than from high density (estimate = 0.33 ± 0.11, $t = 3.12$, $p = 0.003$, Table 1, Figure 1). Females from low-density rearing were also guarded for longer than high-density females (estimate = 0.24 ± 0.11, $t = 2.28$, $p = 0.026$, Figure 1). Male and female body weights did not significantly predict mate-guarding duration (Table S4), and the interaction between male and female density was not significant either (estimate = −0.24 ± 0.15, $p = 0.11$), indicating largely additive effects of the two density treatments on mate guarding.

Table 1: Summary statistics for mate-guarding duration (minutes) across combinations of male and female developmental density. Sample size (n), mean, standard deviation (SD), standard error (SE), and median are shown for each treatment combination.

Male density	Female density	n	mean	SD	SE	median
high	high	16	35.1	15.9	3.97	33.8
high	low	24	43.3	10.5	2.15	40.0
low	high	25	47.5	14.2	2.83	46.1
low	low	19	46.6	13.6	3.11	46.6

The piecewise structural equation model showed good overall fit to the data, with no evidence of violated independence claims (Fisher’s $C = 1.12$, $p = 0.57$). Mate guarding duration had a strong positive direct effect on female feeding time of the nuptial gift (estimate = 0.59 ± 0.10, $p < 0.001$, Figure 2, S2) as well as on ampulla retention time (estimate = 0.32 ± 0.11, $p = 0.005$, Figure 2, S2), independent of feeding time and other covariates. In addition, feeding time was strongly correlated with ampulla retention time (estimate = 0.55 ± 0.11, $p < 0.001$, Figure 2, S2).

Bootstrapping confirmed a significant indirect effect of mate guarding duration on ampulla retention via feeding time (indirect effect = 0.32 ± 0.09, 95% CI 0.16–0.52), indicating that longer mate guarding prolongs nuptial gift feeding, which in turn increases ampulla retention.

In the piecewise structural equation model, female body mass had a significant positive effect on PCT (estimate = 0.31 ± 0.11, p = 0.008, S3), after accounting for mate guarding duration, feeding time, male body mass, and density treatments (Table S5).

In a complementary linear model where mate guarding duration was the response variable, guarding duration was positively associated with ampulla retention time (estimate = 0.36 ± 0.13, p = 0.009) and feeding time (estimate = 0.32 ± 0.14, p = 0.023). Mate guarding duration was also longer under low male density conditions (estimate = 0.43 ± 0.19, p = 0.023), a result consistent with the first linear model. No significant associations were detected with PCT or body mass.

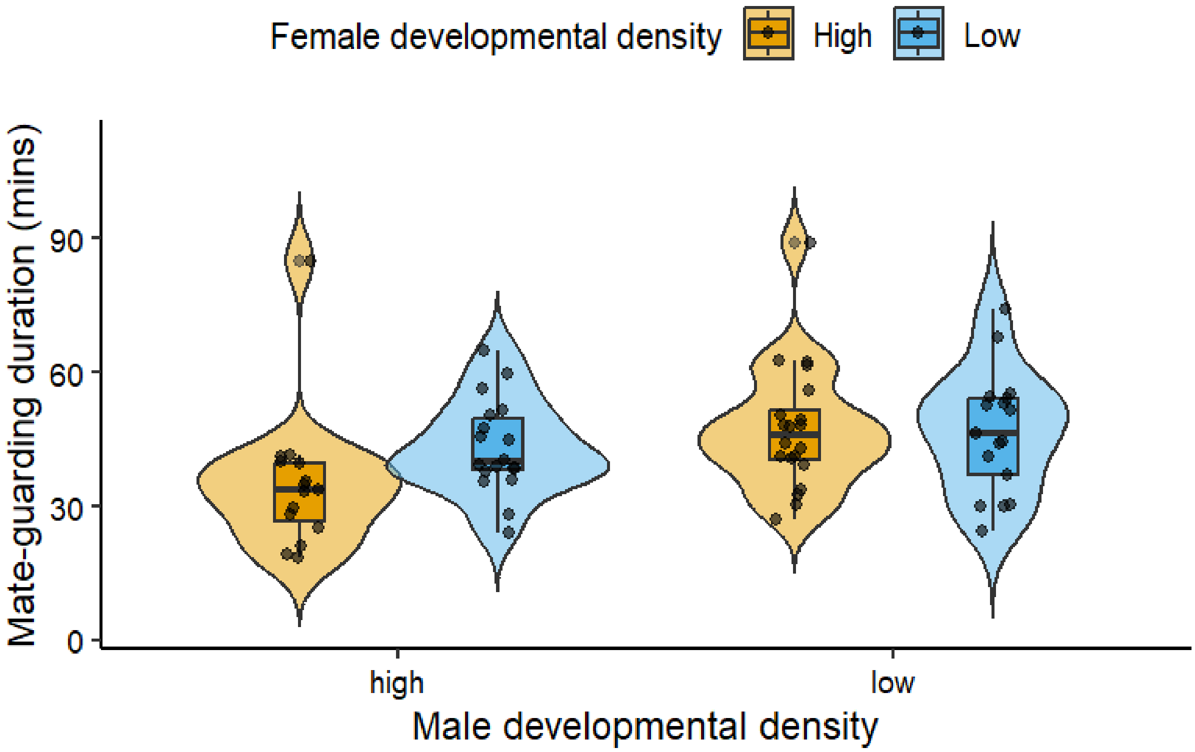

Figure 1: Effects of male and female developmental density on post-copulatory mate-guarding duration. Violin plots show the distribution of mate-guarding duration (minutes), with boxplots indicating medians and interquartile ranges and points representing individual observations. Mate-guarding duration was significantly longer in males reared under low developmental density, with a weaker modifying effect of female developmental density.

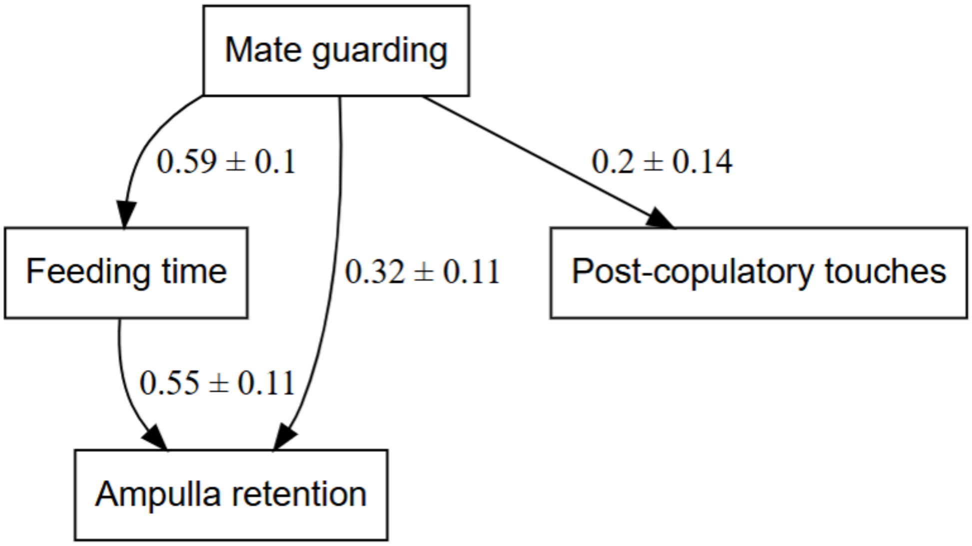

Figure 2: Path diagram summarising the piecewise structural equation model describing relationships among post-copulatory behavioural traits. Arrows indicate hypothesised causal pathways, and values along arrows represent standardized path coefficients ± SE from the component models of the piecewise structural equation model. Mate-guarding duration had strong positive effects on feeding time and ampulla retention, with feeding time further positively associated with ampulla retention. A weaker positive effect of mate guarding on post-copulatory touches is also shown.

Discussion

Our results demonstrate that post-copulatory mate guarding is strongly shaped by the social environment experienced during development and is functionally linked to key post-mating processes that influence sperm transfer in *G. sigillatus* (Figure 2). Contrary to our prediction, males reared at low density guarded females for significantly longer than males from high-density environments, and females reared at low density were also guarded for longer, with largely additive effects of the two treatments. Further analyses revealed that guarding duration affected female feeding time on the spermatophylax (the nuptial gift) and subsequent ampulla retention, consistent with the paternity-assurance function of guarding. In particular, the positive association between guarding duration and ampulla retention supports the hypothesis that males mate guard to prevent premature removal of the ampulla and thereby secure sperm transfer. Together, these findings suggest that mate guarding is a plastic reproductive behaviour shaped by developmental social cues and not solely the immediate adult mating environment.

This pattern of male control can be explained by the stronger selection pressure in males for controlling mate guarding duration. The fitness costs to males of reduced guarding are likely substantial, as shorter guarding durations increase the risk of premature ampulla removal and exposure to stronger sperm competition. In addition, the costs of prolonged guarding are also expected to vary with social environment; under low male density, costs associated with guarding such as opportunity to encounter more mates are reduced (33), making longer guarding less costly for males. Previous studies have shown that density and social context modulate harassment, guarding, and mating propensity, thereby changing how strongly male and female interests conflict over mating rates and guarding duration (34,35).

A previous study disentangling relative control of sexes on mate guarding behaviour in the same species also showed evidence for stronger male control achieved through a different experimental design (36) which adds to the robustness of our result. Unlike, Haneke-Reinders et al (2020), we did not find a correlation between male weight and guarding duration. This difference likely reflects variation in experimental context and analytical approach. In our study, rearing density exerted a strong influence on male investment in mate guarding. When density treatment, female feeding behaviour, and ampulla retention were included in our structural models, male weight explained minimal additional variation. This suggests that behavioural plasticity and functional drivers of guarding may be more important determinants of guarding duration in our conditions than morphological traits. We did, however, find a positive correlation between female weight and the number of postcopulatory touches during guarding behaviour (S2). This can be attributed to the possibility that heavier or larger females might have a greater tendency to prematurely remove the ampulla or that males guard them more vigorously as they perceive heavier females as more fecund (37).

One potential mechanism for the detected female effect on mate-guarding duration could be density-dependent variation in female chemical signalling, particularly cuticular hydrocarbon (CHC) profiles, which may alter male behavioural responses.

CHCs act as short-range pheromonal cues in many insects, allowing males to discriminate among females and adjust courtship and mating effort based on female CHC composition (38). If females reared at high density carry cues indicating the presence of other females through their CHC profiles, this can inform males to seek out other mates, reducing the guarding duration. In *Gryllodes sigillatus*, female CHCs transferred during mating have been shown to influence subsequent mate discrimination, highlighting their importance as chemosensory signals mediating reproductive behaviour (39,40). More broadly, environmental modulation of CHC expression is well documented and can arise from variation in social contact during development (41,42). While we did not characterize CHC profiles of individuals in our system, the hypothesis that density-dependent CHC variation could affect male perception of females seems plausible and thus may contribute to differences in mate-guarding duration.

Our study provides robust evidence that post-copulatory mate guarding in a nuptial gift–giving species is under stronger male control relative to female control. We further show that mate guarding is a plastic behaviour shaped by developmental density and mediated through a set of interacting post-mating traits. In particular, the close association between guarding duration and ampulla retention time underscores the functional importance of mate guarding as a post-mating strategy that ensures effective sperm transfer. More broadly, our findings highlight how early social environments can shape adult reproductive tactics through cascading effects on behavioural interactions. Such tactic shifts can change which individuals are successful under varying densities, such that sexual selection may favour strategies rather than a fixed optimum. Therefore, these behavioural context-dependent variations can have important implications for understanding sexual selection in systems where mating interactions extend beyond copulation. optimum.

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Data and code availability statement

Data and code are publicly available on Zenodo: https://doi.org/10.5281/zenodo.18301357

Author contributions

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

Conflict of interest

The authors declare no conflict of interest.

References

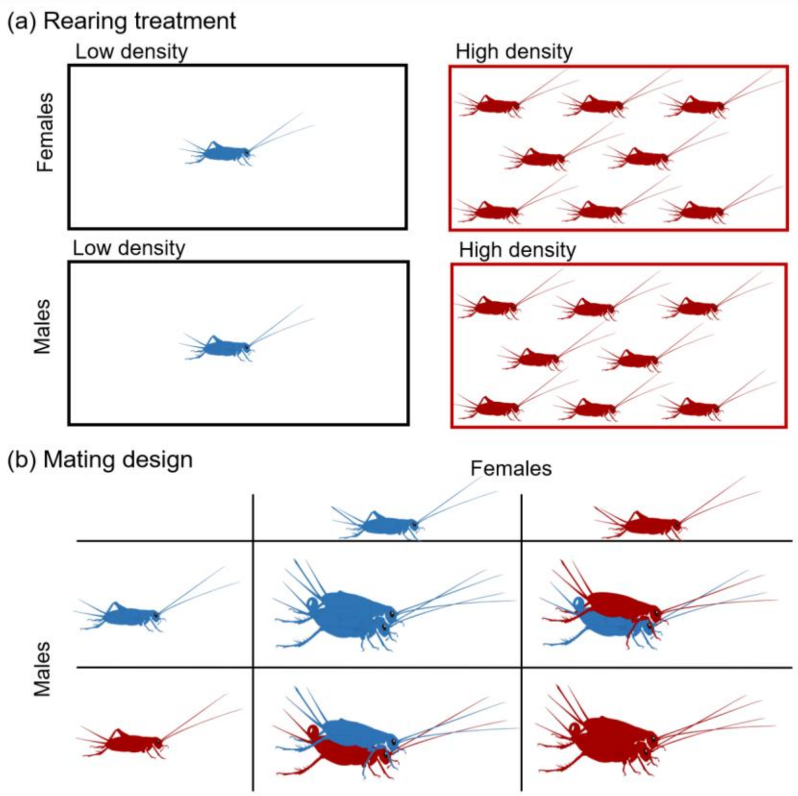
1. Pizzari T, Wedell N. 2013 The polyandry revolution. *Phil. Trans. R. Soc. B* **368**, 20120041.
2. Birkhead TR, Møller AP. 1998 *Sperm competition and sexual selection*. London, UK: Academic Press.
3. Alcock J. 1994 Postinsemination associations between males and females in insects: the mate-guarding hypothesis. *Annu. Rev. Entomol.* **39**, 1–21.
4. Komdeur J. 2001 Mate guarding in the Seychelles warbler is energetically costly and adjusted to paternity risk. *Proc. R. Soc. B* **268**, 2103–2111.
5. Simmons LW. 2001 *Sperm competition and its evolutionary consequences in the insects*. Princeton, NJ: Princeton University Press.
6. Vitta ACR, Lorenzo MG. 2009 Copulation and mate guarding behavior in *Triatoma brasiliensis* (Hemiptera: Reduviidae). *J. Med. Entomol.* **46**, 789–795.
7. Saeki Y, Kruse KC, Switzer PV. 2005 The social environment affects mate guarding behavior in Japanese beetles, *Popillia japonica*. *J. Insect Sci.* **5**, 18.

8. Parker DJ, Vahed K. 2010 The intensity of pre- and post-copulatory mate guarding in relation to spermatophore transfer in the cricket *Gryllus bimaculatus*. *J. Ethol.* **28**, 245–249.
9. Benvenuto C, Knott B, Weeks SC. 2009 Mate-guarding behavior in clam shrimp: a field approach. *Behav. Ecol.* **20**, 1125–1132.
10. Ancona S, Drummond H, Zaldívar-Rae J. 2010 Male whiptail lizards adjust energetically costly mate guarding to male–male competition and female reproductive value. *Anim. Behav.* **79**, 75–82.
11. Dowling J, Webster MS. 2017 Working with what you've got: unattractive males show greater mate-guarding effort in a duetting songbird. *Biol. Lett.* **13**, 20160682.
12. Krietsch J, Valcu M, Cragnolini M, Forstmeier W, Kempenaers B. 2024 Mutual mate guarding with limited sexual conflict in a sex-role-reversed shorebird. *Behav. Ecol.* **35**, arad084.
13. Komdeur J, Kraaijeveld-Smit F, Kraaijeveld K, Edelaar P. 1999 Explicit experimental evidence for the role of mate guarding in minimizing loss of paternity in the Seychelles warbler. *Proc. R. Soc. B* **266**, 2075–2081.
14. Schubert M, Schradin C, Rödel HG, Pillay N, Ribble DO. 2009 Male mate guarding in a socially monogamous mammal, the round-eared sengi: costs and trade-offs. *Behav. Ecol. Sociobiol.* **64**, 257–264.
15. Sherman PW. 1989 Mate guarding as paternity insurance in Idaho ground squirrels. *Nature* **338**, 418–420.
16. Edward DA, Stockley P, Hosken DJ. 2015 Sexual conflict and sperm competition. *Cold Spring Harb. Perspect. Biol.* **7**, a017707.
17. Del Matto LA, Macedo-Rego RC, Santos ESA. 2021 Mate-guarding duration is mainly influenced by sperm competition risk and not female quality in a golden orb-weaver spider. *PeerJ* **9**, e12310.
18. Girard-Buttoz C, Heistermann M, Rahmi E, Agil M, Fauzan PA, Engelhardt A. 2014 Costs of and investment in mate guarding in wild long-tailed macaques (*Macaca fascicularis*). *Int. J. Primatol.* **35**, 701–724.
19. Kamimura Y, Yoshizawa K, Lienhard C, Ferreira RL, Abe J. 2021 Evolution of nuptial gifts and their coevolutionary dynamics with female persistence traits. *BMC Ecol. Evol.* **21**, 164.
20. Amano H, Hayashi K. 1998 Costs and benefits for water strider (*Aquarius paludum*) females of carrying guarding males. *Ecol. Res.* **13**, 263–272.
21. Rodríguez-Muñoz R, Bretman A, Tregenza T. 2011 Guarding males protect females from predation in a wild insect. *Curr Biol* **21**, 1716–1719
22. Rizvi T, Tuni C, Reinhold K. 2025 From mating to sperm storage: density-dependent plasticity in pre- and post-copulatory shared mating traits. *EcoEvoRxiv*. https://doi.org/10.32942/X2KH2T

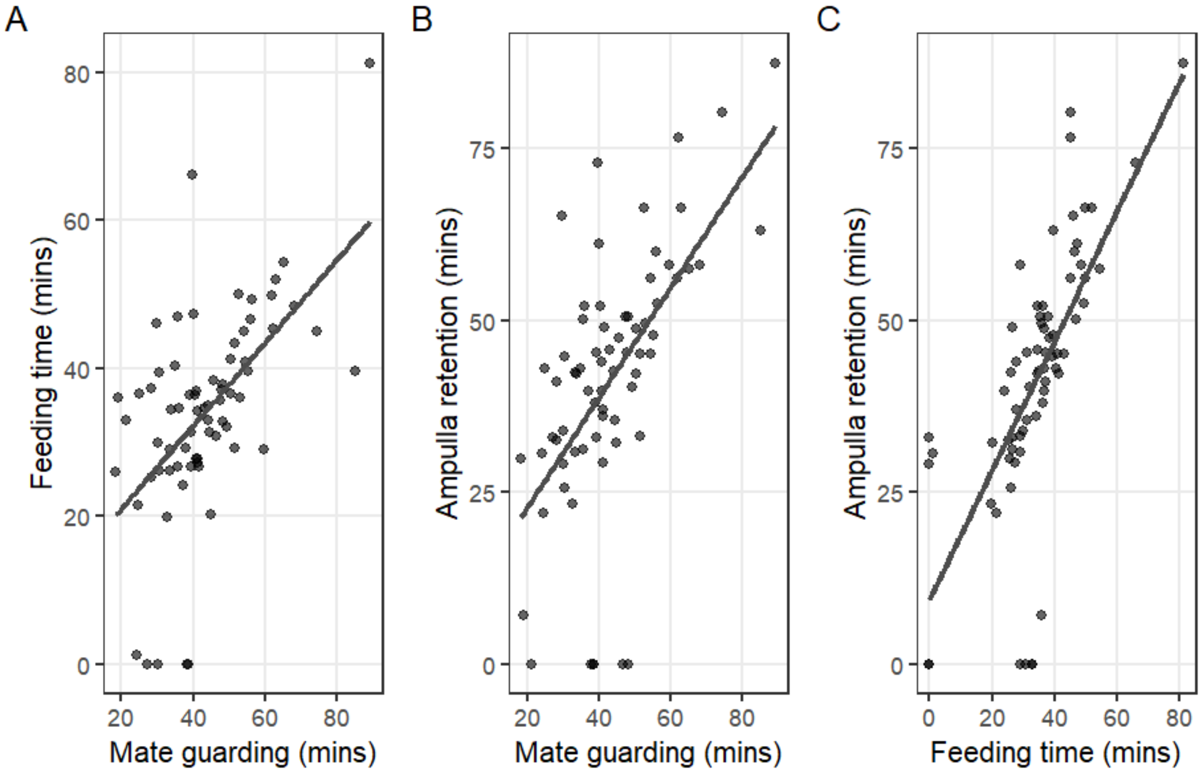
23. Sakaluk SK. 1991 Post-copulatory mate guarding in decorated crickets. *Anim. Behav.* **41**, 207–216.
24. Bateman PW, MacFadyen DN. 1999 Mate guarding in the cricket *Gryllodes sigillatus*: influence of multiple potential partners. *Ethology* **105**, 949–957
25. Foquet B, McKermitt JT, Hunt J, Sadd BM, Sakaluk SK. 2025 Evolution of mate guarding intensity in crickets as a possible adaptation to sexual conflict over sperm transfer. *J. Zool.* **326**, 45–53.
26. Calbacho-Rosa L, Córdoba-Aguilar A, Peretti A. 2010 Occurrence and duration of post-copulatory mate guarding in a spider with last sperm precedence. *Behaviour* **147**, 1267–1283
27. Frankino WA, Sakaluk SK. 1994. Post-copulatory mate guarding delays promiscuous mating by female decorated crickets. *Animal Behaviour*, **48**, 1479–1481.
28. Sakaluk SK, Duffield KR, Rapkin J, Sadd BM, Hunt J. 2019. The troublesome gift: The spermatophylax as a purveyor of sexual conflict and coercion in crickets. In M. Naguib (Ed.), Advances in the study of behavior (Vol. 51, pp. 1–30). Academic Press
29. Bates D, Mächler M, Bolker B & Walker S. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. **67**, 1–48
30. Hartig F. 2022 DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. *J. Open Source Softw.* **7**, 3651.
31. Lenth RV. 2025 *emmeans*: estimated marginal means, aka least-squares means. R package version 1.10.0.
32. Lefcheck JS. 2016 piecewiseSEM: piecewise structural equation modelling in R for ecology, evolution and systematics. *Methods Ecol. Evol.* **7**, 573–579.
33. Kokko H, Rankin DJ. 2006 Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philos Trans R Soc Lond B Biol Sci*. **28**, 319-334
34. Maeno KO, Piou C, Ould Ely S, Ould Mohamed S, Jaavar MEH, Ghaout S, Babah Ebbe MAO. 2021 Density-dependent mating behaviours reduce male mating harassment in locusts. *Proc. Natl Acad. Sci. U.S.A.* **118**, e2108275118.
35. McCullough EL, Buzatto BA, Simmons LW. 2018 Population density mediates the interaction between pre- and postmating sexual selection. *Evolution* **72**, 893–905.
36. Haneke-Reinders M, Mazur AP, Zyma O, Ramm SA, Reinhold K. 2020 Disentangling a shared trait: male control over mate guarding duration revealed by a mate exchange experiment. *Behav. Ecol. Sociobiol.* **74**, 54.
37. Howard RW, Blomquist GJ. 2005 Ecological, behavioral and biochemical aspects of insect hydrocarbons. *Annu. Rev. Entomol.* **50**, 371–393.
38. House CM, Duffield K, Rapkin J, Sakaluk SK, Hunt J. 2024 Transfer of male cuticular hydrocarbons provides a cue of sperm competition risk in decorated crickets. *Evolution* **78**, 1606–1618.

39. Weddle CB, Steiger S, Hamaker CG, Ower GD, Mitchell C, Sakaluk SK, Hunt J. 2013 Cuticular hydrocarbons as a basis for chemosensory self-referencing in crickets. *Ecol. Lett.* **16**, 346–353.
40. Ingleby FC. 2015 Insect cuticular hydrocarbons as dynamic traits in sexual communication. *Insects* **6**, 732–742.
41. Vernier CL, Krupp JJ, Marcus K, Hefetz A, Levine JD, Ben-Shahar Y. 2019 Cuticular hydrocarbon profiles of honey bee workers develop via a socially modulated innate process. *eLife* **8**, e41855.
42. Gordon JM, Šobotník J, Chouvenc T. 2020 Colony-age-dependent variation in cuticular hydrocarbon profiles in subterranean termites. *Ecol. Evol.* **10**, 10095–10104.

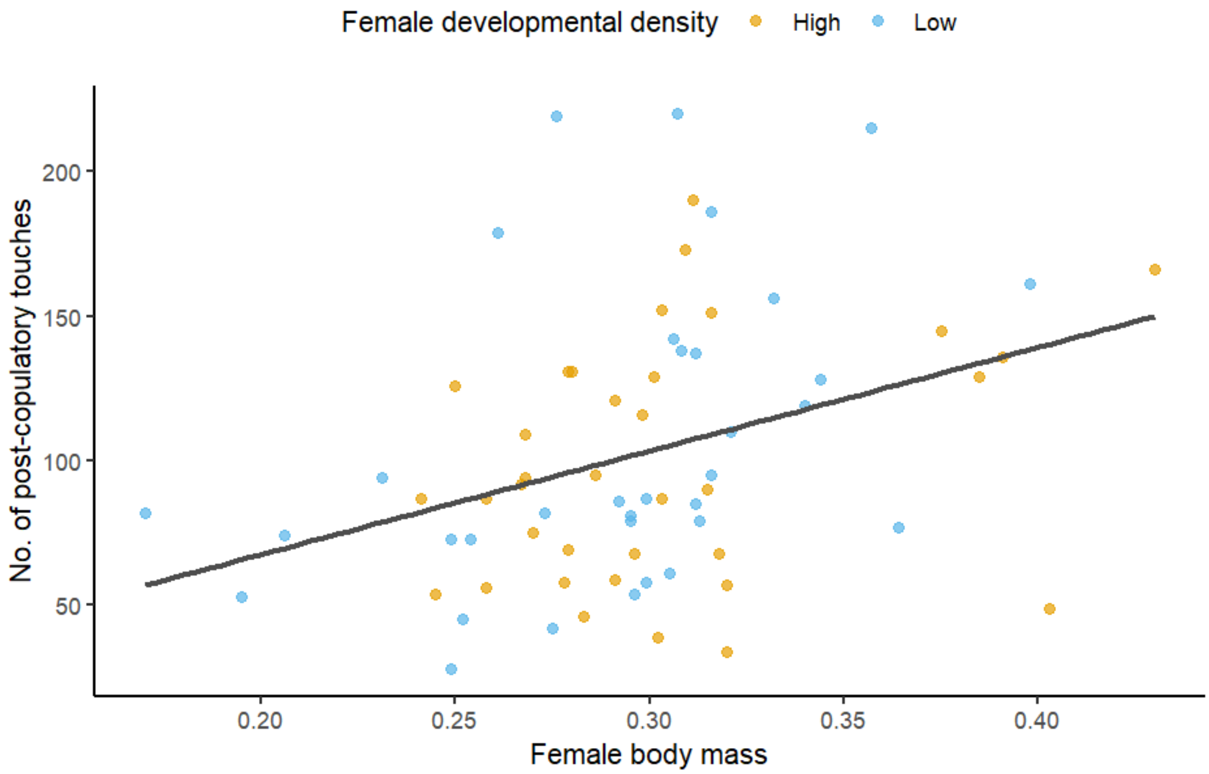
Supplementary Material



S1: 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 post-copulatory mate guarding.



S2: Pairwise relationships among post-copulatory behavioural traits. (A) Mate-guarding duration was positively associated with feeding time (standardised β = 0.59, $p < 0.001$). (B) Mate-guarding duration was also positively associated with ampulla retention time (β = 0.32, $p = 0.005$). (C) Feeding time was positively associated with ampulla retention time (β = 0.55, $p < 0.001$). Points represent individual mating trials, and lines show fitted linear relationships.



S3: Scatterplot showing a positive association between female body mass and post-copulatory touches (PCT), with points coloured by female developmental density. The solid line represents the fitted linear relationship, indicating a positive effect of female body mass on PCT (β = 0.31, *p* = 0.008).

Table S4. Results of the linear model testing the effects of male and female developmental density (high vs. low), body mass, and their interaction on mate-guarding duration. Shown are regression coefficients (Estimate), standard errors (SE), t-values, and p-values.

Predictor	Estimate	SE	t	p-value
(Intercept)	3.302	0.317	10.424	**0.001**
Male density (low)	0.329	0.105	3.124	**0.002**
Female density (low)	0.245	0.107	2.278	**0.026**
Male weight	0.729	1.217	0.598	0.551
Female weight	0.101	0.841	0.120	0.905

Predictor	Estimate	SE	t	p-value
Male density (low) x female density (low)	-0.242	0.149	-1.616	0.111

Table S5. Path coefficients from the piecewise structural equation model (SEM) examining relationships among mate-guarding duration (MG), feeding time on the spermatophylax (FT), ampulla retention time (AR), and post-copulatory touches (PCT). Shown are unstandardized estimates, standard errors (SE), and p-values for all fixed effects included in the component models. Density effects are reported relative to the reference category. Statistically significant effects ($p < 0.05$) indicate key pathways linking mate guarding to post-mating females behaviours.

Response	Predictor	Estimate	SE	p_value
PCT	MG	0.202	0.140	0.154
PCT	MG	-0.178	0.140	0.208
PCT	Male weight	0.024	0.117	0.841
PCT	Female weight	0.305	0.112	**0.009**
PCT	Male density = high	-0.104	0.325	0.795
PCT	Male density = low	-0.040	0.324	0.918
PCT	Female density = high	-0.167	0.327	0.685
PCT	Female density = low	0.023	0.324	0.954
AR	MG	0.318	0.109	**0.005**
AR	FT	0.548	0.109	**0.001**
AR	Male weight	-0.116	0.088	0.193
AR	Female weight	0.122	0.088	0.168
AR	Male density	-0.009	0.119	0.941
AR	Male density = high	0.029	0.123	0.813
AR	Female density = low	-0.011	0.121	0.931

Response	**Predictor**	**Estimate**	**SE**	**p_value**
AR	Female density = high	0.031	0.122	0.799
FT	MG	0.587	0.103	**0.001**
FT	Male weight	0.028	0.102	0.787
FT	Female weight	0.030	0.102	0.766
FT	Male density = low	-0.061	0.138	0.661
FT	Male density = high	0.138	0.143	0.335
FT	Female density = low	-0.156	0.139	0.264
FT	Female density = high	0.234	0.139	0.098