Received: 7 January 2021 | Revised: 14 March 2021 | Accepted: 19 March 2021

DOI: 10.1002/ece3.7719

ORIGINAL RESEARCH

Ecology and Evolution Open Access WILEY

Costly mating delays drive female ornamentation in a capital breeder

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Funding information
Academy of Finland, Grant/Award Number: 294664; Waldemar von Frenckells stiftelse

Abstract

1. How fecundity might be traded off with mate attraction and other aspects of reproduction in females remains poorly understood.
2. We investigated these allocation trade-offs using the common glowworm (*Lampyris noctiluca*), a lampyrid beetle, in which flightless, sedentary females only use resources gathered during the larval stage to attract flying males by glowing.
3. While sexual signaling was not found to have a significant fecundity cost, a delay in successfully attracting a mate greatly increased the risk of reproductive failure, with fecundity losses being more severe in small females.
4. These findings are among the first to show that failure to quickly attract a mate can decrease female fecundity. The results also show how the length of delay before mating can drive the evolution of female sexual ornamentation.

KEYWORDS
capital breeder, delayed mating, fecundity, female ornament, *Lampyris noctiluca*, sex role, sexual selection, signaling cost

1 | INTRODUCTION

Because male mating success usually varies more than that of females, males typically gain more from investment into sexual ornaments (Clutton-Brock, 2007; Kokko & Jennions, 2008; Tobias et al., 2012). Sexual ornamentation is also thought to be much rarer in females because of their lower potential reproduction rates and typically higher overall costs of reproduction (Bateman, 1948; Fitzpatrick et al., 1995; Kokko & Monaghan, 2001), meaning that selection on male and female ornaments can fundamentally differ. In this respect, ornamentation in females is expected to evolve only if its costs are low (Tobias et al., 2012), males provide important resources, male availability is particularly limited, or female–female competition is otherwise intense (Arnqvist & Nilsson, 2000; Gwynne, 1991; Jennions & Petrie, 2007; Kokko & Mappes, 2005; LeBas et al., 2003).

These circumstances are more likely met in animals that reproduce using only stored resources, that is, capital breeders (Houston et al., 2007). In capital breeders, the finite amount of stored resources has to cover the costs of all aspects of reproduction and self-maintenance (staying alive and active) until successful mating and egg deposition. Therefore, capital breeding females are often in more of a rush to mate than income breeders, which may result in competition for males among capital breeding females. Capital breeding can also induce stronger resource allocation trade-offs than income breeding because any investment in signaling (to increase the likelihood of mating) should reduce resources available for boosting fecundity or survival. It is therefore surprising that, as far as we know, no studies to date have

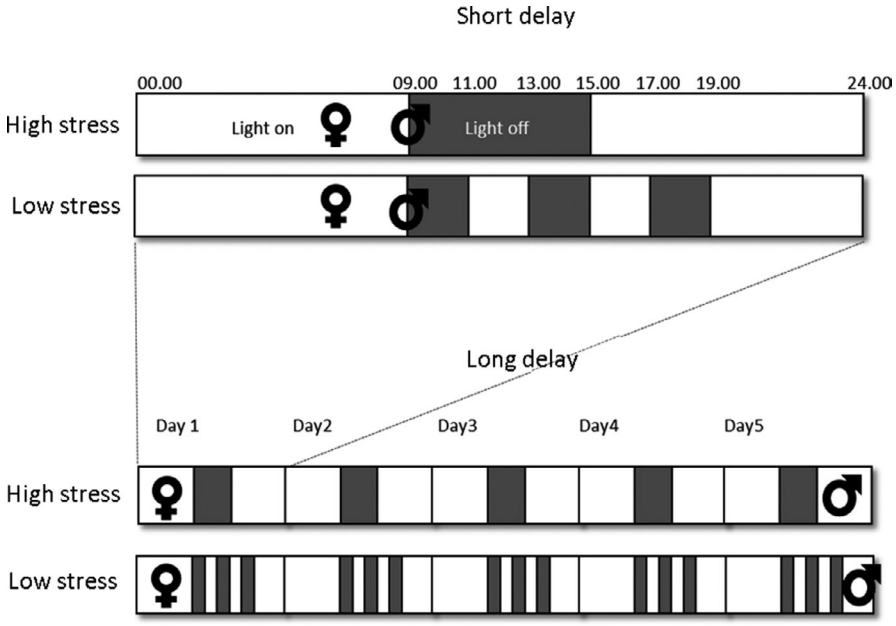

FIGURE 1 Diagram showing the light rhythm in the different treatments of the experiment

addressed the trade-offs between sexual signaling, self-maintenance, and fecundity in capital breeders.

The common glowworm (*Lampyris noctiluca*) is a capital breeding beetle, in which sedentary females emit light from an organ on their abdomen to attract flying, nonglowing males. The benefits of this female ornament are evident: Males use it to locate females, while a bright female is more likely to attract a mate (De Cock et al., 2014; Hopkins et al., 2015; Lehtonen & Kaitala, 2020). The extent of female glowing effort is defined by the time it takes for her to attract a mate, after which she quickly stops glowing, lays all her eggs, and dies (Dreisig, 1971; Tyler, 2002). Female fecundity correlates with body size and typically varies from 25 to over 200 eggs (Hopkins et al., 2015; Tyler, 2002). Some field observations suggest that a majority of females manage to mate during their first or second night of glowing, while longer mating delays are not rare and mating delays up to three weeks have been documented (Dreisig, 1971; Hickmott & Tyler, 2011; Tyler, 2002; personal observations). Female glowworms are not known to receive any nuptial gifts (Lewis & Cratsley, 2008) and are not expected to get any other direct benefits from multiple matings. The act of mating is prolonged and the male usually stays with the female at least until the end of the night (Tyler, 2002; personal observations), which limits opportunities for multiple matings not only in females but also in males. Because glowworms do not eat as adults and the demands of reproduction need to be met by using only the resources gathered during the larval stage, adult females face a potential trade-off between the different aspects of reproduction, including survival until successfully attracting a mate.

Besides the risks and costs related to the length of time passed until mating, glowing effort during that time is another cost to glowworm females, given that glowing requires some energy (Woods et al., 2007). In addition, switching the glow on and off might be costly. One potential mediator of these costs is the use of energy from unlaid eggs (see Wing, 1989). Therefore, we expected that both a costly signaling environment (and/or an increased signaling output) and a time delay before mating could decrease fecundity and that larger females may be more capable of coping with these costs. Accordingly, we experimentally tested the potential trade-offs between fecundity, sexual signaling, and self-maintenance in females of different sizes. This was done by investigating female fecundity in response to experimental exposure to delayed mating and temporally patchy light conditions, with the latter presumably inducing an increased signaling effort.

2 | MATERIALS AND METHODS

2.1 | Study site and collection of specimens

Females for the study were collected as adults in several sites around Tvärminne Zoological Station (N59°50′, E23°15′) and a lakeside in Lohja (N60°12′, E24°0′), southern Finland, in June and July 2015. This was done by one to two persons walking at night in areas with prior glowworm sightings and collecting all the females they were able to find. All collected females were used in the experiment. Because females usually cease glowing soon after they have mated, and lay eggs (and then die) soon after (Dreisig, 1971; Tyler, 2002), it is unlikely that any of the collected females had mated (or laid eggs) prior to capture. Because females were sampled every night in the same locations, it is unlikely that any female collected for the study had glowed on any night before it was collected. We also collected 71 males for the experiment using funnel traps, each with a green LED as a lure (Hopkins et al., 2015). Whether males had mated earlier was not known.

2.2 | Experimental setup

Adults were brought to the laboratory, located at the field station, where the experiment was started immediately. The laboratory had

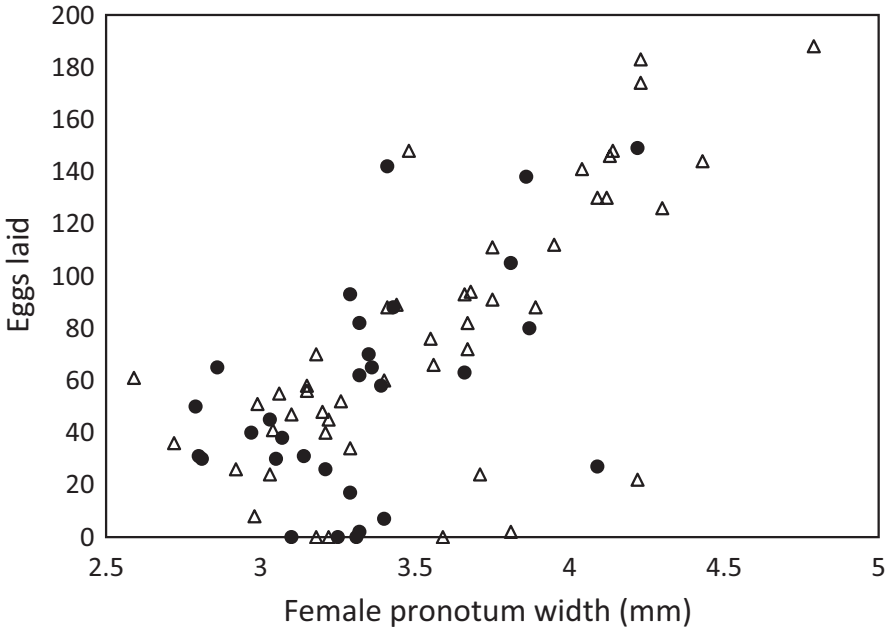

FIGURE 2 Number of eggs glowworm females laid after mating with respect to female size (pronotum width) in the short (closed circle, solid regression line) and long (open triangle, dotted regression line) delay treatments

a temperature of ~20–24°C and ambient indoor humidity. Each individual was kept singly in 7 cm × 7 cm × 10 cm plastic containers with damp paper towels to hide in. To study resource allocation tradeoffs between signaling, other aspects of reproduction, and self-maintenance, we measured fecundity (the number of eggs) of focal females in a 2 × 2 factorial design with two treatment categories, signaling effort and mating delay, both with two levels.

The signaling effort treatment involved manipulation of the number of dark periods the focal female experienced within each 24-hr cycle. In particular, females typically glow for ~2 hr during the darkest period of the night, or until finding a mate (Dreisig, 1971; Tyler, 2002; personal observations). Females assigned to the low effort treatment were kept in a 18:6-hr light:dark rhythm (Figure 1), which corresponds to the natural day:night cycle in the region at the time of the study. The high effort treatment, in turn, had 3 separate periods of 2 hr of darkness (light:dark cycle of 14:2:2:2:2:2, Figure 1). We expected this signaling environment to increase signaling output, induce stress, or both.

To assess the effect of delayed mating, we also had a mating delay treatment with two levels. In the short delay treatment, a haphazardly chosen male was placed in the same container with the female at the start of each replicate (Figure 1). Males do not usually mate with nonglowing females (personal observations) so it is unlikely that mating took place prior to the start of the dark period. In addition, females stop glowing soon after mating (Dreisig, 1971; Tyler, 2002; personal observations). In the long delay mating treatment, the focal female was given a male, as described above, but only at the end of the fifth 24-hr cycle (Figure 1). Females that laid eggs before being provided a male, or died during the experiment, were disregarded (both cases indicating that the female had probably mated before the start of the replicate). The sample sizes for successful replicates were as follows: $n_{\text{low effort, short delay}} = 15$, $n_{\text{low effort, long delay}} = 25$, $n_{\text{high effort, short delay}} = 15$, and $n_{\text{high effort, long delay}} = 22$. Females were randomly assigned to the treatments.

In all treatments, females were monitored throughout the dark periods and their brightness was estimated at approximately five-minute intervals by comparison to LEDs of known brightness, allowing us to estimate both the duration and brightness of their glow. The investigator aimed for maximal objectivity, but due to the nature of the setup, was not blind to group allocations during these assessments. We also counted the number of eggs laid by each female during the experiment. Females died soon after laying their eggs, and once they had died, we measured the pronotum (dorsal exoskeletal plate) width of each female and counted any eggs that might have been viable but not laid by the female. The total glowing output of each female was estimated by using the length of time it was observed to glow and its estimated brightness at each measurement. We calculated the average brightness of two consecutive measurements and multiplied it by the amount of time between the measurements. Then, we summed all such values together for each female. This allowed us to estimate the total glowing output as μmol photons × m^{-2}

2.3 | Statistical analysis

Analyses were conducted in R 3.2.3. As appropriate for overdispersed count data, we applied a generalized linear model (GLM) with a negative binomial distribution ("glm" function, with the parameter theta calculated using the "glm.nb" function of the "MASS" package) to assess the treatment and body size effects on the number of eggs laid after mating. In particular, the initial model included effort treatment, mating delay treatment, and body size (pronotum width) and all three first-order interactions. We then simplified the model by removing nonsignificant interaction effects, using $\alpha = 0.10$.

Of the 77 females, 10 experienced a breeding failure in the sense that they laid fewer than 10 eggs after mating. We chose <10 as the definition for a failure because it corresponded with a gap in egg numbers in our dataset (see Figure 2) and because the literature suggests that females typically lay at least 20 eggs (Hopkins et al., 2015; Tyler, 2002). Our conclusions (see the results) remain the same if we use 20 (or any number lower than 20) as the definition. We used Barnard's exact test ("Barnard" package) to analyze whether the treatments differed in the occurrence of breeding failures. This analysis was done separately for signaling effort and mating delay treatments.

Finally, we assessed whether the signaling effort treatment significantly affected female glowing output. This was done by comparing the measured glowing out in the two signaling effort treatments with a Kruskal–Wallis test.

3 | RESULTS

The "effort treatment × mating delay treatment" (GLM comparison, $\chi^2 = -0.960$, $df = 1$, $p = .82$) and "effort treatment × pronotum width" (GLM comparison, $\chi^2 = -3.59$, $df = 1$, $p = .65$) interaction effects were nonsignificant, and the model was refitted without them. While the effort treatment did not have a significant effect on the number of eggs a female laid after mating (GLM, $t_{72} = -0.102$, $p = .92$), the mating delay treatment × pronotum width interaction did (GLM, $t_{72} = -2.84$, $p = .006$). In particular, the fecundity of small females was more affected by delayed mating than that of large ones (Figure 2). These results remain qualitatively the same if we use the total egg number (i.e., the number of eggs successfully laid after mating + the number of any eggs not laid) as the response variable. In particular, the simplified model in this case also showed a significant mating delay treatment × pronotum width interaction (GLM, $t_{72} = -2.25$, $p = .028$).

Five out of 40 females in the low effort treatment and 5 out of 37 females in the high effort treatment laid fewer than 10 eggs (Figure 3), implying no significant difference between the two effort treatments (Barnard's test, $p = .95$). None of the 30 females in the short delay treatment laid fewer than 22 eggs (Figure 2), while ten out of 47 females in the long delay treatment laid fewer than ten eggs (of which six did not lay any, Figure 2). This implies that females had a significantly higher likelihood of breeding failure in the long than short mating delay treatment (Barnard's test, $p = .007$). All females that suffered reproductive failure were relatively small, having pronotum widths of 3.82 mm or less (Figure 2).

The signaling effort treatment did not have a significant effect on the measured glowing output (Kruskal–Wallis test, $\chi^2 = 0.077$, $df = 1$, $p = .78$). The conclusions regarding fecundity (the number of laid eggs) remain qualitatively the same if we use the glowing output, instead of the effort treatment, in the models.

4 | DISCUSSION

Our results show that while the signaling effort treatment did not have a significant effect on the number of eggs laid, delayed mating did. This was especially the case for small individuals, as demonstrated by a significant interaction between the effects of the delayed mating treatment and body size. The result suggests that the cost of delayed mating can be an important driver of female sexual ornamentation. A mating delay of a duration that is relatively common in the wild (Dreisig, 1971; Tyler, 2002; personal observations) can have a catastrophic outcome to the glowworm female, as demonstrated by a relatively high proportion of females experiencing

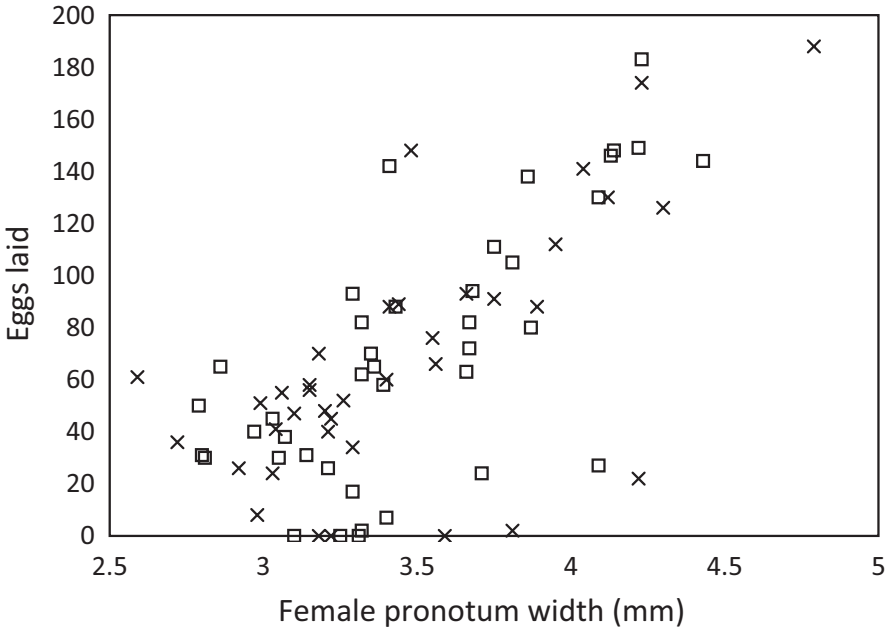

FIGURE 3 Number of eggs glowworm females laid after mating with respect to female size in the high effort (squares, solid line) and low effort (crosses, dotted line) treatments

complete reproductive failure when mates were unavailable for only five days.

We found that the costs of mating delays were higher for small females. This could be due to females needing to trade-off between eggs, signaling, and self-maintenance. Because glowworms are capital breeders, feeding to replenish lost resources is impossible. Small females are likely to have both fewer eggs and smaller energy reserves (Honěk & Honek, 1993; Hopkins et al., 2015; Tyler, 2002), and therefore, any trade-offs should be much more marked in them. Regardless of the physiological mechanism, our results suggest that delayed mating affects small females more than large females. This effect is amplified by the fact that small females are less attractive to males in many species (Bateman & Fleming, 2006; Byrne & Rice, 2006; Rosenqvist & Berglund, 2011; Svensson et al., 2010), including glowworms (Hopkins et al., 2015), and therefore likely to experience longer mating delays.

Our signaling effort treatment did not have a significant effect on fecundity. The result suggests that signaling poses lower costs to capital breeders than mating delays do. The result is in accordance with the prediction that female ornaments should be associated with low costs (Tobias et al., 2012). The finding is also in line with the earlier estimate that glowing increases energy use by 37% in a related species (compared to over 50% for walking) (Woods et al., 2007). While this may seem to be a large increase, given that females in our experiment had an opportunity to glow for a maximum of six hours a day, the increase would be equivalent to roughly two and a quarter hours of extra time per day before mating (6 hr × 0.37 increase = 2.22 hr), or less than half an extra day of self-maintenance during the course of five days in the long delay treatment.

We show that while glowing does increase energy use, the costs of extended self-maintenance (staying alive for a longer period without mating) clearly outweigh the costs of trying to attract males. Similarly, a female ornament has been found to be inexpensive enough to be positively associated with fecundity in some species that are not capital breeders, such as the wide-bodied pipefish (*Stigmatopora nigra*) and the fowl (*Gallus gallus*) (Cornwallis & Birkhead, 2007; Mobley et al., 2018). It is worth noting that our signaling effort treatment that manipulated the light:dark cycle may have had only very limited effect on signaling costs and/or effort, at least within the time frame females were observed. In particular, the treatment did not significantly affect the length of the overall time females were seen glowing, indicating that a temporally patchy, presumably stressful, glowing environment has limited effects on the female glow. Similarly, the costs of turning the signal on and off are currently not known and may be lower than what we assumed.

5 | CONCLUSION

To conclude, our results indicate that besides the costs of developing and using sexual signals, also those linked to the time passed until successful mating can be important in the evolution of female sexual ornamentation, especially in capital breeders. The findings imply that the evolution of female sexual ornaments may in part be driven by the need to reduce premating time, with the cost of mating delays being particularly harsh for small individuals. Our results therefore suggest that future considerations of optimal reproduction strategies should include costs of mating delays and trade-offs in mate attraction, especially as experienced by capital breeders and females of different sizes.

ACKNOWLEDGMENTS

This study was funded by the Academy of Finland (Grant 294664) and Waldemar von Frenckells stiftelse. The authors thank Phill Watts, Laura Härkönen and anonymous reviewers for valuable comments to the manuscript.

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTION

Juhani Hopkins: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Writing-original draft (equal); Writing-review & editing (equal). **Gautier Baudry:** Conceptualization (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Writing-review & editing (equal). **Topi K. Lehtonen:** Formal analysis (equal); Investigation (equal); Visualization (equal); Writing-review & editing (equal). **Arja Kaitala:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Supervision (equal); Writing-review & editing (equal).

DATA AVAILABILITY STATEMENT

The full dataset for this study is available via Dryad (https://doi.org/10.5061/dryad.8931zcrqk).

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How to cite this article: Hopkins J, Lehtonen TK, Baudry G, Kaitala A. Costly mating delays drive female ornamentation in a capital breeder. *Ecol Evol*. 2021;11:8863–8868. https://doi.org/10.1002/ece3.7719