

Does foreplay matter? *Gammarus pulex* females may benefit from long- lasting precopulatory mate guarding

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Precopulatory mate guarding (PCMG) is generally assumed to be costly for both sexes. However, males may gain by displaying long-lasting mate guarding under strong male–male competition. Surprisingly, the potential for females to benefit from being held by males has been largely overlooked in previous studies. In *Gammarus pulex*, an amphipod crustacean, PCMG lasts several weeks, yet females are described as bearing only cost from such male mating strategy. We investigated potential female benefits by assessing the effect of mate guarding on her intermoult duration. Unpaired females had longer intermoult duration than paired females. Intermoult duration clearly decreased when paired females engaged in early and long-lasting mate guarding. In addition, short intermoult and long-lasting mate guarding had no effect on egg number. These results highlight a potential benefit associated with PCMG for *G. pulex* females, suggesting that the strength of an intersexual conflict over its duration may be overestimated.

Keywords: amplexus; precopula; intermoult duration; sexual conflict

1. INTRODUCTION

In many crustacean species, males are in competition for access to females which are only receptive for a short period of time following their reproductive moult. This, associated with a lack of female reproductive synchrony, is assumed to be responsible for the evolution of precopulatory mate guarding (PCMG, also called precopula or amplexus [1]) during which a male generally grabs a female. This behaviour is considered to be costly for both sexes although some costs are sex-specific. Males, for instance, endure reduced mating opportunities (reviewed in Jormalainen [2]), reduced foraging efficiency [3], higher drift in currents [4] and injuries resulting from interference with other males [5]. Females, on the other hand, suffer increased cannibalism by males [6]. Other costs are endured by both sexes and may have limited effects on fitness asymmetry between sexes. Most notably, higher predation risk [7] and energy deprivation [5,8] are expected when paired. More obvious fitness benefits related to

long-lasting PCMG exist, but, to our knowledge, are solely described for males. In populations with a male-biased operational sex-ratio [9] and, thus, a strong male–male competition, early guarders gain mating advantages [1,10]. Surprisingly, no study has thus far investigated potential fitness benefits for females engaged in long-lasting amplexus. As a consequence, males are assumed to display long amplexus to ensure copulation, while females are presumed to prefer short precopula to avoid associated costs [2]. Consequently, PCMG is typically assumed to lead to intersexual conflict over its duration [11].

In *Gammarus pulex*, a freshwater amphipod, a male guards a female during her intermoult (time between two moults) before she becomes receptive for copulation. Species exhibiting strong intersexual conflict over PCMG duration are usually characterized by short PCMG periods and female resistance to males' early guarding attempts [2]. On the contrary, *G. pulex* amplexus durations are surprisingly long-lasting (up to 20 days), while females do not seem to exhibit any adaptations to shorten it [12]. Most studies have typically focused on costs incurred by females (reviewed in [2]), thereby ignoring the possibility that long-lasting PCMG may also be beneficial for them. This study explores the potential benefits of PCMG for *G. pulex* females. We tested for PCMG effects on female intermoult duration (i.e. delay between two reproductions) and discussed our results in the context of intersexual conflict over precopula duration.

2. MATERIAL AND METHODS

Using kick sampling and a hand net [13], individuals were collected once a week between 18 March and 22 April 2009 in the Suzon river in Burgundy (N: 47°24,215'; E: 4°52,974') and immediately taken to the laboratory. Couples were isolated and maintained in 100 ml cups under a constant photoperiod (12 : 12 h) in ultraviolet-treated water at 15°C. Five days after moult, each female was assigned to one of the three treatment groups: (i) 121 females were individually put with a male previously in PCMG with a different female; (ii) 22 females were housed with a male (previously in PCMG) that had had its gnathopods removed, thereby preventing amplexus [14]; and (iii) 42 were placed alone in cups. These three treatment groups were maintained simultaneously in the controlled conditions described above. Each cup was checked daily for female moult by the presence of an exuvium. Intermoult duration (number of days between two consecutive moults), number of days spent in PCMG and egg number were recorded. Male and female body size were estimated after female moult by measuring the fourth coxal plate [15] using a Nikon SMZ-10A stereoscopic microscope and a VTO 232 video-measure system from Linkam Scientific Instruments Ltd.

Every female moulted during the experiment. Among the 121 females of the first treatment, only 105 engaged in amplexus. For the purpose of statistical analysis, females were assigned to one of three categories: females (P) observed in precopula for at least one day ($n = 105$); females (NP) with a male never observed in precopula ($n = 38$); and single females (S) ($n = 42$). Females from the three categories did not significantly differ in size ($F_{2,182} = 2.96$, $p = 0.054$) or date of collection ($\chi^2_{1,183} = 0.41$, $p = 0.52$).

A Cox proportional hazards regression [16] was performed to assess the effect of the three categories and female size on female intermoult duration. PCMG was considered to be discontinuous when the female was observed at least one time alone since the beginning of PCMG. Using P females solely, a second Cox regression assessed the effect of female size, continuous nature of PCMG, with either time to first PCMG or PCMG duration fitted as covariates, on female intermoult duration. Schoenfeld residuals were examined to assess proportional hazards assumption of the Cox regressions [17]. We used a multiple linear model to test for the effect of male and female size, with either intermoult duration, time to first PCMG, PCMG duration or continuous nature of PCMG fitted as covariates, on female egg number. Homogeneity of variance was verified using a Bartlett test. A Shapiro–Wilks' test was performed to assess the normality of the residuals.

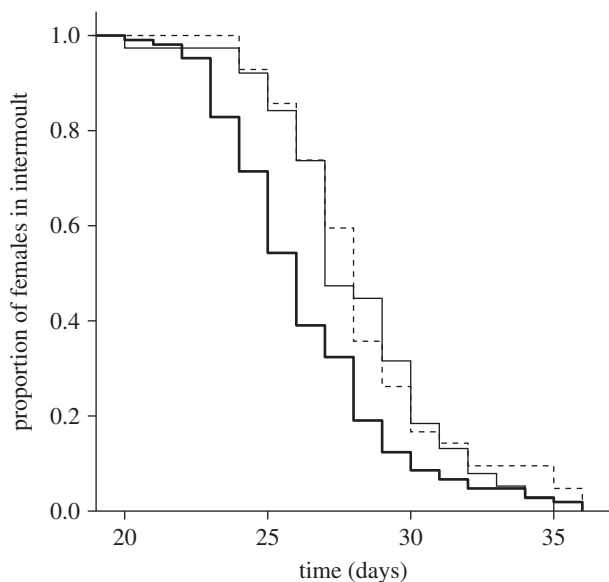


Figure 1. Proportion of females in intermolt as a function of time for the three categories of females. Bold line: P, females in precopula ($n = 105$); thin line: NP, females with a male without precopula ($n = 38$); dashed line: S, single females ($n = 42$).

3. RESULTS

Larger females showed longer intermoult ($\chi^2_{1,183} = 13.12$, $p < 0.001$). There was also a strong effect of female categories (P, NP or S) on their intermoult duration (Cox regression, $\chi^2_{2,182} = 14.86$, $p < 0.001$; figure 1). Intermoult duration of females in PCMG (26.3 ± 3.1 days) was on average 2 days shorter than those of S females (28.4 ± 3 days, contrast post hoc test, $z = 2.63$, $p < 0.01$) and NP females (28.1 ± 3 days, $z = 3.32$, $p < 0.001$) which showed similar intermoult duration ($z = 0.77$, $p = 0.44$). Everything else being equal, females displaying PCMG increased their probability of moulting by 62 per cent (exponentiated regression coefficient 1.62) when compared with single females.

P females spent on average 7.1 ± 3.4 days in PCMG (range: 1–18). Sixty-one per cent of females were guarded without interruption, but there was no difference in total time spent in PCMG between females engaged in continuous or discontinuous PCMG ($F_{1,103} = 0.98$, $p = 0.32$). The continuous or discontinuous nature of PCMG did not have an effect on female intermoult duration ($\chi^2_{1,103} = 0.56$, $p = 0.45$). Female intermoult duration was shortened by both early PCMG initiation ($\chi^2_{1,103} = 28.75$, $p < 0.001$) and long-lasting PCMG ($\chi^2_{1,103} = 5.79$, $p < 0.05$) but there was no effect of male size ($\chi^2_{1,103} = 1.16$, $p = 0.28$). On the other hand, the longer the time before first amplexus, the shorter the PCMG ($F_{1,103} = 87.6$, $p < 0.001$). Larger females carried more eggs in their brood pouch after fertilization than smaller ones ($F_{1,103} = 19$, $p < 0.001$) and none of the other variables tested had an effect on egg number (intermoult duration: $F_{1,103} = 0.59$, $p = 0.441$; PCMG duration: $F_{1,103} = 1.3 \times 10^{-5}$, $p = 0.99$; PCMG continuous nature: $F_{1,103} = 0.41$, $p = 0.52$; time to first PCMG: $F_{1,103} = 0.14$, $p = 0.70$; male size: $F_{1,103} = 0.90$, $p = 0.34$).

4. DISCUSSION

Paired females showed shorter intermoult when compared to unpaired ones. In addition, their intermoult were shortened by both early and long-lasting PCMG, without decreasing the number of eggs laid. Intermoult duration directly reflects female reproductive time-out in *G. pulex*. Females with short intermoult have higher mating rates. Early and long-lasting PCMG may then confer potential benefits to females. Further investigations are still required to clarify to what extent it is related to higher lifetime reproductive success. More generally, it is still not clear whether high mating rate contributes positively to overall female fitness [18]. We do not know if PCMG has an effect on other female components of fitness such as survival or fecundity. It could have a deleterious effect on vitellogenesis and thus on offspring number and survival [2]. However, in our experiment, PCMG had no effect on egg number, which is the first assessment of *G. pulex* female fecundity. A recent study on another amphipod species even suggests that pairing behaviour could reduce predation risk [7]. More generally, these results are consistent with previous findings of Ward [19]. Early PCMG has also been shown to shorten female intermoult duration in other crustacean species (*Armadillidium vulgare* [20], *Paracerceis sculpa* [21] and *Eogammarus oclairi* [22]), although there is no evidence whether it has an effect on female reproductive success.

Potential benefits gained by female *G. pulex* during PCMG argue against a strong intersexual conflict over precopula duration, as both males and females should favour early and long-lasting PCMG. Besides, in species where a clear conflict exists, females generally resist early guarding attempts [23]. In *G. pulex*, resistance displayed by females has been interpreted as a form of mate choice [24,25] rather than a way to shorten PCMG duration [12,26]. We suggest that future studies should focus on females' potential benefits from long-lasting PCMG. Precise evaluation of fitness costs and benefits for both sexes, especially regarding energy intake of paired and single individuals, should clarify the potential for intersexual conflict over PCMG duration.

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