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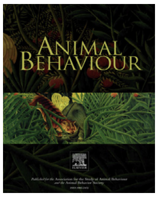

The curse of being single: both male and female *Gammarus pulex* benefit energetically from precopulatory mate guarding

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Precopulatory mate guarding (PCMG) is frequently presented as a classic case of sexual conflict between partners. For instance, long-lasting PCMG is regarded as an adaptive male strategy to secure a female in a context of strong intrasexual competition, while females guarded for a long time are assumed to bear many costs. This assumption has been derived from guarding systems where females obviously resist males' attempts to initiate early guarding. However, females of some species such as the freshwater amphipod *Gammarus pulex* do not seem to possess adaptations to reduce PCMG duration, which remains to be explained from an evolutionary perspective. In this model organism for sexual conflict research, a male grasps a female several days before her sexual receptivity. Here we tested the hypothesis that *G. pulex* females might benefit from being passively transported by their partner during PCMG, whereas the male alone bears the costs of swimming while carrying his mate. We therefore compared the energetic states of paired and single individuals and found that, after 5 days of PCMG in controlled conditions, paired individuals contained more protein, lipid and glycogen reserves than single individuals in both sexes. Our results suggest that PCMG might be energetically beneficial not only to the female, but also to the male. We discuss overall fitness consequences of PCMG for both partners given the mutual benefits we highlighted here. We plead for a more precise estimation of the cost/benefit ratio for each sex to improve our understanding of how sexual conflict shapes guarding patterns.

In arthropods as well as vertebrates, reproduction may involve precopulatory mate guarding (PCMG, also called amplexus or precopula), during which a male monopolizes a female until copulation (Bowcock, Brown, & Shine, 2009; Jormalainen, 1998). PCMG is particularly widespread when the number of sexually active males exceeds the number of females available for reproduction (Parker, 1974). In many species (especially among crustacean taxa such as amphipods and isopods), the male-biased operational sex ratio results from the short period of sexual receptivity of females, which typically lasts a few hours after their reproductive moult (Jormalainen, 1998; Parker, 1974). Consequently, receptive females are rare at any time, whereas almost all mature males are sexually active.

The evolutionarily stable strategy in response to such male–male competition is to precociously pair with a female long before her sexual receptivity (Grafen & Ridley, 1983; Jormalainen & Merilaita, 1993; Jormalainen, 1998). In that context, PCMG is defined as the time a male is willing to spend guarding a female before copulation instead of searching for another one (Yamamura & Jormalainen, 1996). It was theoretically shown that a male able to rely on slightly longer PCMG than his competitors will access a larger number of unguarded females, hence improving his chance to secure reproduction (Grafen & Ridley, 1983). Males are thus expected to irreversibly increase PCMG duration (Jormalainen, 1998; Jormalainen, Tuomi, & Yamamura, 1994; Yamamura & Jormalainen, 1996). However, the male optimal guarding duration is also constrained by associated costs, such as loss of other opportunities of reproduction (Parker, 1974) or decreased food intakes (Robinson & Doyle, 1985). Guarded females are, on the other hand, expected to receive no benefits and rather only incur costs from such a male-competitive strategy. Several studies have put forward higher energetic expenditure as a prominent cost for guarded

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females, for instance due to the transport of a passive male during PCMG in the water strider *Aquarius remigis* (Watson, Stallmann, & Arnqvist, 1998). Additionally, energetic depletion could result from reduced food intake documented for females and hermaphrodites engaged with a male in PCMG (Benvenuto & Weeks, 2012; Bowcock et al., 2009). Other costs endured by guarded females include increased vulnerability to some predators, as shown in the amphipod *Hyalella* sp. (Cothran, 2004) or reduced locomotor efficiency, as shown in the cane toad, *Bufo marinus* (Bowcock et al., 2009). These costs increase proportionally with the guarding duration. Optimal guarding duration is thus predicted to be shorter in females than in males (Jormalainen, 1998; Jormalainen et al., 1994; Yamamura & Jormalainen, 1996).

Because of this asymmetry between partners in the costs and benefits associated with PCMG, the duration of PCMG is now widely accepted to result from the conflicting interests of both partners. In other words, it can be considered as an example of intersexual conflict (Jormalainen, Merilaita, & Härdling, 2000; Plaistow, Bollache, & Cézilly, 2003; Yamamura & Jormalainen, 1996). This contrasts with early approaches considering PCMG as a purely male decision-making problem (Parker, 1974). Progress in the conceptual framework dealing with the evolution of PCMG can be achieved with additional information concerning females' gains resulting from this reproductive behaviour. A precise evaluation of the relative costs and benefits of PCMG for both partners is therefore needed to estimate more accurately the intensity of sexual conflict and how it acts as a selective pressure shaping the evolution of mate-guarding strategies (Chapman, Arnqvist, Bangham, & Rowe, 2003; Cothran, Chapman, Stiff, & Relyea, 2012; Karlsson Green & Madjidian, 2011). Recently, there has been mounting evidence that females' gains associated with PCMG could be more common than initially supposed. For instance, females experiencing long-lasting PCMG show reduced predation risks in the wild cricket *Gryllus campestris* (Rodríguez-Muñoz, Bretman, & Tregenza, 2011), but also increased reproductive rates in the freshwater amphipod *Gammarus pulex* (Galipaud, Dechaume-Moncharmont, Oughadou, & Bollache, 2011). As these latter authors argued, assessments of the effect of PCMG on the energetic budget should help researchers to understand more deeply the fitness consequences for both partners, especially for the female's reproductive output.

Gammarus pulex is a model organism for the study of sexual conflict (Galipaud et al., 2011; Plaistow et al., 2003). In this freshwater amphipod, males start initiating guarding when females are undergoing vitellogenesis (e.g. egg biosynthesis). Females can be inseminated within the 12 h following their reproductive moult, after which eggs are moved to a ventral brood pouch where they are fertilized and incubated until hatching (Jormalainen, 1998; Sutcliffe, 1992). For three main reasons, this species is a good candidate to put forward the link between benefits for guarded females, intensity of sexual conflict and the evolution of the related mating strategies. First, PCMG duration is much longer in *G. pulex* than in most other related taxa exhibiting PCMG (Jormalainen, 1998). In laboratory conditions, *G. pulex* amplexus last 217 h at 10 °C (up to 600 h at 1 °C). This remains relatively longer than closely related isopod species (*Idotea baltica*: 34–46 h; *Asellus aquaticus*: 104 h; *Lirceus fontinalis*: a few days) and amphipod species (*Gammarus zaddachi*: 100 h; *Gammarus lawrencianus*: 91 h; *Hyalella azteca:* 26–115 h; Jormalainen, 1998). From field observations, Birkhead and Clarkson (1980) reported that PCMG in *G. pulex* could even last up to 20 days, which remains to be explained from an evolutionary perspective. Second, *Gammarus* females lack the behavioural adaptations (female resistance behaviour in response to male pairing attempts) observed in other crustacean taxa to shorten PCMG duration, which seems to contradict the classic evolutionary outcomes of a sexual conflict (Birkhead & Clarkson, 1980; Jormalainen & Merilaita, 1995; Yamamura & Jormalainen, 1996). Third, paired females of this species are passive and transported by the male during PCMG. Indeed, it has been shown that the swimming performance of the pair is positively related to the size of the male relative to the size of the female. This implies that only the male contributes to the locomotion effort of the pair, the female being just a load when the pair swims (Adams & Greenwood, 1983). We therefore hypothesized a sex-specific energetic cost arising from the pair locomotion in *G. pulex*: the female should suffer lower energetic losses than the male. The female could even benefit from being carried during PCMG through energetic savings. Surprisingly, only one study has examined the energetic consequences of PCMG in this species, and focused only on males, revealing absolute energetic losses for those that were guarding, attributed to pair formation (Plaistow et al., 2003). However, this study did not compare the energetic reserves of single and paired individuals. Such a comparison is now needed to explore more deeply the energetic consequences of PCMG in this species.

Here, we investigated the impact of PCMG on the energetic states of both sexes in *G. pulex* by comparing single and paired individuals. Owing to their passive transport by males, we expected guarded females to save energy compared to single females. This should translate into higher lipid and protein contents, as these metabolites are stored during vitellogenesis, the crucial stage of egg production (Sutcliffe, 1992). On the other hand, paired males actively transporting their partners are thought to bear alone the energetic costs of the pair's locomotion, affecting glycogen and soluble carbohydrates, which are known to fuel short-term activities such as locomotion (Plaistow et al., 2003).

METHODS

Ethical Note

This work followed the ASAB/ABS guidelines for the treatment of animals in behavioural research. Information about individuals' origin, collection, housing conditions and killing are described below. Transport between sampling site and laboratory, as well as daily monitoring of experimental units, were devised to reduce stress and maximize animals' welfare.

Biochemical Assays

Precopula pairs of *G. pulex* were collected on May 2016 in the River Suzon (Burgundy, France). As energy reserves of females may vary with their reproductive status, we visually selected only pairs whose females were undergoing vitellogenesis, with black dorsolateral ovaries (Sutcliffe, 1992). Each pair was gently split and the resulting single individuals were randomly placed under controlled conditions (14 °C, 12:12 h light:dark cycle), either alone (40 males or 40 females) or with their former partner (240 pairs) in glass crystallizers (diameter = 6 cm, height = 4.5 cm) filled with 70 ml of water coming from the sampling site. No food was provided to avoid the potential compensation of PCMG costs through energy intakes. The pairing status of each individual was monitored four times a day for 5 days. This duration was determined by preliminary experiments: it was sufficiently long to observe an effect of the pairing status on energy budgets of both sexes, but sufficiently short to limit the risk of missing data due to pairs splitting after copulation. Individuals that did not pair within the first 30 min of the experiments ($N = 44$) or that split before the end of the 5 days ($N = 135$) were excluded from molecular analyses. We also excluded individuals that moulted ($N = 46$ pairs and one single female), as ecdysis strongly influences the body reserves in gammarids (Charron et al., 2014). At the end of the 5 days, 61 pairs, 39 isolated females and 40 isolated males were used for energetic assessments. Each individual was blotted dry, frozen at −80 °C and weighed (±0.1 mg) with a microbalance Sartorius Quintix 35-1S before biochemical assays of four metabolites potentially involved in energy production: proteins, lipids, glycogen and soluble carbohydrates. The total amount of available soluble proteins was estimated using a DC Protein Assay kit (Bio-Rad, Hercules, CA, U.S.A.), and the other measurements (lipids, glycogen and soluble carbohydrates) were based on the colorimetric technique described by Foray et al. (2012). We ran two replicates per individual to assess organic compounds and we excluded individuals for which the coefficient of variation exceeded 25%. The repeatability of these measurements was high, since we retained 87% of the individuals in the protein data set, 96% in the lipid data set, 99% in the glycogen data set and 97% in the soluble carbohydrate data set. All the chemical measurements were expressed in mass per unit of individual mass (μg/g).

Activity Scores and Body Size Measurements

The difference in energetic states between paired and unpaired individuals may result from differences in locomotor activity. Following the method of Bollache, Kaldonski, Troussard, Lagrue, and Rigaud (2006), we thus compared the activity scores of 37 pairs and 63 single individuals (25 males and 38 females) collected in the same sampling site as described above but assigned to a different experimental group. Four times a day, we randomly selected pairs and single males and females on day 0 to day 5 after collection. We then gently put a glass cylinder (diameter = 3.5 cm) in the middle of the crystallizer to confine their movement to the boundary of the crystallizer. A diameter line had been previously drawn below the crystallizer. After 5 min of acclimation, we estimated the activity score of the individual or the pair as the number of times the gammarids crossed the diameter line during a 5 min observation period. To control for a size effect on this activity score, we estimated body size of each individual as the length of the fourth coxal plate (Bollache, Gambade, & Cézilly, 2000) using a stereoscopic microscope Nikon SMZ-10A and a video analysis system VT0 232 from Linkam Scientific Instruments Ltd (Tadworth, Surrey, U.K.).

Statistical Analyses

We first compared the body size and the percentage of moulted individuals in the two groups (pairs and single gammarids) to test whether, by chance, certain kinds of individuals had been selected. We then carried out two-way analysis of variance (ANOVA) to assess the effects of sex, pairing status and their interaction on the weight-corrected contents of proteins, lipids and glycogen, which were normally distributed. As it was skewed towards low values, the soluble carbohydrate content, c, did not follow a normal distribution. We modelled it using a generalized linear model (GLM) with beta distribution, with classical transformation $c' = ((n-1)P + 0.5)/c$, with n being the sample size and P the ratio between the value of the corrected carbohydrate content and the highest value of this content observed in our data set (Smithson & Verkuilen, 2006). This transformation complied with the beta regression assumption, stating that the data are within the interval]0; 1[. Cohen's d with its bootstrapped 95% confidence interval ($CI_{95\%}$, 10 000 iterations) is reported as the measure of effect size (Nakagawa & Cuthill, 2007). As discrete count data, the activity score was analysed using a GLM with negative binomial distribution to compare the locomotor activities of paired and single individuals, with the individual's body size as a covariate. Reported values are means and their bootstrapped 95% CI (10 000 iterations). All the statistical analyses were performed in R version 3.3.2 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). For all tests, we chose an alpha threshold of 0.05.

RESULTS

Biochemical Assays

Our selection process led to a significant difference in body mass between paired and single males (t test: $t_{99} = -2.62$, $P = 0.01$), but not between paired and single females ($t_{98} = -1.58$, $P = 0.12$). Cohen's d remained in accordance with these statistical conclusions (males: Cohen's $d = 0.51$, $CI_{95\%} = [0.13;\ 0.94]$; females: Cohen's $d = 0.34$, $CI_{95\%} = [-0.08;\ 0.78]$). Single males were bigger than paired ones: they had mean body masses of 44.1 mg ($CI_{95\%} = [41.9;\ 46.3]$, $N = 40$) and 39.7 ($CI_{95\%} = [37.4;\ 42.0]$, $N = 61$), respectively. Single and paired females had mean body masses of 19.5 mg ($CI_{95\%} = [18.0;\ 21.0]$, $N = 39$) and 18.0 mg ($CI_{95\%} = [16.9;\ 19.0]$, $N = 61$), respectively. We also found differences in the percentage of moulted individuals in the two groups (chi-square test: $X^2_1 = 13.97$, $P < 0.001$). The percentage of individuals removed due to moult was significantly higher in the paired group (19.2%) than in the single one (1.2%).

Almost all metabolite contents were significantly influenced by pairing status in the same way: paired individuals of both sexes had generally higher reserves than single ones (Fig. 1, Table 1). This difference was significant for proteins in males (ANOVA: $F_{1,87} = 28.55$, $P < 0.001$, Cohen's $d = 1.16$, $CI_{95\%} = [0.72;\ 1.69]$) and in females ($F_{1,83} = 4.76$, $P = 0.032$, Cohen's $d = 0.51$, $CI_{95\%} = [0.04;\ 1.06]$), for lipids in males ($F_{1,94} = 63.78$, $P < 0.001$, Cohen's $d = 1.68$, $CI_{95\%} = [1.31;\ 2.18]$) and in females ($F_{1,94} = 50.25$, $P < 0.001$, Cohen's $d = 1.50$, $CI_{95\%} = [1.16;\ 1.96]$), and for glycogen in females ($F_{1,98} = 4.07$, $P = 0.046$, Cohen's $d = 0.42$, $CI_{95\%} = [0.09;\ 0.75]$). We observed a similar value of the effect size for glycogen in males, although the difference was nonsignificant: paired males tended to possess more glycogen reserves than single ones ($F_{1,98} = 2.41$, $P = 0.12$, Cohen's $d = 0.32$, $CI_{95\%} = [-0.10;\ 0.82]$). For none of these metabolites was the interaction term between sex and pairing status statistically significant (Table 1). For soluble carbohydrates, there was no significant effect of sex (beta regression: $X^2_1 = 2.60$, $P = 0.11$), pairing status ($X^2_1 = 3.17$, $P = 0.075$) and their interaction ($X^2_1 = 0.37$, $P = 0.54$; Fig. 1).

Activity Scores

Activity score did not differ significantly according to body size (GLM: $X^2_1 = 3.37$, $P = 0.07$), pairing status ($X^2_1 = 1.68$, $P = 0.20$) and sex for single individuals ($X^2_1 = 0.56$, $P = 0.45$). This implies that pairs, single males and single females moved equally. Males had a mean activity of 15.7 lines crossed per 5 min ($CI_{95\%} = [10.4;\ 21.6]$, $N = 25$), females had a mean activity of 18.9 lines ($CI_{95\%} = [14.7;\ 23.2]$, $N = 38$) and pairs had a mean activity of 13.2 lines ($CI_{95\%} = [9.5;\ 17.4]$, $N = 37$).

DISCUSSION

This study aimed at investigating the energetic consequences of PCMG for both partners in the freshwater amphipod *G. pulex*. Our initial prediction was that the active transport of the female by the male is energetically beneficial to the female and detrimental to the male. Contrary to these expectations, we found that both sexual partners had significantly higher body reserves when paired than when single. In addition, the effect sizes of this difference in

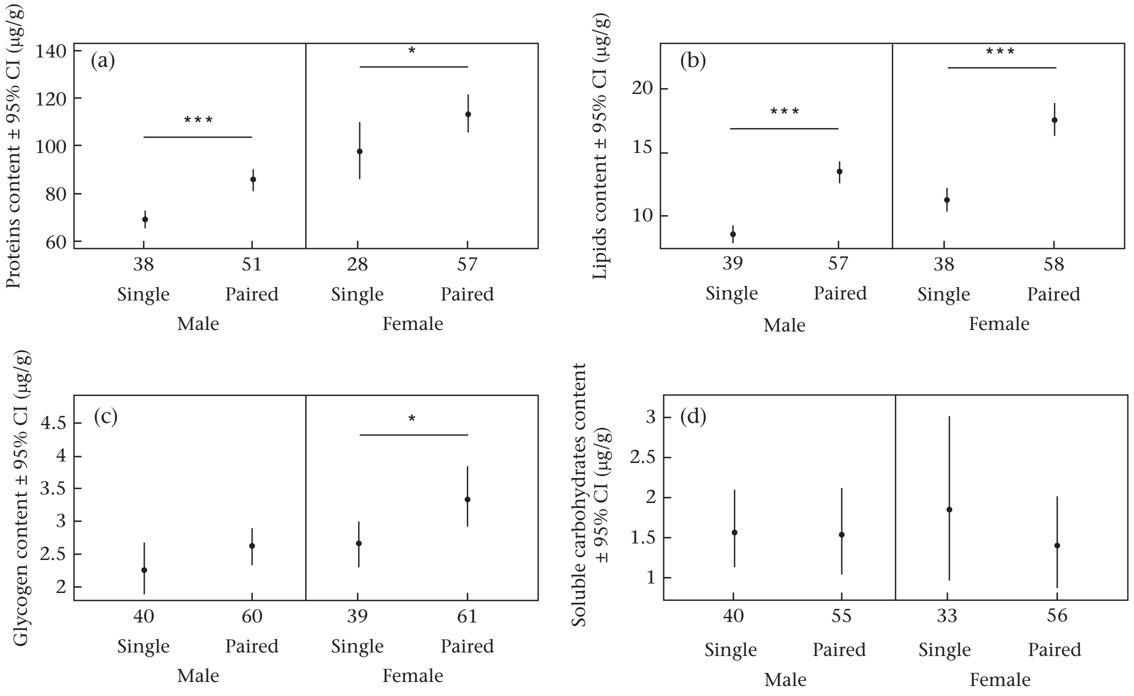

Figure 1. Effect of the pairing status (single/paired) on the weight-corrected body contents of four metabolites for both sexes, after 5 days in laboratory conditions. (a) Proteins, (b) lipids, (c) glycogen and (d) soluble carbohydrates. Dots represent mean body content values, and the associated bars represent bootstrapped 95% confidence intervals of these means. The sample sizes are reported below the *x* axis. $^{*}P < 0.05$; $^{***}P < 0.001$.

Table 1
Results of the ANOVAs testing the effect of sex, pairing status (single/paired) and sex∗status interaction on the weight-corrected contents of the three metabolites that were normally distributed

	df	*F*	*P*
Proteins			
Sex	1, 171	56.33	<0.001
Status	1, 171	18.46	<0.001
Interaction	1, 170	0.019	0.890
Lipids			
Sex	1, 189	44.75	<0.001
Status	1, 189	106.8	<0.001
Interaction	1, 188	1.85	0.175
Glycogen			
Sex	1, 198	8.63	0.004
Status	1, 198	6.49	0.012
Interaction	1, 197	0.53	0.466

reserves according to the pairing status did not differ significantly between males and females.

We first tested whether the energetic differences we highlighted might be the by-product of the artificial selection that we performed. We found a significant difference regarding males' body mass: single males weighed more than guarding ones. Although we corrected the quantified reserves by the individual's body weight, this factor could indirectly impact energy reserves through an effect on locomotor activity. However, we found no significant relationship between body size and activity score. These differences in body mass seem therefore unlikely to be involved in the strong impact of pairing on energetic budgets we highlighted. Additionally, we found a higher percentage of moulting animals in the paired group than in the single one. As Galipaud et al. (2011) pointed out, PCMG tends to reduce the female's intermoult duration. This acceleration of the female's moulting cycle could explain why paired individuals moulted significantly more than single ones after 5 days of PCMG. Hence, we can presumably hypothesize that guarded females were closer to their reproductive moult than single ones. According to a previous study, *Gammarus* females store energetic reserves just before ecdysis (e.g. during late intermoult and premoult stages), probably in relation to the inhibition of some digestive enzymes (Charron et al., 2014). We can thus reasonably expect that single females further from their reproductive moult consumed their reserves more intensely than paired ones, which remains in accordance with our main findings.

In addition to this physiological effect, PCMG could affect energetic states of individuals through an impact on locomotion activity. Comparing our activity measurements and energetic analyses, it seems that, for a similar activity score, single males and females spent more energy than paired ones, which is not fully in line with our initial prediction. The most parsimonious explanation of these energetic differences would involve additional costs for single individuals, probably related to mate-searching activity. Our results show that these additional costs did not translate into higher activity scores during daytime. However, our metrics excluded nocturnal activity of gammarids, which has been recently documented in three species including *G. pulex* (Perkin, Hölker, Heller, & Berghahn, 2014). Locomotor activity of single individuals could increase at night because of mate-searching costs. This activity pattern could decrease the predation risk, as single gammarids actively seeking a mate are expected to be more exposed to predators (Jormalainen et al., 1994). Interestingly, although previous theoretical work made the assumption that mate-searching costs were mainly paid by males (Jormalainen, 1998; Jormalainen et al., 1994), we found no differences between the sexes in swimming activity, suggesting that females can also take part in mate sampling. As females were initially thought to remain hidden while males were actively searching for a mate, some additional information would be required to confirm our statement, for instance by measuring activity in more complex environments providing shelters.

Energetic states in natural conditions are also expected to vary according to energy intakes, which can be disrupted if paired individuals are unable to feed (Bowcock et al., 2009; Robinson & Doyle, 1985) or if food is scarce in the hiding places used by pairs (Sparkes, Keogh, & Pary, 1996). Most notably, sexual conflict might arise if PCMG impedes the foraging activity of one partner but not the other. For example, hermaphrodites of the androdioecious clam shrimp *Eulimnadia texana* close their carapace during PCMG and are therefore unable to keep filter feeding, whereas guarding males are not affected at all (Benvenuto & Weeks, 2012). This potential source of conflict was not addressed in the present study, since no food was provided during the experiments. Gammarids are frequently presented as omnivorous shredders feeding on dead plant material, carrion and living animals (Kelly, Dick, & Montgomery, 2002; MacNeil, Dick, & Elwood, 1997). Gnathopods have been recognized as key structures involved in food acquisition; hence males firmly grasping guarded females with these body parts could suffer from reduced food intakes (Robinson & Doyle, 1985). Despite this potential negative effect of PCMG on males' food absorption, field data revealed accumulation of lipids and glycogen in paired males as shown in our study (Plaistow et al., 2003). Other appendages such as antennae, antennulae and the third pair of uropods are also involved in food collection, and could enable males to keep feeding while holding on to a female (Mayer, Maas, & Waloszek, 2012). It thus seems unlikely that PCMG might severely hinder the feeding rate of one sex in this species, as it does not immobilize these body parts. Therefore, the dynamics of the intense depletion and accumulation of reserves we highlighted for single individuals and pairs, respectively, probably reflects those found in nature.

The most relevant energetic expenses for single individuals mainly involved lipids and proteins, which are crucial precursors of egg biosynthesis (Sutcliffe, 1992). Hence, it appears that PCMG could avoid such energetic losses and increase the female's reproductive output. This is in accordance with the conclusions drawn by Galipaud et al. (2011) who reported higher reproductive rates for long-guarded *G. pulex* females. On the other hand, paired males saving lipids and proteins are likely to take advantage of these reserves for their future episode of growth following PCMG. Body size is indeed strongly related to male competitive abilities in male—male competition over female access in *G. pulex*: larger males are able to guard larger females (Elwood & Dick, 1990). As the female's fecundity increases with increasing body size, larger males are consequently likely to fertilize more eggs (Elwood & Dick, 1990; Elwood, Gibson, & Neil, 1987; Grafen & Ridley, 1983). An experiment comparing growth rates of paired and single males would, however, be welcome to test such a hypothesis of differential resources allocation with pairing status.

To our knowledge, this study is one of the few to assess simultaneously the fitness consequences of PCMG for both partners (see Jormalainen, Merilaita, & Riihimäki, 2001 for another example). Such a holistic view of the cost/benefit ratios related to PCMG for both partners should become more systematic, since it may greatly improve our understanding of the intensity of sexual conflict shaping guarding patterns. Indeed, sexual conflict is assumed to stem from asymmetry between the sexes in the costs and benefits of sexual interactions, in the present case PCMG duration (Chapman et al., 2003). The similar energetic savings we found for paired males and females indicates, from an energetic point of view, a reduced asymmetry in these costs and benefits, and, consequently, that sexual conflict over PCMG duration is limited. This could explain the absence of female adaptive behaviour aiming to avoid long-lasting PCMG. In the marine isopod *I. baltica*, females pay important costs of immobilization; hence their cost/benefit ratio associated with PCMG strongly tends towards losses compared with males. It is thus in their interest to shorten PCMG duration, which could explain why they vehemently resist males' attempts to initiate guarding (Jormalainen et al., 2001). Such behavioural or morphological adaptations have been frequently observed in species where females suffer considerable costs as a consequence of guarding interactions (Arnqvist & Rowe, 1995; Jormalainen & Merilaita, 1995, 1993). Female resistance to early guarding attempts could reveal strong sexual conflict over PCMG duration. Such resistance behaviour has rarely been observed in gammarid species, however (Jormalainen & Merilaita, 1995). Most notably, *G. pulex* females appear to be particularly passive during PCMG which lasts much longer (up to 20 days) than in *I. baltica* (6 days; Birkhead & Clarkson, 1980; Jormalainen et al., 2001). Additionally, *G. pulex* females have large pores at the surface of their cuticle, presumably involved in a lock-on mechanism facilitating the clasp of the male on their back during amplexus (Platvoet, Song, Li, & van der Velde, 2006). Such behavioural and morphological cues indicate there is probably little sexual conflict over guarding duration in *G. pulex*, which is supported by the present results.

From the male's point of view, imposing higher energetic expenditures on the female would have a detrimental effect on her egg production, which would, in turn, be prejudicial for his own fitness (Jormalainen et al., 1994). This is especially true when males have only a few reproductive opportunities in their life. In *G. pulex*, males guard females for a long time before copulation, thereby forgoing other reproductive opportunities. The lifetime reproductive success of a male greatly depends on the egg production of each of the relatively few females he manages to guard. In other words, in species where male—male competition leads to long-lasting PCMGs, we should expect these PCMGs to have limited or no detrimental effect on female energetic resources. According to our results, PCMG even seems beneficial to females in terms of energy budgets. If, overall, long-lasting PCMG increases females' lifetime fitness, it could be considered as mutually beneficial for both partners (West, Griffin, & Gardner, 2007). That long-lasting PCMGs are mutually beneficial does not necessarily imply that they evolve in males because it benefits females, in which case PCMG would be considered as a cooperative behaviour (West et al., 2007). Instead, it more probably implies that the optimal guarding durations differ only weakly between the sexes, leading to decreased resistance of females to mating attempts and possibly facilitation of pairing.

Conclusion

From an energy point of view, it seems that both sexual partners mutually benefit from PCMG, a surprising result under the prevalent hypothesis of a sexual conflict over PCMG duration in amphipods (Jormalainen et al., 1994; Yamamura & Jormalainen, 1996). We here aimed at highlighting the need for simultaneous assessments of cost/benefit ratios associated with male sexually selected traits for both partners. No apparent sign of sexual conflict has been found in *G. pulex*, and our study brings new information about the energetic benefits of guarding for both partners. As Trivers (1972) pointed out, the interests of males and females are rarely identical and sexual conflict is probably ubiquitous. Its intensity may, however, vary greatly among species and especially ecological contexts (Arbuthnott, Dutton, Agrawal, & Rundle, 2014; Tregenza, Wedell, & Chapman, 2006). In the case of PCMG, the costs and benefits for females proved to be reliable indicators of that intensity, as well as the differences in the cost/benefit ratio between the sexes. Similar to what we observed here from an energetic perspective, when PCMG is beneficial for females, the partners' interests may converge over its duration, hence limiting the intensity of sexual conflict. To understand the evolution of PCMG and the variation in its duration among species (e.g. Jormalainen, 1998), we plead for further investigations regarding the costs and benefits of PCMG for both sexes, particularly those linked to predation risks (Cothran, 2004; Cothran et al., 2012), foraging efficiency (Bowcock et al., 2009; Robinson & Doyle, 1985), interference with other conspecifics such as harassment by males (Amano & Hayashi, 1998) or loss of mating opportunities (Jormalainen, 1998).

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