



Disentangling a shared trait: male control over mate guarding duration revealed by a mate exchange experiment

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Abstract

Shared behavioural traits result from the interaction of two or more individuals, making it difficult to discern which individual is in control of the behaviour of interest. Especially in the case of shared reproductive traits such as mating duration or mate guarding duration is this an important issue to resolve, because these are potentially closely connected to fitness and are likely to exhibit sexual conflict. Here, we sought to disentangle which sex controls mate guarding duration in the tropical house cricket *Gryllobates sigillatus*, a species in which mate guarding and nuptial feeding by the male have been proposed to prevent premature removal of the transferred spermatophore by the female. To do so, we performed a series of mating experiments in a paired design, in which the first mating dyad was allowed to start mating some time before the second dyad. Once both dyads were in the mate guarding phase, we then interrupted them and exchanged partners, enabling us to determine whether the remaining guarding duration depended more on the duration of guarding already performed by the male in the new dyad (implying male control) or on the guarding already received by the female (implying female control). We found that the time a female was guarded overall was significantly affected by how long the exchanged male had already engaged in mate guarding with the previous female, but conversely, the total time males guarded both females was unaffected by the duration of guarding that the exchanged female had previously received. Our data thus clearly demonstrate that males rather than females control mate guarding duration and adjust the duration according to females' weight.

Significance statement

It is not easy to determine which individual is in control of a shared behavioural trait (SBT). This information could provide insight into selection pressure on one sex and could help us understand differences in SBTs between related species or between different dyads of one species. We used a relative novel but simple method to disentangle a SBT in a cricket. We performed mating experiments and exchanged the mating partners after copulation within the mate guarding phase and measured the total guarding duration. Our analyses showed males were not influenced by the exchange and guarded as long as expected regardless how long the female were guarded before by another male. Our data suggest males are likely in control of mate guarding duration, and they have no ability to recognize post-copulatory mate exchange.

Keywords Interaction phenotypes · Mate guarding · Mate exchange · Sexual conflict · Mating behaviour · *Gryllobates* · Cricket

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Introduction

Males and females of almost all animal species have fundamentally different interests and strategies to optimize their own fitness, ultimately resulting from anisogamy (Bateman 1948; Parker et al. 1972; Schärer et al. 2012; Arnqvist and Rowe 2013; Parker 2014; Schärer et al. 2014; Janicke et al. 2016; Lehtonen et al. 2016). Therefore, although recent work has established the evolutionary significance of both female competition (Rosvall 2011) and male mate choice (Bonduriansky 2001), it is typically the case that female

reproductive success is maximized by resource availability and by obtaining the best mates (through either pre- or post-copulatory choice), whereas male reproductive success usually depends more on maximizing mating and fertilization rates (Bateman 1948; Trivers 1972; Parker 1979; Andersson 1994; Chapman et al. 2003). Given these fundamentally different routes through which males and females maximize fitness, it is not surprising that the interests of males and females frequently differ during reproductive interactions.

As a result of differential selection on males and females, sexual conflict is often expected for traits such as copulation frequency, copulation duration and mate guarding duration (Jormalainen et al. 1994; Schneider et al. 2006; Blanckenhorn et al. 2007). Such traits are ‘shared’, in that at least two individuals together display a single behavioural trait (e.g. copulation duration). Sexual conflict will frequently occur for shared traits, as optimal values for these traits are often closely connected to the fitness of the involved individuals and at the same time are likely to differ between the sexes (Simmons 2001; Edward et al. 2014). In post-copulatory mate guarding, for example, both sexes should have an interest to influence the duration towards their own optimum, because both sexes have costs and benefits (Jormalainen 1998). The benefit of extended mate guarding to males seems quite clear, because guarding may prevent or delay re-mating of polyandrous females and is thereby likely to increase the fertilization success of the guarding male in the face of sperm competition (e.g. in spiders Schneider and Lesmono 2009; Córdoba-Aguilar et al. 2010; Peretti and Eberhard 2010, and in insects Alcock 1994; Edvardsson and Arnqvist 2000; Bussière et al. 2006; and see Peretti and Aisenberg 2015 for a review). Guarding is nevertheless costly to males, through time and energetic costs and through missed additional potential mating opportunities and potentially increased predation risks, especially in species in which the guarding males exhibit a conspicuous behaviour or ornament (Parker 1974; Alcock 1994; Dickinson 1995; Rodríguez-Muñoz et al. 2011).

Females may also benefit from extended mate guarding. Tuni et al. (2013), for example, showed that females of the cricket *Teleogryllus oceanicus* assess relatedness during post-copulatory mate guarding, bias storage of sperm towards unrelated males, and thus diminish the risk of inbreeding. Other studies have shown that females can benefit from pre- and post-copulatory mate guarding in the form of a reduced predation risk (Zeiss et al. 1999; Rodríguez-Muñoz et al. 2011; Cothran et al. 2012) or in the form of reduced male harassment (Davis 2002). Nevertheless, guarded females also incur costs with increased guarding duration. Perhaps the most widespread cost of mate guarding to females may be the delay in re-mating it causes when females can benefit from polyandry, for example by increased genetic diversity in offspring, beneficial seminal proteins and more nuptial gifts (Birkhead and Møller 1998; Jormalainen 1998; Cothran 2008; Rodríguez-

Muñoz et al. 2011; Elias et al. 2014). Being guarded may also have negative consequences, for example lowering food intake rate (Parker 1979; Chapman et al. 2003) or energetic costs of exerting resistance behaviours (e.g. somersaults in water striders (Arnqvist 1989) or the ‘kicking behaviour’ shown in many grasshoppers (e.g. Hartmann and Loher 1996)).

Because of the various costs and benefits of mate guarding in both sexes, it is not trivial to estimate in which sex we should expect a higher selection pressure to have more control over this trait. Even when it is relatively clear which sex should be under a higher selection pressure, it does not naturally follow that individuals of that sex are more able to control that trait and thus we should not automatically expect the trait to be at one sex-specific optimum (Parker 1979). Moreover, for many traits, it is likely that both sexes exert some degree of control over the ultimately expressed trait value, meaning we will often not be asking the binary question of which sex is in control, but rather about the relative degree of influence exerted by males and females (Jormalainen 1998).

Here, we aim to determine the extent to which males and females control the duration of post-copulatory mate guarding in the cricket *Gryllodes sigillatus*. In this species, males produce a nuptial gift in the form of a spermatophylax (Sakaluk 1984). (Please note: Sakaluk and other authors used the name *G. supplicans* until 1992 in some studies but *G. supplicans* seems to be a synonym of *G. sigillatus* (see (Sakaluk et al. 1992)). The spermatophylax is attached to a smaller sperm-containing ampulla, and males transfer these two components together to the female genital opening during copulation (Alexander and Otte 1967). Males usually guard the female after copulation for an extended period. Soon after copulation, females usually start feeding on the spermatophylax and frequently they remove the ampulla only after they finish feeding on the spermatophylax. Mate guarding is also likely to serve in preventing competitor males from moving the ampulla during mating attempts (Sakaluk 1991). Probably as a side effect of mate guarding, females thus can usually feed on the spermatophylax without harassment by other males. As an alternative explanation, Bateman and MacFadyen (1999) interpreted the mate guarding of *G. sigillatus* as a strategy to prevent the female from prematurely removing the ampulla. Both interpretations suggest benefits for males from extended mate guarding, because under both hypotheses, the sperm-containing ampulla is attached to the female genital opening for longer and can therefore transfer more sperm. Furthermore, the whole mate guarding process likely prevents other males from copulating with the female and reduces the sperm competition risk. Females, on the other hand, may benefit from guarding but may also incur costs that increase with guarding duration, such as reduced opportunities for mate choice and/or foraging. Most studies that examined mate

guarding in crickets discuss the benefits of extended mate guarding for males (especially the reduced sperm competition risk), and they mainly agree that selection pressure to control mate guarding duration should be higher in males (see, e.g. Alcock 1994; Frankino and Sakaluk 1994; Hockham and Vahed 1997; Bateman and MacFadyen 1999; Bussière et al. 2006; Parker and Vahed 2010, but also see Zeiss et al. 1999; Rodríguez-Muñoz et al. 2011; Cothran et al. 2012 for female benefits).

Mate guarding in field crickets can be recognized by the following behaviours: (1) standing close to the female with the cerci in her direction, (2) frequently antennating the female, (3) searching rapidly whenever the female wanders out of range of the male's antennae or cerci, (4) producing aggressive chirps upon any movement by the female or upon intrusion by another male and (5) physically attacking males that intrude (after Khalifa 1950 and Alexander and Otte 1967). Sometimes, some of these elements are observable at the same time (e.g. 1, 2 and 4). In our study, we measured mate guarding duration as the time from starting behaviours 1 or 2 after mating and included short phases of behaviour 3 if the guarding was thereafter continued.

To test which sex controls the mate guarding duration in *G. sigillatus*, we performed time-shifted experimental matings with a reciprocal mate exchange component. Tuni et al. (2013) showed that post-copulatory mate recognition appears to be absent at least in the field cricket *Teleogryllus oceanicus* and an experimental mate exchange is possible during the guarding phase, motivating us to use this method for our species. As a result of our mate exchange method, males and females received a partner that had experienced a different mate guarding duration with the previous partner. If females are in control of mate guarding duration, e.g. influence the termination of mate guarding, males that receive more recently mated females can be expected to guard longer than if they receive a female that mated longer ago. If instead males are primarily influencing mate guarding duration, females that receive recently mated males as new partners can be expected to be guarded for longer than females receiving males that mated prior to them and have thus already guarded for longer. By considering the difference between the total guarding durations for the males and females in the two dyads that were reciprocally exchanged, we could therefore use this paired design to distinguish whether males, females or both influence mate guarding duration.

Material and methods

Experimental design and animals

We purchased adult individuals of *Grylloides sigillatus* from a pet shop (Reptilienkosmos) in mixed sex packs and kept them

sorted by sex (in containers with a size of 30 × 20 × 25 cm, around 25 to 40 insects per container) for 1 week after arrival to avoid further uncontrolled matings. We kept the animals in the following conditions: 28 °C at daytime, 24 °C at night, 12 h artificial day-night rhythm, about 60% humidity, oat flakes and fish food as food and water ad libitum. Due to the nocturnal activity of *G. Sigillatus*, we performed the experiments during the artificial night time and observed all behaviours under red light.

Before we started the matings, we weighed all animals to be able to test for body mass effects, because some studies have shown positive correlations between mass/size and guarding duration (e.g. in birds (Møller 1987), in a grasshopper (Cueva del Castillo 2003) and in the cricket *Gryllus bimaculatus* (Simmons 1986)), and Sample et al. (1993) clearly showed that weight and body size are strongly correlated in insects. To start the experimental matings, we put one randomly chosen dyad into a small circular plastic arena (diameter 163 mm, height 124 mm) and observed the start of the copulation. The arena was large enough to provide females the opportunity to escape from mating attempts, and as an additional option for an escape, we placed a cylindrical carton of about 4 cm length and 4 cm diameter in each arena. We recorded all start and end times of copula and guarding period (also after the exchange) for the dyads. We performed the experiments as described in the following steps:

1. Copulation phase: After copulation attempts started in the first dyad, which from here on we always call dyad A, we put another dyad (defined as dyad B) into another arena and allowed them to copulate (difference in copulation start between dyads 9–56 min; mean 26.5 min, largely resulting from varying mating latencies of the dyads).
2. Exchanging: When both dyads had finished copulation, which we defined as separation of partners after the comparatively quick spermatophore transfer, the males invariably started mate guarding within a minute. We used the re-positioning of the male so that its cerci were very close to the female as the start of mate guarding. Waiting for at least 5 min after the second dyad had started mate guarding (6–21 min, mean 9.9 min; timepoint depended on a favourable situation to remove the male carefully), we simultaneously exchanged the males and let them both guard the female that initially mated with the other male (see scheme in Fig. 1).
3. Observing: We observed the mate guarding behaviour on the basis of the typical aspects of this behaviour described above (after Khalifa 1950; Alexander and Otte 1967) and stopped the time either when the males ceased guarding behaviour or else when mate guarding stopped because the female successfully moved away or hid herself under or behind the cardboard.

4. Data collection and calculation: We calculated the total guarding duration for each male and female involved. We determined how long each female was guarded by adding up the guarding performed by males A and B, and for each male, we measured how long in total it guarded the females A and B.

We used this time-shifted paired design so that differences in copulation start times also result in differences in guarding start times. In addition, with this approach, we controlled for any effects of variation regarding temperature, air pressure, observer disturbances and time since the separation of the sexes. In total, 21 quartets (dyads A1 and B1 to dyad A21 and B21) copulated and the males were exchanged as described above. On average, the B males started mate guarding 26.5 min after the A males. The exchange was in all cases performed by A-P. Mazur and O. Zyma by tickling males A and B simultaneously with small paintbrushes so they moved a few centimetres into small plastic containers held besides them and were then immediately exchanged between the containers so that they were adjacent to the females that was initially guarded by the other male when tickled out of the plastic containers. We are aware the tickling with paintbrushes and the exchange itself could influence the total guarding duration, but we think the reciprocal nature of the exchange cannot affect the main hypothesis we are testing here. All animals were used randomly without any prior selection based on size, weight or behaviour.

After the experimental matings, we calculated the differences between total guarding durations of the A and B females and called this variable *observed difference* ($f_A - f_B$). According to our null hypothesis ($H_0 =$ females are in control), we defined for our statistical test, we would expect to find no

significant differences between the total mate guarding durations of the two females. On the other hand, according to our alternative hypothesis ($H_a =$ here defined as males are in control), we would expect significant differences between the total time females are guarded (defined as: time guarded by male A + time guarded by male B) because the later mated males B will likely guard earlier mated female A for longer than the original male A would have done. If, however, males are in control of mate guarding duration, total male guarding time (defined as: time guarding female A + time guarding female B) should not differ between first and second males. Assuming sole male control, our design allows to predicted and *expected differences* between the total time females are guarded as twice the time difference between the start points of guarding for the two dyads:

$$\text{expected diff.} = 2 \times (\text{start guarding in pair B} - \text{start guarding in pair A}) \quad (1)$$

This assumes that we can find the ‘lost’ guarding time of female B in the total time the female A is guarded. To standardize deviations from the *expected differences*, we devised formula 2 with the *observed differences* in females (see above and Fig. 1). We called the calculated variable *standardized difference*:

$$\text{standardized difference} = \frac{\text{observed difference } (f_A - f_B)}{\text{expected difference}} \quad (2)$$

From this transformation, we obtained relative values of deviation from our *expected difference*, where 1 means the value is as expected for sole male control. Values lower than 1 represent guarding durations that were less influenced by the exchange than expected under male control. We would interpret values between 1 and 0 as the result of males and females

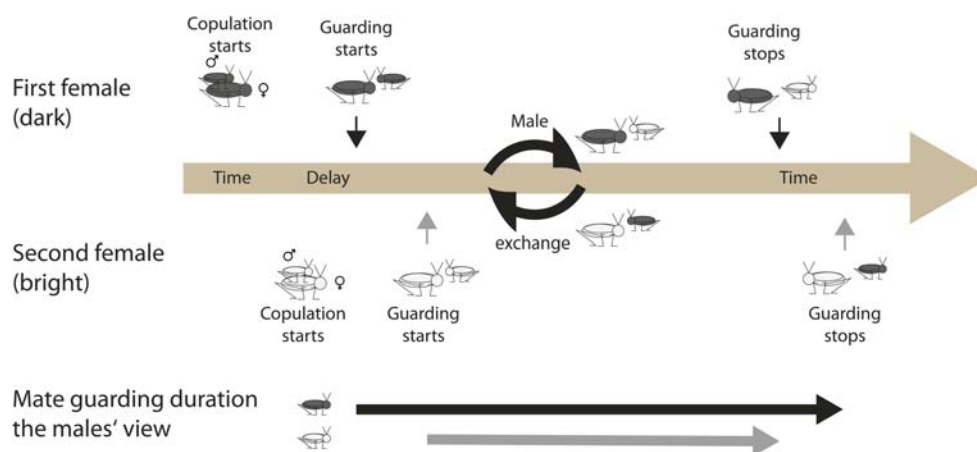


Fig. 1 Scheme of our experimental design. We always used two dyads in parallel but with a different start time and gave them the opportunity to mate. After copulation ended and mate guarding started, we exchanged the two males and let them guard until they stopped or the female

successfully escaped mate guarding. The scheme shows the result predicted by our null hypothesis (females in control), i.e. the same guarding duration from female perspective and a significant difference in mate guarding duration from the male perspective

influencing mate guarding duration and values close to 0 would fit with our null hypothesis and thus indicate that (solely) females control mate guarding duration.

Statistical analyses

Using a general linear model, we estimated the influence of differences in guarding starting time (start time dyad B – start time dyad A) and the influence of weight differences between male A and male B (weight *male A* – weight *male B*) and also between female A and female B (weight *fA* – weight *fB*) on the standardized differences of the expected mate guarding durations. With *t* tests, we compared the total guarding time between mate A and A in females and males. With a one-sample *t* test, we tested whether the *standardized difference* is different from 0 (i.e. different from our null hypothesis). All statistical tests were performed with R version 3.4.2 (R Core Team 2017).

Ethical note

All animals in this behavioural study were handled in accordance with animal welfare guidelines. After observing the animals in the experiments, they were kept in large containers (30 × 20 × 25 cm) in groups of 20–40 animals (mixed sexes) with hiding opportunities, at room temperature, with a natural day night-rhythm and with food and water ad libitum. After finishing the whole experiment, the surviving insects were killed by freezing.

Results

We measured the total mate guarding duration, which ranged between 8 and 160 min for males ($N = 42$, mean 71.48 min, $SD \pm 39.08$) and between 7 and 164 min for females ($N = 42$, mean 71.48 min, $SD \pm 43.37$). Consistent with the male control hypothesis, total guarding duration differed significantly between A and B females ($t = 3.5$, $df = 21$, $p = 0.001$; Fig. 2), but total guarding duration did not differ between A and B males ($t = 0.94$, $df = 21$, $p = 0.35$; Fig. 2). Males continued guarding after the exchange between 0.5 and 118 min ($N = 42$, mean 48, $SD \pm 37.69$).

The mean value of the standardized difference was 0.916, and the median was 0.900, which is significantly different from 0 but not from 1 (one-sample *t* tests, $p < 0.001$ and $p = 0.63$, respectively). All but three of the 21 A females were guarded for longer than the B females (see values above 0 in Figs. 3 and 4). The start time difference between the two copulations showed no significant effect on the standardized differences (Table 1).

Examining potential effects of body size on mate guarding duration, we found a positive correlation between a male's

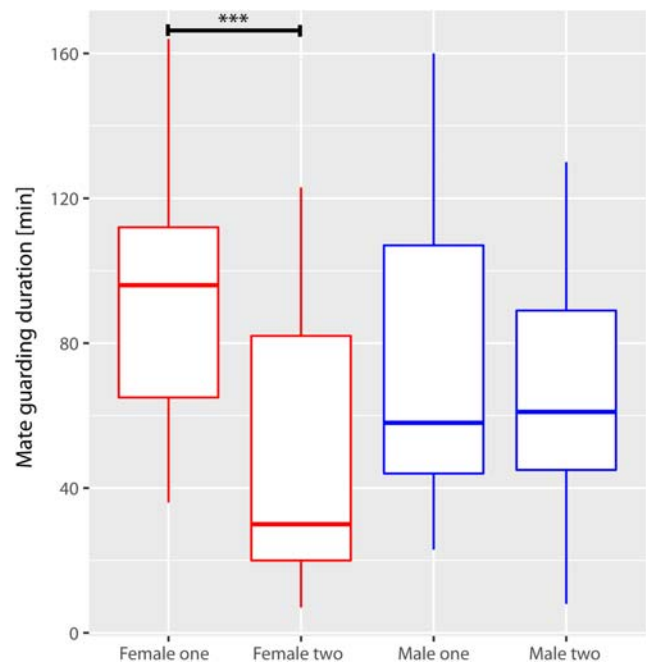


Fig. 2 Boxplot of differences in mate guarding duration between A and B females and between A and B males. We found highly significant differences in the mate guarding duration between the females (red boxes on the left, $t = 3.5$, $df = 21$, $p = 0.001$). The B females were guarded for significantly shorter periods. However, we found no significant differences between the A and B males (blue boxes on the right, $t = 0.94$, $df = 21$, $p = 0.35$). (Standard boxplot with median, whiskers depict upper and lower quartile of the data)

weight and his mate guarding duration ($t = 2.55$, $df = 40$, $r = 0.37$, $p = 0.015$; Fig. A1a in the appendix) but not between a female's weight and her mate guarding duration ($p = 0.927$; Fig. A1b in appendix), in which we must consider that female's guarding time is the sum of guarding duration of two different males. In contrast, we found a negative correlation between weight differences (female A – female B) and standardized differences (see “Material and methods”) in female guarding duration. That is, the heavier the female A was (compared to the female B in the quartet), the longer female B was guarded in comparison to female A ($t = -2.16$, $df = 19$, $r = -0.44$, $p = 0.044$; Fig. 3b). We found no such correlation in males ($p = 0.137$; Fig. 3a).

Discussion

We performed mate exchange experiments with two dyads of crickets that had mated some minutes apart to examine the average male and female contribution in determining mate guarding duration. We found no differences in the average mate guarding duration between the males of the first and the second mating dyads, which we called A and B dyads throughout, despite the fact that we exchanged them. In contrast, we found large differences in the females' total mate

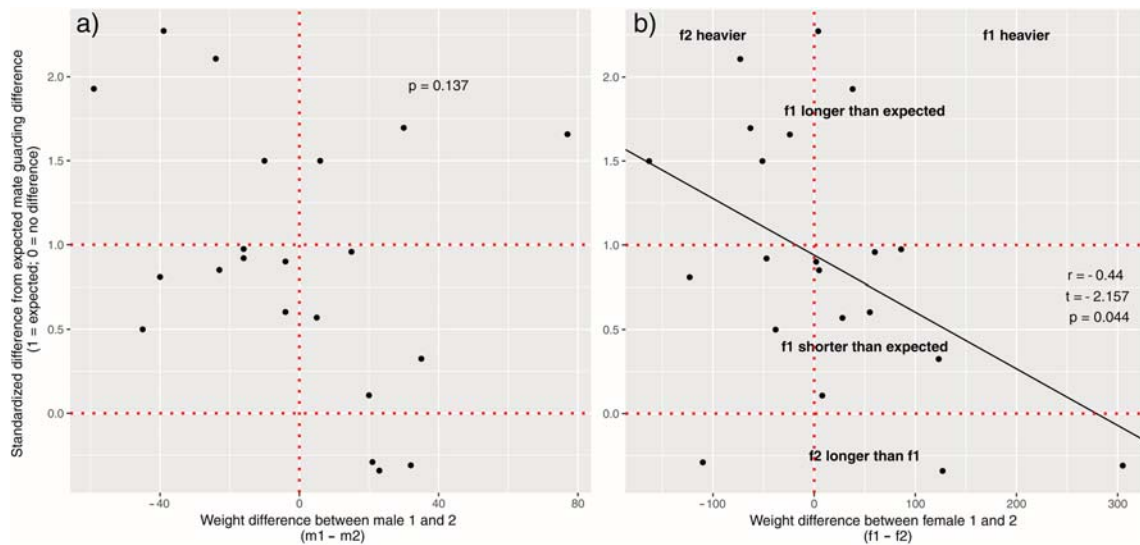


Fig. 3 Distribution of standardized differences in relation to weight differences in males and females. **a** We found no correlation between standardized differences and weight differences in males **b** but a negative correlation in females. Almost all the A females were guarded longer than the B females within a quartets (data points above horizontal zero-line). Only three B females were guarded longer than the A females

guarding duration between the A and B dyads and thus concluded that mate guarding duration is to a large extent controlled by males. As we also found a negative correlation between female weight differences and guarding time, we conclude that males adjust the guarding duration according to the females’ weight. Moreover, since the males continued guarding the new female after the exchange, post-copulatory mate discrimination by males appears to be absent in this species.

We found that A females were usually guarded for longer than B females, with no corresponding differences between

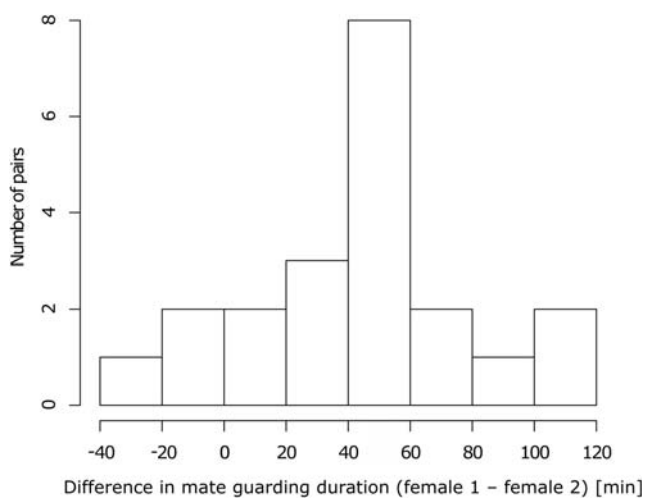


Fig. 4 Distribution of differences in mate guarding duration calculated for females. In only 3 out of 21 cases, the B females were guarded longer than the A females (bars left from 0). In all the other cases, the A females were guarded longer

within the same quartet (see data points below horizontal zero-line). (Explanations for 3b: *f1 longer than expected*: the A females were guarded longer than expected; *f1 shorter than expected*: A females were guarded shorter than expected but still longer than the B females; *f2 longer than f1*: only in these cases the B females were guarded longer than the A females)

males. In addition, the observed differences did not on average differ from the predicted ‘expected difference’ (see “[Material and methods](#)” for calculations of these terms). We could therefore reject the hypothesis that females alone control mate guarding duration and concluded that our alternative hypothesis, which males control mate guarding duration, is more likely. In other words, had females controlled the duration, we would not expect differences between total guarding time of females A and B because the time they would take to stop the guarding period, for example, by moving away or kicking the male, should not be influenced by their mate’s mate guarding duration prior to the mate exchange, but only by the total amount of mate guarding they had experienced. Assuming female control of mate guarding duration, we would therefore expect significant differences in mate guarding time between the A and B males, which we did not find.

Table 1 Linear model fit to estimate the influence of weight differences in males and females and of the start time differences on the *standardized difference* in mate guarding. No effects were found for weight differences in males or for the start time difference between the pairs. However, a negative effect for weight difference in females was found. That is, the greater the weight difference, the smaller the standardized difference

	Estimate	SE	t	p
(Intercept)	1.623	0.397	4.09	0.001
Weight difference females	-0.003	0.002	-2.13	0.048
Weight difference males	-0.002	0.005	-0.51	0.615
Start time difference	-0.026	0.014	-1.87	0.079

Here, we should consider we did not test the guarding behaviour under male-male competition conditions. Females' motivation to short the guarding duration by kicking the male or by escaping could be higher when other males are present. As we already mentioned in the introduction, females can also benefit from polyandry by increased genetic diversity in offspring, beneficial seminal proteins and more nuptial gifts (Birkhead and Møller 1998; Jormalainen 1998; Cothran 2008; Rodríguez-Muñoz et al. 2011; Elias et al. 2014) and they possibly could raise their mating rate by shortening the guarding time. Therefore, the absence of male competitors could be one explanation for the male control pattern we found.

Another potential explanation for this pattern of male control over guarding duration is that the selection pressure for controlling mate guarding duration is higher in males, because their costs resulting from a shorter guarding duration likely are much higher than the females' costs resulting from a longer guarding duration. Males will likely have reduced chances in sperm competition if reduced mate guarding duration leads to females prematurely removing the ampulla or this happening during mating attempts of other males. Females, on the other hand, may only lose time during mate guarding they could use for foraging or further matings and thus may only benefit slightly from a reduced mate guarding duration (Sakaluk and Cade 1980). The shared trait 'guarding duration' can be seen as an extended phenotype (Dawkins 1982) as a combination from genes of two different individuals. This phenotype has an evolutionary influence of the fitness of both partners and therefore both partners should have an interest to influence it. The higher the influence and therefore the fitness benefits of partner A, the lower could be the fitness benefits of partner B and counter wise. This impact of the sexual conflict has natural borders because at one point the fitness decrease of partner B also decreases the fitness of partner A. That means the potential fitness of both partners can decline because of the conflict (Queller 2014), and it is likely that no sex is in absolute control of the trait.

Usually, mate guarding is, however, interpreted as a male strategy to reduce sperm competition (Thornhill and Alcock 1983; Simmons 2001). Most field cricket species are highly promiscuous, and the risk of sperm competition is quite high (e.g. Backus and Cade 1986; Mallard and Barnard 2003; Simmons et al. 2007; Thomas and Simmons 2007, 2009), which is also the case in *G. sigillatus* (Sakaluk 1986). Not surprisingly, mate guarding is also quite common in field crickets and is usually associated with sperm competition avoidance (e.g. Hockham and Vahed 1997; Bateman and MacFadyen 1999; Parker and Vahed 2010; Rodríguez-Muñoz et al. 2011). The amount of transferred sperm, as well as sperm competition avoidance, plays an important role in sperm competition. In species with an attached spermatophore, the amount of transferred sperm is usually positively

correlated with the spermatophore attachment duration (e.g. Sakaluk 1984; Simmons 1987; Wedell and Arak 1989; Reinhold and Heller 1993). Several studies in different cricket species have shown that spermatophores were attached significantly longer when females were guarded, whereas unguarded females removed the ampulla (the sperm-containing portion of the spermatophore) earlier (Loher and Rence 1978; Evans 1988; Bussière et al. 2006; Parker 2009; Bateman and MacFadyen 1999 and see chapter 11 in Vahed 2015 for an overview). However, other studies found no differences in attachment time between guarded and unguarded female crickets (Khalifa 1950; Sakaluk and Cade 1980).

The fact that *G. sigillatus* provide nuptial gifts and guard their mates after copulation is quite unique in the suborder Ensifera and the question is why this species show both behaviours. It has already been demonstrated that the spermatophylax prevents the female from removing the ampulla before the ejaculate is transferred (Alexander and Otte 1967; Sakaluk 1984), and the bigger the spermatophylax, the more ejaculate is transferred (Sakaluk 1984, 1985, 1986). Because of these findings, Sakaluk (1991) interpreted the mate guarding in *G. sigillatus* as a strategy to avoid dislocation of the spermatophore due to mating attempts of other males and not as a strategy to prevent the guarded female from removing the spermatophore too early. But he also found a positive correlation between ampulla attachment duration and mate guarding in guarded females compared to females that were isolated by removing the males, as was also shown by Bateman and MacFadyen (1999). However, as discussed above, mate guarding cannot prevent a premature removal of the ampulla in every case, but the nuptial gift in this *Gryllodes* species at least also provides some protection against premature ampulla removal.

In addition to the longer guarding duration of A females compared to B females, we found a negative correlation between weight differences ($fA - fB$) and guarding duration in females (Fig. 3b). It may seem that heavier females were guarded less, but we should consider that we changed the mating partners after mating. Given that female mass often correlates strongly with fecundity in insects (Kozłowski 1992; Stearns 1992; Roff 2002), we would expect males to invest more guarding when mating with heavier females. So, if males are in control of guarding duration and decide about it before or during mating, and do indeed invest more into guarding heavier females, this would—due to the mate exchange element—appear in our experiment as the lighter female (guarded after the mate exchange by the male who mated with the heavier female) being guarded for longer, which is exactly what we saw. Our results further suggest that the assessment of the mating partner was not readjusted after the mate exchange took place.

We also found a positive correlation between mate guarding duration and the male's weight. A correlation

between weight (or body size) and mate guarding duration in males has already been shown in some other species of different taxa (e.g. in birds (Møller 1987), in a grasshopper (Cueva del Castillo 2003) and in the cricket *Gryllus bimaculatus* (Simmons 1986)). One possible explanation for the correlation could be that larger males guard more efficiently because females are not able to relieve themselves from guarding or, another explanation, that extended guarding provides greater benefits for large males (Simmons 1991). As neither male mass nor weight differences between the exchanged males had significant effects when it was added as a covariate in our linear model, we assume that the paired design we used probably controlled well for these differences during the course of the experiment.

Besides our main results, our experiment also showed either that individual *G. sigillatus* males do not have the ability to recognize their mating partner or that a post-copulatory mate discrimination has not evolved in males of this species. This would make sense because there are no reasons to expect an exchange of the mating partner in nature. Nevertheless, cuticular hydrocarbons play an important role in mate recognition in other reproductive contexts (Tregenza and Wedell 1997; Mullen et al. 2007; Thomas and Simmons 2009). Furthermore, Tuni et al. (2013) clearly showed that females of *Teleogryllus oceanicus* can distinguish between close relatives (siblings) and unrelated animals during post-copulatory mate guarding, and the authors suggested that females do so by using cuticular hydrocarbons. Still, neither males nor females seemed to be disturbed by a mate exchange because the females in the cited study used cryptic female choice to store more sperm when they were guarded by an unrelated male even when they copulated with a sibling. It seems they decided about the sperm storage due to the relatedness with the guarding male and not due to the relatedness with the copulation partner. Also, our results show there is certainly no post-copulatory mate discrimination outgoing from males and given there should be no benefits to guarding a non-mate, the fact males still do it likely implies also no mate recognition.

It is generally difficult to investigate which sex controls a shared behavioural trait. Nevertheless, some studies could show indirect evidence for male control of copulation duration in different insects and spiders (e.g. Hughes et al. 2000; Wilder and Rypstra 2007; Mazzi et al. 2009; Vahed et al. 2011; Bretman et al. 2013; Engqvist et al. 2014; Haneke-Reinders et al. 2017). On the other hand, there are studies concluding that there is female control over mate guarding duration (e.g. Rowe 1992 in a water strider and Jormalainen and Merilaita 1995 in the isopod *Idotea baltica*, see also Eberhard 1996; Simmons 2001 for other examples), although the males in these species should have similar benefits of extended guarding as in other species (e.g. reduced polyandry, lower sperm competition risk, higher amount of transferred sperm, higher fertilization rate). While these studies used

experimental manipulations of the operational sex ratio or of female condition, we used an experimental manipulation of guarding duration itself and found males to be in control. These contrasting results show that one cannot predict with certainty which sex is in control of mate guarding duration from the assumed costs and benefits of mate guarding or from the results of a related model species. To understand the effects phylogeny and ecology have on this question, we have to perform more specifically tailored experiments. In conclusion, our data show that in *G. Sigillatus*, females have no measurable influence on mate guarding duration and that males are likely in control of this shared behavioural trait. Our results further indicate that males probably adjust mate guarding duration to the female's weight and potentially also to their own weight. Mate guarding and the resultant longer attachment of the ampulla likely have positive effects for male fitness because the amount of transferred sperm increases with time (Sakaluk 1984), which leads to increased fertilization chances (Sakaluk 1986; Simmons 1987). We therefore postulate that the benefits of controlling mate guarding duration are higher for males in *G. sigillatus* than for females.

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Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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