

## Research article

# Pre- and post-copulatory mate choice in *Platygyrillus primiformis*: cryptic female choice and sexual conflict

Darren Parker\*

University of Derby, Derby, UK.

\* **Corresponding author:** 45 Wild Street, Derby DE1 1GP, UK. Tel: +44 7929528965. Email: dazparker14@hotmail.com

**Supervisor:** Dr Karim Vahed, N610, University of Derby, Kedleston Road, Derby DE22 1GB, UK.

The effect of sexual conflict upon mating systems is a controversial topic. The aim of this study was to determine whether post-copulatory choice by females (spermatophore removal) reinforces pre-copulatory choice with respect to male body size and fighting ability, and whether such post-copulatory female choice is influenced by post-copulatory mate guarding by males using *Platygyrillus primiformis* (Orthoptera: Gryllidae; Gryllinae). A no-choice test was used to determine the attractiveness of males and spermatophore attachment time was recorded as a measure of cryptic female choice. Females maintained a pre-copulatory mate choice for large males that were more successful in fighting, shown by a shorter latency to copulation. Larger, males that were more successful in fighting were also preferred by post-copulatory cryptic female choice, shown by a longer spermatophore attachment time, reinforcing pre-copulatory mate choice. Males attempted to counter this selection by guarding females, which increased their spermatophore attachment time. Interestingly, spermatophore attachment time increased similarly for all sizes of male as a result of mate guarding, meaning that females maintained their original choice.

**Key words:** *Platygyrillus primiformis*, mate choice, sexual conflict, cryptic female choice, male manipulation, sexually antagonistic coevolution.

## Introduction

In the animal kingdom, females are typically the choosy sex due to the effects of anisogamy.<sup>1, 2</sup> Females prefer to mate with males which best increase their fitness and classically this is thought to be for larger males. However, some recent studies have shown that females of various taxa may mate indiscriminately or even actively discriminate against larger males.<sup>3–6</sup>

Active discrimination against larger males is likely to be the result of either a direct fitness cost to the female (e.g. increased the risk of injury)<sup>7</sup> or an indirect (genetic) cost (e.g. large size genes may reduce fitness when in female offspring).<sup>8</sup> Indiscriminate mate choice (in non-lekking species) may evolve for a number of reasons: to increase female survivorship if the costs generated by resisting a male are higher than by accepting mating,<sup>9</sup> leading to the adoption of a convenience polyandry mating system,<sup>10</sup> or to gain direct fitness

benefits from males (e.g. from donation of a prey item (material benefit polyandry)).<sup>11</sup> Indiscriminate mate choice seems particularly likely to evolve if females can subsequently bias paternity towards males with superior genes using post-copulatory mate choice, as this allows females to gain both from direct benefits (or reduced costs) and indirect genetic benefits by biasing paternity towards genetically desirable males.<sup>10</sup>

The majority of studies on Gryllid crickets (but not all),<sup>3</sup> however, show that females prefer to mate with larger males.<sup>12–14</sup> In many taxa, choice for large males is put down to one of two major explanations. The first is that females may gain more direct benefits from mating with larger males and thus by preferring to mate with larger males they will gain from an increase in fitness. This is unlikely to affect Gryllid mating systems, as males provide little nutritional resources to the female (Gryllid spermatophores are generally small, and thus consumption is unlikely to

provide any increase in female fitness<sup>12, 13, 15</sup> (but refer Wagner and Harper<sup>16</sup> and Discussion section). The second explanation suggests that females gain from mating with larger males via indirect genetic benefits.<sup>12, 13, 17</sup> Size is thought to be highly correlated with genotypic fitness, as it measures an individual's ability to withstand environmental pressures (from parasites, etc.). Individuals that are best able to deal with environmental pressures are able to allocate more resources to growth and therefore are able to achieve a larger size.<sup>18</sup> Thus, by selecting males who are large, a female is able to gain genes for her offspring which could then aid them to successfully withstand environmental pressures, enhancing the female's fitness.<sup>18, 19</sup> A large size is also thought to secondarily increase the fitness of both sexes of offspring via increased egg production in females<sup>13</sup> and greater access to females for males via enhanced fighting ability (rival displacement)<sup>3, 20</sup> (the latter was tested by this study as it has not been demonstrated in the literature for *Platygyrillus primiformis* (Prediction 1)). Thus, as genes for a large size may provide offspring with both increased environmental robustness<sup>18, 19</sup> and increased reproductive success<sup>3, 13, 20</sup> female *P. primiformis* are expected maintain a preference for large males who are more successful at fighting<sup>8</sup> (Prediction 2).

Selection for purely indirect benefits, however, is a controversial issue,<sup>21</sup> as it is often seen as a weak selective force in comparison to selection for direct benefits.<sup>22, 23</sup> This means that if indirect benefits are opposed by a direct cost, selection should act to reduce the direct cost and not favour selection for indirect benefits (producing a convenience polyandry mating system).<sup>23</sup> Investigation into this area is often confounded by direct benefits,<sup>24</sup> however, Gryllid crickets such as *P. primiformis* remove this confounding influence as the direct costs to the female associated with choosing a male (reduced foraging,<sup>25</sup> increased predation<sup>25, 26</sup> and the signal itself<sup>27</sup>) are large with little chance of compensation via any nutritional benefits gained from mating<sup>12, 13, 15, 17</sup> (but refer Wagner and Harper<sup>16</sup>). Thus, as there is a direct cost associated with choosing a male any pre-copulatory mate choice in *P. primiformis* will be maintained to gain from indirect benefits (despite the direct cost).<sup>17</sup>

### Cryptic female choice

Cryptic female choice is a controversial topic<sup>28–30</sup> due to the challenges of formally demonstrating its existence.<sup>12, 31</sup> Gryllid crickets allow cryptic female choice to be demonstrated as the male's spermatophore is attached externally onto the female's ovipositor,<sup>13</sup> meaning spermatophore attachment time (the length of time the spermatophore is attached to the females ovipositor) can be recorded. A greater spermatophore attachment time means increased paternity for the male as a greater amount of sperm is transferred to the spermatheca where it mixes randomly with stored sperm to generate a 'fertilization set' which the

female then uses to fertilize her eggs (fair raffle system).<sup>13, 32</sup> Thus, males with greater spermatophore attachment times gain a greater share of the fertilization set and thereby gain from increased reproductive success. As females can remove the spermatophore at any time, they can easily bias paternity towards certain male phenotypes,<sup>12, 14, 15, 33</sup> most likely discriminating against smaller males (Prediction 3).

A further unresolved question is whether post-copulatory mate choice acts to reinforce pre-copulatory mate choice<sup>12, 13</sup> or oppose it.<sup>34</sup> One reason for reinforcing pre-copulatory mate choice may be to 'double up' choice to counter any male manipulation at either the pre-copulatory or post-copulatory mating stages. By 'doubling up' mate choice females are able to reduce the effects any male manipulation may have. In contrast, it is difficult to see why post-copulatory mate choice should oppose pre-copulatory mate choice<sup>9</sup> (Prediction 4).

Males should attempt to oppose female post-copulatory choice by premature spermatophore removal by guarding the female (staying in close proximity after copulation and attacking the female if she attempts to move away/remove the male's spermatophore)<sup>35</sup> in order prolong spermatophore attachment (shown by a number of studies<sup>12, 14, 15, 33</sup> but not all)<sup>36</sup> (Prediction 5). An increase in the male's spermatophore attachment time due to mate guarding, however, may be a product of either male manipulation (where males prevent spermatophore removal by the female) or female choice (where females choose to extend spermatophore attachment time on the basis of the male's guarding behaviour (vigour hypothesis)). In the literature, there is support for both the vigour hypothesis<sup>15, 35, 37</sup> (where preferred males (as determined from female spermatophore attachment times when the male is prevented from guarding) guard females most vigorously) and the alternative sexual conflict/male manipulation hypothesis,<sup>12, 38</sup> (disfavoured males guard more vigorously, as they stand to lose most from cryptic female choice).<sup>12, 38</sup> The two hypotheses can be resolved by observation of mate guarding behaviour (which males guard with most vigour (vigour was not recorded by this study, see<sup>12, 35</sup> for details on the quantification of mate guarding vigour)) or by calculation of relative gains from increased attachment time as a result of guarding (as if guarding is manipulative, disfavoured males would be expected to gain more from mate guarding, but if females use mate guarding to assess a male's quality, favoured males should gain most from mate guarding)<sup>12</sup> (Prediction 6).

This study investigated how pre- and post-copulatory sexual selection has shaped mating behaviour of *P. primiformis* (Orthoptera: Gryllidae; Gryllinae)<sup>39</sup> by investigating female pre-copulatory mate choice, cryptic female choice, and possible male counter-adaptation to female choice. In addition, this investigation sheds light on a number of currently unresolved issues: if females choose

large males, if cryptic female choice exists and if post-copulatory mate choice acts to reinforce pre-copulatory mate choice, all of which are currently contested in the literature.<sup>4, 10, 29</sup>

### Predictions

The following predictions were tested by this study.

- (1) Larger males will have a higher fighting success than smaller males.
- (2) Males that are large and more successful at fighting will have a shorter latency to mating time.
- (3) Spermatophore attachment time will be longer for larger, more successful males.
- (4) Post-copulatory mate choice will enforce pre-copulatory mate choice.
- (5) Spermatophore attachment times will be longer when a male is allowed to guard.
- (6) Disfavoured males will gain relatively more benefits from mate guarding (via extension of spermatophore attachment time).

## Materials and methods

### Study animals

The stock of *P. primiformis* in this study was taken from a maintained population at the University of Derby originally collected from Westville, Durban, Natal in South Africa (Lat: 29°49'60S, Lon: 30°55'60E).<sup>37</sup> The colony of *P. primiformis* was maintained in semi-natural conditions (28°C, 12 h light: 12 h dark cycle, in plastic aquaria (30 cm × 20 cm × 15 cm) and fed on dried rat and rabbit pellets with the addition of vegetables (as a vitamin supplement).

Before all experiments, both male and female *P. primiformis* were mated once with a stock *P. primiformis* (stock males and females used were of similar size ( $0.32 \pm 0.08$  and  $0.37 \pm 0.07$  g, respectively) to avoid any effects that variation in previous mate quality may have) in order to make sure males were sexually competent<sup>14, 40</sup> and to better parallel the *in vivo* situation as females are unlikely to be virgins for the majority of copulations.<sup>13</sup> It has also been demonstrated that virgin females behave differently on their first mating as insurance against a lack of other mating opportunities<sup>14, 36, 41</sup> to the extent that males may skip part of their courtship.<sup>10</sup> Thus, by using mated females, this study minimizes the risk of misunderstanding important aspects of *P. primiformis*' mating behaviour.<sup>10</sup> This is particularly important for understanding male manipulative behaviour as on the first mating females will be less choosy<sup>10</sup> and also leave the males spermatophore attached for longer<sup>36</sup> meaning males will have less need to

manipulate the female on the first copulation than on subsequent copulations.

### Assessing male fighting ability

Eighty males were used to assess male fighting ability, split randomly into 10 groups of 8 and then weighed. Males were then chilled for 15 min at 8–10°C allowing them to be marked easily on their pronotum using non-toxic enamel paint. Males were then left for 24 h to reacclimatize before experimentation.

Two males from a marked set of eight were then placed into a small container (15 cm × 8 cm × 6 cm) and left to fight. The winner was determined by the male that initiated three attacks in which the opponent retreated (the fighting behaviour in *P. primiformis* is highly ritualized (similar to *Gryllus bimaculatus*)<sup>20</sup> with the winning male producing a victory song). The remaining six males were then paired and fought in the same way to produce four winners and four losers. The four winners were then paired up to fight and the same for the losers. Following the second fight, crickets with the same fight outcomes from the two fights were then placed together for a third fight, allowing the males to be placed into fighting classes based on number of fights won (3,2,1, or 0).<sup>3</sup> The weight of the males in different fighting classes was then compared using one-way ANOVA to test Prediction 1. Pairing males with the same fight history controls for any order effects fight history may have had on a males fighting ability.<sup>42</sup> The males used here to assess fighting ability were then subsequently used in mate trials (below).

### Mate trials

The *P. primiformis* used in this study were all of a similar age, as a number of studies have shown that female crickets prefer older males, regardless of size.<sup>18, 43</sup> Before the beginning of the mate trials, each male was placed into a container with a mesh barrier which physically separated the male from the female (subsequently excluded from mate trials) but still allowed visual and chemical communication. This was to ensure each male had a spermatophore ready to transfer (indicated by the male stridulating) to ensure that none of the males used would be delayed in mating as a result. Female weight was standardised ( $0.34 \pm 0.02$  g) as males have been shown to exhibit mate choice for larger females in a number of Orthopteran taxa including *G. bimaculatus*<sup>44</sup> and *P. primiformis*<sup>13</sup> possibly related to the cost of spermatophore production.<sup>45</sup> As this study is concerned with female choice, this variable was eliminated.

### Pre-copulatory choice

Seventy-four males that had been ranked into fighting classes (based on the number of fights won (3, 2, 1, 0) (above)) were used to investigate pre-copulatory choice. This was done by introducing a ranked male to a randomly selected stock

female ( $0.34 \pm 0.02$  g) into a small container (15 cm  $\times$  8 cm  $\times$  6 cm) and recording the latency to mating time (from introduction to copulation) to test Prediction 2. Six males from the 80 used in the fighting trials (above) were not used to establish this relationship due to death (meaning only 74 of the males (10, 29, 26, and 9 males in fighting Classes 3, 2, 1, and 0, respectively) could be used to establish this relationship.

This study used latency to copulation as a measure of attractiveness, with short latency times indicating greater attractiveness. This method of assessing attractiveness is closer the situation faced by females *in vivo*<sup>46</sup> than studies that determine female preference by presenting two males simultaneously<sup>47, 48</sup> as male Gryllid crickets do not tolerate the presence of rivals.<sup>40, 46, 49</sup>

### Post-copulatory choice

Spermatophore attachment time was also measured after recording the latency to mating time for 50 of the males used in the pre-copulatory trials (above) (spermatophore attachment time was not recorded for three sets of males used in the pre-copulatory mate trails (above) due to time constraints) to test Predictions 3 and 4.

The male's presence was varied during this study, either removing the male directly after spermatophore transfer or leaving the male with the female until the spermatophore was detached, to test the effects of mate guarding (Predictions 5 and 6). Each male was used in each treatment only once with 24 h in-between to recover, females however were not reused. Whether a male was left to guard a female first or removed first was varied in each set of mating trials to counter any order effects (e.g. ageing of the males).<sup>25, 43</sup>

### Statistical analysis

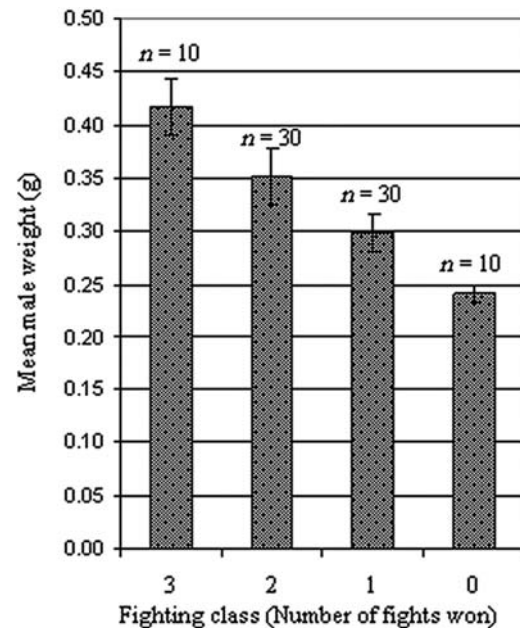
All statistical analyses (Dunn-Sidak, one- and two-way ANOVAs) were performed using SPSS v16.

## Results

### Fighting ability of male *P. primiformis* in relation to weight

Heavier males were found to have a higher fighting success (Fig. 1) supporting Prediction 1. One-way ANOVA showed this result was statistically significant ( $F_{3,76} = 139.59$ ,  $P < 0.01$ ).

*Post hoc* (Dunn-Sidak) analysis showed that the males' weights were significantly different in each fighting class ( $P < 0.01$  in all instances), showing that a male's weight is an accurate predictor of his fighting success.



**Figure 1.** Relationship of male weight (mean  $\pm$  standard deviation) to the number of fights won, showing that heavier males were more likely to win fights.  $n$  = number of individual males in each treatment (total number of males used = 80).

### Attractiveness of male *P. primiformis* as determined by a no-choice test

The most successful fighting males (those that won the most fights) were shown to have shorter 'latency to mating' times (Fig. 2) and thus considered to be more attractive to females (Prediction 2).<sup>3, 12</sup> One-way ANOVA showed this relationship to be statistically significant ( $F_{3,70} = 170.75$ ,  $P < 0.01$ ).

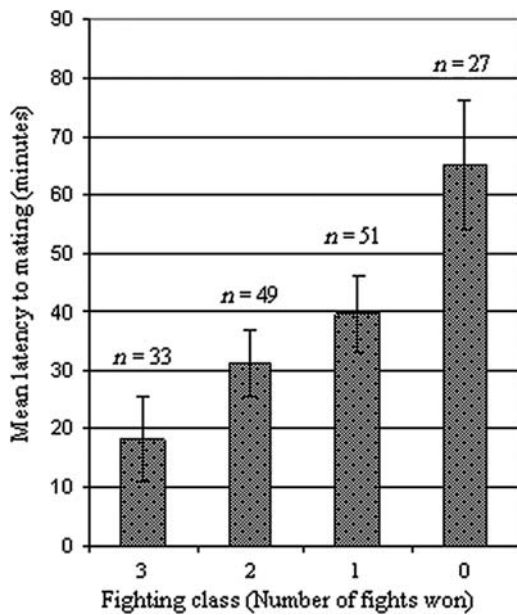
*Post hoc* (Dunn-Sidak) analysis showed that there was a significant difference in latency to mating time between each fighting class ( $P < 0.01$  in all instances).

### Spermatophore attachment time in relation to male fighting ability and mate guarding

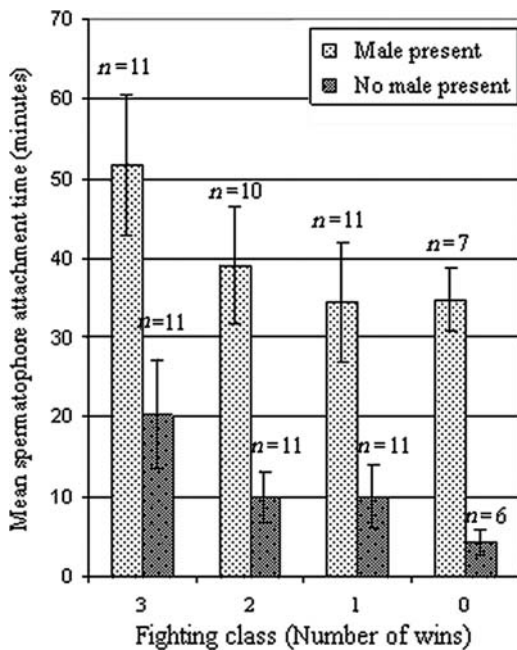
Females left spermatophores from males in higher fighting classes attached for longer (Prediction 3) (Fig. 3), reinforcing pre-copulatory mate choice (Prediction 4). This study also found that females left spermatophores attached for longer with a male present (Prediction 5) (Fig. 3). Two-way ANOVA showed both mate guarding ( $F_{1,92} = 451.42$ ,  $P < 0.01$ ) and fighting class ( $F_{3,92} = 20.27$ ,  $P < 0.01$ ) has a significant effect on spermatophore attachment time. Please note each male was used once in each treatment (guarding or not guarding) to ensure data was independent and comparable across the treatments (see Materials and Methods section for details).

Dunn-Sidak analysis showed that there was only a significant difference in spermatophore attachment time between fighting Class 3 and the other classes ( $P < 0.01$ ), with no





**Figure 2.** Relationship between fighting class and latency to mating time (mean  $\pm$  standard deviation) showing a shorter latency to mating times for more successful fighting males. *n* = number of individual males in each treatment. Total number of individual males used = 74.



**Figure 3.** Relationship of spermatophore attachment time (mean  $\pm$  standard deviation) to both fighting class and male presence (after spermatophore attachment), showing a decrease in attachment time, with a decrease in fighting class and with male absence. *n* = number of males in each treatment (each male was used in each treatment once). Number of individual males and females used in trials = 50.

**Table 1.** Average increase in spermatophore attachment time as a result of mate guarding

	Fighting class (number of wins)			
	3	2	1	0
Average increase in spermatophore attachment time (min)	31.4	29.11	24.49	30.41
Average increase in spermatophore attachment time (% increase)	154	291	245	703

significant difference between the other classes ( $P > 0.05$ ). This trend was shown both when males were present or absent after spermatophore attachment, showing that the two factors (fighting class and male presence) are independent (interaction was found not to be statistically significant in the two-way ANOVA ( $F_{3,92} = 1.47, P = 0.23$ )).

This study also found no significant difference between fighting classes in the amount spermatophore attachment time increased as a result of mate guarding using one-way ANOVA ( $F_{3,46} = 1.78, P = 0.164$ ); each class of male gained a similar increase in spermatophore attachment time as a result of guarding (Table 1). A significant difference was, however, found between fighting classes in the relative gain in spermatophore attachment time (per cent increase in attachment time) as a result of mate guarding using one-way ANOVA ( $F_{3,46} = 14.18, P < 0.01$ ), with relative gains being significantly higher for less successful, smaller males (Table 1), providing support for Prediction 6. Please note each male was used once in each treatment (guarding or not guarding) to ensure data was independent and comparable (as above).

## Discussion

### Male-male Interactions

As predicted larger males won more fights<sup>3, 20</sup> and can therefore potentially gain from increased access to females by exclusion of smaller rivals. Size is also likely to be highly correlated with genotypic fitness<sup>18, 19</sup> and increased egg production in females<sup>13</sup> which may account for why females maintain a strong selection for large males (below).<sup>8</sup>

### Pre-copulatory mate choice

Males that were more successful at fighting had shorter latency to mating times, supporting the classic idea that females prefer to mate with larger males.<sup>13, 45, 50</sup> This study also supports the idea that female preference for large males is maintained to gain from indirect genetic benefits, as it seems highly unlikely that females gain any direct benefits from mating, due to the small size of the males' spermatophore<sup>13</sup> (if benefits are gained they are unlikely to compensate costs caused by the males'

courtship,<sup>25–27, 51, 52</sup> and frequently spermatophores were simply detached and left on the substrate rather than consumed). Thus, as females were shown to mate discriminately for no known direct benefits, the most plausible reason for female preference for large males in *P. primiformis* is to gain from indirect genetic benefits (but see below).

### Pre-copulatory sexual conflict

Female choice for large males creates a selection pressure on smaller, disfavoured males to override a female's choice and manipulate the female into mating sub-optimally.<sup>53</sup> This is supported by this study because if females were free to simply choose the best males, then males of a lower quality should have been ignored indefinitely (as females were not virgins).<sup>54</sup> As even the smallest males were able to secure matings it suggests that female choice may be compromised by male manipulation, however further study is required to confirm this.

### Problem of indirect benefits

As already stated females in this study are likely to be choosing males for indirect genetic benefits, however indirect benefits are seen as a weak selective pressure and thus easily opposed by direct fitness costs.<sup>22, 23, 38</sup> As female crickets appear to suffer greatly from the effects of male courtship,<sup>37, 52, 55</sup> incurring a number of direct fitness costs (e.g. reduced foraging<sup>25</sup>, increased risk of parasitism (particularly from phonotactic dipterans),<sup>51, 56</sup> predation,<sup>25, 26</sup> or simply by the signal itself<sup>27, 52</sup>), selection should act against the maintenance of pre-copulatory mate choice (favouring convenience polyandry).<sup>13</sup>

An explanation for why female *P. primiformis* maintain a pre-copulatory mate choice for indirect benefits is thought to be due to one of the following: (i) copulation with males is costly and thus females reduce direct costs by being selective, (ii) females gain direct benefits from mating with superior males, or (iii) the direct costs from mating are low.

The total cost of mating in *P. primiformis* was not quantified in this study nor available in the literature, however studies on similar species (e.g. *G. bimaculatus*)<sup>27</sup> have shown that the largest cost associated with mating arises from courtship (rather than copulation or the ejaculate) suggesting that mate choice is not maintained to avoid mating costs. It is possible that females may gain direct benefits from larger males and not smaller males (benefits must differ between sizes of male otherwise a convenience polyandry system would be expected to evolve) from greater hatching success, however, where this is the case it is often as a result of larger males providing fertility benefits to females<sup>16, 57</sup> which *P. primiformis* do not appear to provide,<sup>13</sup> making it unlikely that females gain direct benefits from large males (although further study is still needed to confirm this). As this study was conducted *in vitro*, females and males were kept in close proximity during experiments

meaning females could not escape from disfavoured males. If females were free to move away from disfavoured males (as *in vivo*), it is likely that costs of resisting disfavoured males would be greatly reduced. Thus, this study suggests that *P. primiformis* females select large males for indirect benefits, despite possible direct costs however these are likely to be much reduced *in vivo*.

### Post-copulatory mate choice

Males that were more successful at fighting were shown to have longer spermatophore attachment times both in the presence and absence of a guarding male,<sup>12–14, 37</sup> and thus are likely to gain from increased paternity.<sup>32, 58</sup> This shows that females can bias paternity using premature spermatophore removal,<sup>12, 14, 15, 33</sup> and in doing so amplify pre-copulatory mate choice.<sup>12, 13</sup> There does not appear to be any direct costs or benefits to the female associated with spermatophore attachment time in Gryllid crickets, either from the transfer of the full ejaculate<sup>27</sup> or premature spermatophore removal (e.g. via reduced clutch hatching success,<sup>13</sup> although this may play a role in other species)<sup>16</sup> supporting the idea that female choice is for indirect benefits (although further work is needed to confirm this).

### Post-copulatory sexual conflict

In absence of a mate guard, only the largest males that had been the most successful at fighting (Class 3) had a significantly increased spermatophore attachment time, showing that females discriminate highly against smaller males, creating a selection pressure on disfavoured males to oppose female choice. This study showed that mate guarding increased spermatophore attachment time and thus appears to represent a male counter-adaptation to cryptic female choice.<sup>12, 14, 15, 33</sup> There are two hypotheses suggested for how this behaviour influences the female to prolong the attachment of the spermatophore, the vigour hypothesis or sexual conflict hypothesis (see Introduction section), and this study supports the latter. This is because smaller males gained relatively more from mate guarding (703% increase in spermatophore attachment time compared 154% for the largest males, despite the fact that absolute increase in attachment time is similar for all males), contrary to what would be expected if females used mate guarding to further assess a male's quality.<sup>12, 38</sup> Mate guarding intensity (the amount of mate guarding behaviours (e.g. body judders)<sup>35</sup> performed over a particular time period) is also predicted to be higher in smaller males which could be confirmed by quantification of mate guarding intensity (as has been demonstrated in *Teleogryllus commodus*).<sup>12</sup>

As mate guarding was found to increase spermatophore attachment time by a similar amount for all classes of male it appears that female *P. primiformis* may have evolved resistance to the males' counter-adaptation. The reasons for suspecting this is that although males do actively guard

females, they do not seem to be able to prevent spermatophore removal. Often when the female attempts to remove the male's spermatophore it goes unheeded, and on the occasions when it is challenged (by aggressive attack from the male) the spermatophore is often removed anyway (usually by female rubbing her ovipositor on the substrate). This shows that males do not directly affect the females' choice of when to remove the spermatophore. This disagrees with several other studies, which show that males can actively prevent females from removing their spermatophore,<sup>12, 35, 36</sup> however the mate guarding behaviour for *P. primiformis* has not been detailed in the literature and so may they be unique in this respect. Males may still gain benefits from guarding, despite not altering the female's choice, by preventing rival males from mating with the female (rival exclusion)<sup>36, 55, 59</sup> or possibly from the opportunity to remate with the female (spermatophore renewal).<sup>36, 59</sup>

Thus, even though males transfer more sperm by mate guarding, females are still able to bias paternity towards attractive males. Males continue to guard females, as females discriminate highly against males that do not guard and also may gain from other routes (rival exclusion or spermatophore renewal).

## Conclusion

In conclusion, this study showed that female *P. primiformis* prefer to mate with larger, males that were more successful at fighting. This choice is reinforced by post-copulatory mate choice whereby females left the spermatophores from larger males attached for significantly longer (demonstrating cryptic female choice).

Mate guarding was shown to increase spermatophore attachment time and appears to have evolved due to conflict over spermatophore attachment times (as the optimal spermatophore attachment time for males is longer than that of non-virgin females)<sup>10</sup> rather than a way for a females to assess a male's vigour as a basis to extend his spermatophore attachment time. Thus, mate guarding in *P. primiformis* appears to represent a male manipulation to increase paternity (via increased spermatophore attachment time) by opposing female choice. Females appear to have evolved resistance to the male's manipulation however, as although spermatophore attachment times were increased by mate guarding, it was by a similar amount for all sizes of male, meaning females retained their original choice.<sup>35</sup>

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