



## Can females choose to avoid mating failure in the seed bug *Lygaeus simulans*?



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It is becoming increasingly clear that copulation does not necessarily always lead to offspring production in many organisms, despite fertilization success presumably being under both strong natural and sexual selection. In the seed bug *Lygaeus simulans*, between 40% and 60% of copulations fail to produce offspring, with this 'mating failure' representing a significantly repeatable male-associated trait. Mating has been demonstrated to be costly in this species and, as such, we might expect females to minimize the chance of mating failure by displaying a preference for males with higher insemination success where possible. After assaying males for mating failure, we asked whether females preferred males with a history of successful inseminations versus unsuccessful inseminations in pairwise mate choice trials. Contrary to our expectations, females showed no preference for more successful over less successful males. Moreover, females showed no preference for larger males in the choice trials, even though larger males were significantly more likely to successfully inseminate females in the initial assay. This apparent lack of female precopulatory choice suggests that postcopulatory choice mechanisms may be key to mating failure in this species. However, this does not necessarily explain why females pay the cost of mating with males they will then reject via postcopulatory processes. More generally, our results suggest that mating failure may play a largely underappreciated role in mating systems evolution, influencing both the cost of choosiness, and the costs and benefits of polyandry.

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Successful insemination is under both natural and sexual selection. Despite this, nontrivial levels of infertile matings have been documented across a range of taxa (Garcia-Gonzalez, 2004; Greenway, Dougherty, & Shuker, 2015; Tyler & Tregenza, 2013) and may reach levels of 60% in some species (Tadler, Nemeschkal, & Pass, 1999). Considering that the primary function of mating is the transfer of sperm, this poses somewhat of an enigma. In light of the well-documented costliness of mating (Arnqvist & Nilsson, 2000), if these mating failures incur equivalent costs to fertile matings, but with none of the accompanying reproductive success, then individuals should be under selection to minimize their occurrence and avoid them where possible. Mating failures have a wide range of causes, from morphological and genetic incompatibility between mating partners, through to individual infertility (Garcia-Gonzalez, 2004; Greenway et al., 2015; Hasson & Stone, 2009). As such, precopulatory mate choice for fertile or compatible mates represents a

potentially vital defence against mating failure, preventing the compromise or elimination of an otherwise fertile individual's reproductive success through mating with an infertile or sperm-depleted partner.

Despite the fact that functional fertility itself is often not apparent until mating is underway or over, precopulatory mate choice favouring fertile individuals of both sexes has been documented in some species, perhaps most explicitly in the parasitoid wasp *Nasonia vitripennis*. Male *N. vitripennis* sex pheromone titre correlates with sperm load (Ruther, Matschke, Garbe, & Steiner, 2009), meaning that preference for high pheromone titres enables females to minimize the risk of mating with a sperm-depleted male. Such covariance between phenotype and functional fertility provides a basis for the phenotype-linked fertility hypothesis (Sheldon, 1994), and enables precopulatory mate choice to function as a fertility assurance strategy.

Examples of traits under precopulatory selection also correlating with functional fertility traits include the eye-stalk length of the stalk-eyed fly, *Teleopsis dalmanni*, which correlates with male testes size (Cotton, Small, Hashim, & Pomiankowski, 2010), and male coloration in the Trinidadian guppy *Poecilia reticulata*, which

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is associated with sperm swimming speed (Locatello, Rasotto, Evans, & Pilastro, 2006). In extreme cases such as in species with disassociated sperm transfer, for example, the springtail *Orchesella cincta*, female mate choice may occur directly on the spermatophore itself, bypassing mate assessment via male secondary sexual characteristics altogether (Zizzari, Braakhuis, van Straalen, & Ellers, 2009).

In reality, male reproductive quality is unlikely to be reflected by a single trait and, equivalently, female choice is likely to be more nuanced and based on assessment of a number of traits, some of which may not be assessable until mating is underway (Candolin, 2003). However, mate choice for fertility benefits can still be selected for. This is evident in the field cricket *Gryllus lineaticeps*, in which different elements of male song correlate with different mating benefits. In this species, females express a mating preference based on male chirp rate (correlated with increased fertility benefits), over an alternative signal of chirp duration (associated with longevity benefits; Wagner & Basolo, 2007). Thus, obtaining sufficient high-quality ejaculate appears to be prioritized by females over other direct benefits. Alongside male secondary sexual characters, individuals may use social cues to avoid potentially sperm-depleted mates: *P. reticulata* females have been shown to discriminate against males they have recently observed sexually interacting with another female (Scarponi, Chowdhury, & Godin, 2015), while female cockroaches (*Nauphoeta cinerea*) prefer males that have mated or associated with fewer females (Harris & Moore, 2005).

As outlined by Parker (1983), choosiness should be favoured when there is variation in mate fertility (or 'quality' more broadly) across a population. In addition, precopulatory choosiness should be promoted when both the costs of mating and the mate encounter rate are high (Bleu, Bessa-Gomes, & Laloï, 2012; Gowaty & Hubbell, 2009; Kokko & Monaghan, 2001). However, if there are no salient indicators of fertility or compatibility available to individuals before mating is underway, precopulatory choosiness loses its advantage (Parker, 1983). Under these conditions, postcopulatory mate choice, typically in combination with polyandry, may be required to minimize the likelihood of mating failure and maximize reproductive success (Colegrave, Kotiaho, & Tomkins, 2002; Pai, Bennett, & Yan, 2005; Reding, 2015; Török, Michl, Garamszegi, & Barna, 2003). Determining when mate choice decisions occur over the course of a reproduction event (i.e. before or after mating) and, more importantly why, is crucial to understanding the evolution of mating systems.

Here we test whether female *Lygaeus simulans* use precopulatory choice to discriminate against males that have previously failed to sire offspring in favour of more successful males. Mating failure is very common in this species, with between 38% and 60% of pairings not resulting in sperm transfer or producing offspring (Dougherty & Shuker, 2014; Greenway & Shuker, 2015; Tadler et al., 1999). *Lygaeus simulans* male mating failure is significantly repeatable ( $r = 0.415$ ,  $P = 0.001$ ; Greenway & Shuker, 2015) and there is also high variance in overall male reproductive success, with around 26% of males not fathering offspring given multiple opportunities with independent females (Greenway & Shuker, 2015). In that study, male overall reproductive success was bimodally distributed, as individuals consistently failed or succeeded over the course of up to four matings to father offspring irrespective of mating number, with experience of prior mating interactions having no overall effect on mating outcome (Greenway & Shuker, 2015). Despite being polyandrous, matings are costly to females, reducing both their longevity and fecundity (Evans, 2011; see also Shuker, Ballantyne, & Wedell, 2006 for data from the sibling species *Lygaeus equestris*). In addition to these documented costs, matings in this species are nontrivial in length, typically lasting upwards of

4 h and for as long as 16 h (Micholitsch, Krugel, & Pass, 2000). With these potential drivers of mate choice in mind, we used mating failure assays followed by pairwise choice trials to explicitly test whether females mate nonrandomly with respect to the outcomes of a male's previous mating attempts; put simply, do females avoid males characterized by 'mating failure'?

## METHODS

### Study System and Husbandry

*Lygaeus simulans* is an aposematic species of seed predator, with a European-wide distribution, frequently co-occurring with its sister species *L. equestris* (Solbreck, Olsson, Anderson, & Förrare, 1989). After reaching reproductive maturity at around 7 days posteclosion, females exhibit high levels of polyandry and lay multiple clutches of eggs over the course of their lifetime (for general lygaeid ecology see Burdfield-Steel & Shuker, 2014). In the wild, adults and nymphs are found at high densities (up to 100 individuals per square metre: Solbreck & Kugelberg, 1972). They form aggregations when reproductively active both as the hibernation period comes to an end and once migrated back to feeding and breeding sites, where they feed on patchy and ephemerally distributed host plants (Solbreck & Kugelberg, 1972). Mating rates are high under natural conditions, as in the laboratory, with upwards of 30% of *L. equestris* individuals observed in copula at any one time during peak mating periods in the field (Solbreck, 1972). Copulation in *L. simulans* appears to induce oocyte maturation and oviposition (Tadler et al., 1999), although reproductively mature unmated females occasionally lay unfertilized eggs (Kugelberg, 1973; E. V. Greenway, personal observations).

For this experiment, we used individuals from a *L. simulans* laboratory population, originating from bugs collected in Tuscany, central Italy in 2008 and 2009, and maintained at 29 °C and a 22:2 h light:dark cycle to prevent reproductive diapause. After isolation as fifth-instar nymphs from our stock population, newly moulted adult virgin males and females were sorted by sex and incubated in plastic tubs (108 × 82 × 55 mm) with ad libitum dehusked sunflower seeds (*Helianthus annuus*: supplied by Goodness Direct, [www.goodnessdirect.co.uk](http://www.goodnessdirect.co.uk)) and plastic tubes of demineralized water at maximum densities of 10 bugs per tub until sexual maturity.

### Mating Failure Assay

To assay males for a mating failure phenotype, we paired sexually mature focal males (7–10 days posteclosion) with randomly assigned virgin females on two successive days. On each day, males were placed in individual 55 mm diameter petri dishes with a female. They were then checked at 10 min intervals for 450 min, during which they were classified as being in copula if they were observed in a characteristic back-to-back mating posture. As a minimum mating duration of 30 min is required for complete intromission of the male's genitalia (Micholitsch et al., 2000), only individuals that were observed copulating at three or more consecutive checks were classified as having mated. As soon as copulations had terminated, the male and female were separated to prevent remating. Any copulations that had not terminated naturally by the end of the observation period were ended manually, by gentle brushing with a fine paint brush. All males that mated were kept individually overnight in petri dishes, with three sunflower seeds (representing ad libitum food) and a ball of cotton wool soaked in distilled water for moisture. The following day, every male was then paired with another virgin female and the

procedure repeated. All trials were carried out at room temperature (22–25 °C) under natural light.

After each mating trial, females were placed in individual tubs with a tube of distilled water and ad libitum sunflower seeds to enable oviposition, and then frozen after 7 days. Any eggs produced were counted and returned to the incubator for a further 7 days before being scored for the presence or absence of nymph production, and the number of nymphs if present. Fertilization success (or lack thereof) from these two matings was then used to assign focal males post hoc to one of three statuses: ‘failed’ if they fathered no offspring; ‘intermediate’ if offspring were produced from one mating; and ‘successful’ if offspring were produced from both matings ( $N = 296$  males in total).

#### Quantifying Sperm Transfer

After storage at  $-18$  °C, we dissected out the reproductive tracts of 284 females that failed to produce offspring from the initial mating failure assay to confirm the presence or absence of sperm transfer, along with 41 females that had produced offspring. The spermatheca was removed from each female and placed on a glass slide under a coverslip with a drop of saline solution. It was then examined and scored for the presence or absence of sperm under  $400\times$  magnification using a light microscope, blind to the mating outcome. During dissection, females were also qualitatively scored for the presence or absence of mature oocytes in the ovarioles (see Fig. 1).

#### Mate Choice Trial

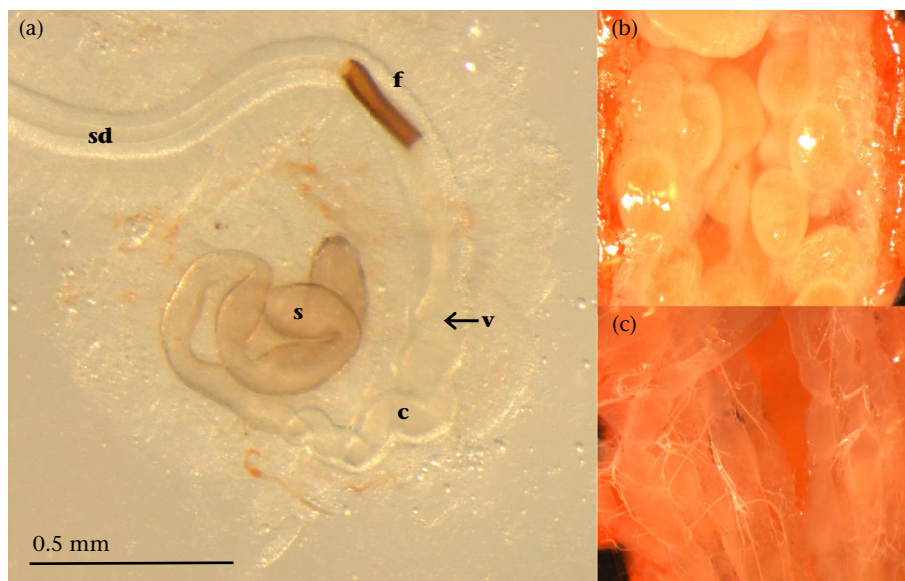
Using a random number generator, males that had mated on both days 1 and 2 were randomly paired together in 55 mm petri dishes on day 3. Through the nature of the experimental design, pairing was blind to prior male reproductive success, as the outcome of the mating failure assay was not apparent for a further 13 days after the choice trial (see above). A spot of white acrylic

paint was applied to either the left or right hand side of the pronotum of each male in order to identify it. A virgin female was then placed in the petri dish with each pair of males ( $N = 142$  trials). We observed bugs continuously for 120 min and scored them at 1 min intervals for mating attempts (genital–genital contact) and matings (back–to–back positioning) between each male and the focal female, before separating all bugs and euthanizing them. As well as the identity of the first male to mate, the delay between the start of the trial and an observation of mating was recorded to determine mating latency.

#### Statistical Analysis

Having assigned males to mating failure status categories dependent on their performance in the mating failure assay, we used binomial tests to establish whether females in the choice trials mated nonrandomly with respect to male failure status (i.e. whether they deviated significantly from the null proportion of matings with higher ranked males of 0.5). In addition, we examined a subset of these choice trials in which females were faced with a choice between ‘failed’ and ‘successful’ males (i.e. males that failed in both of their assays, and males that succeeded in fathering offspring in both of their assays). We then tested whether mating latency (time taken for a mating to occur) in choice trials differed depending on the failure status of the chosen male in relation to its competitor (i.e. more or less successful in the assay phase). Mating latency was non-normally distributed and thus log transformed, before analysis using one-way ANOVA with ‘male status relative to rival’ as the explanatory variable.

To establish whether females displayed any preference based on male size (as in the sister species *L. equestris*: Dougherty & Shuker, 2014), the body lengths of 237 males were measured, from snout tip to the end of the underside of the abdomen, using an eyepiece graticule and dissecting microscope. First, we checked the repeatability of these measurements by remeasuring 52 males blind to the previous measurement, and found it to be very high (ANOVA



**Figure 1.** Components of the female reproductive tract of *Lygaeus simulans*. (a) The spermathecal duct and spermatheca. During copulation, the male processus (intromittent organ) passes along spermathecal duct (sd), reaching a valve-like structure (v), which leads to a convoluted tube (c) anterior to the spermatheca (s) itself. To assess whether sperm transfer had occurred, the spermatheca was placed on a slide under a coverslip and viewed at higher magnification under a light microscope to check for the presence of sperm, which forms visible strands (Micholitsch et al., 2000). In this individual, a female taken from our stock population, the spermathecal duct contains a fragment (f) of the male intromittent organ, which has presumably broken off during copulation. No processus fragments were found when dissecting experimental females, although minor processus breakages appear to be relatively common in this species (Dougherty & Shuker, 2015). (b) Ovarioles containing mature oocytes. (c) Ovarioles containing immature oocytes.

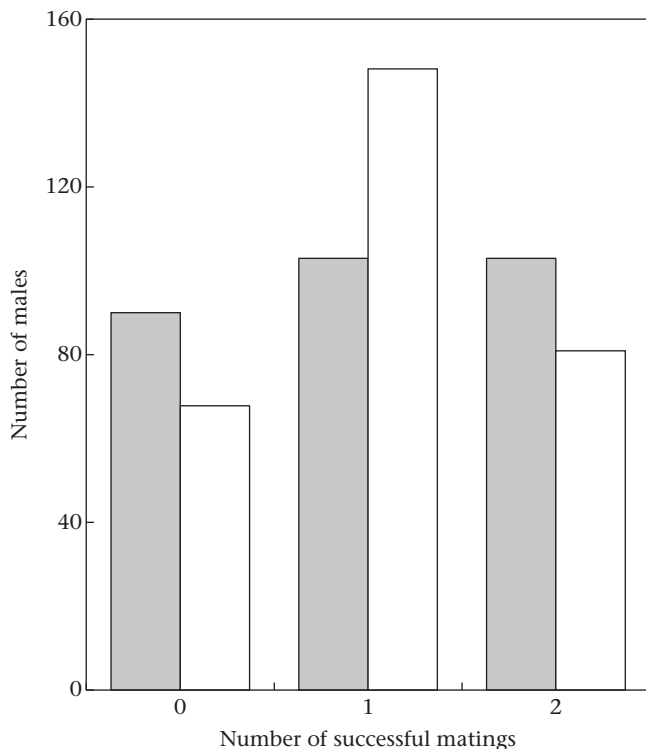
method:  $R = 0.98$ ,  $P = 0.001$ ). To test for potential effects of male body length on mating failure, we used a generalized linear model (GLM) with a quasi-binomial distribution and logit link (accounting for overdispersion), with mating outcome as the response variable. We then performed binomial tests to establish whether females mated nonrandomly with respect to male body length, both in all trials and in a subset of trials in which the two males differed in size by 0.5 mm or more. All statistical analyses were carried out in R v.3.0.2 (R Core Team, 2013).

## RESULTS

### Mating Failure Assay

Of the 592 matings that occurred in the assay phase, 283 failed to result in offspring production (an overall mating failure rate of 47.8%). Of these 283 failed matings, 147 (51.9%) females did not oviposit, while the remaining females laid infertile eggs. Upon dissection, an almost identical proportion of individuals in each of these failure categories had mature oocytes present in their ovarioles (recorded in 53.7% of females that oviposited versus 54.5% of females that did not). Therefore, at least in terms of oocyte production and maturation, there was no difference in female reproductive competence between individuals that failed to oviposit and those that did. However, of all females that failed to produce offspring, only 7.4% had sperm present in their spermatheca, compared to 100% of a sample of females that successfully produced offspring. Thus, reproductive failure appears to be driven by lack of sperm transfer rather than female infertility.

Mating failures were nonrandomly distributed among the 296 double-mated males, with more individuals falling into the 'failed' or 'successful' categories than expected by chance (30.4% of males 'failed', 34.8% were 'intermediate' and 34.8% were 'successful', i.e.

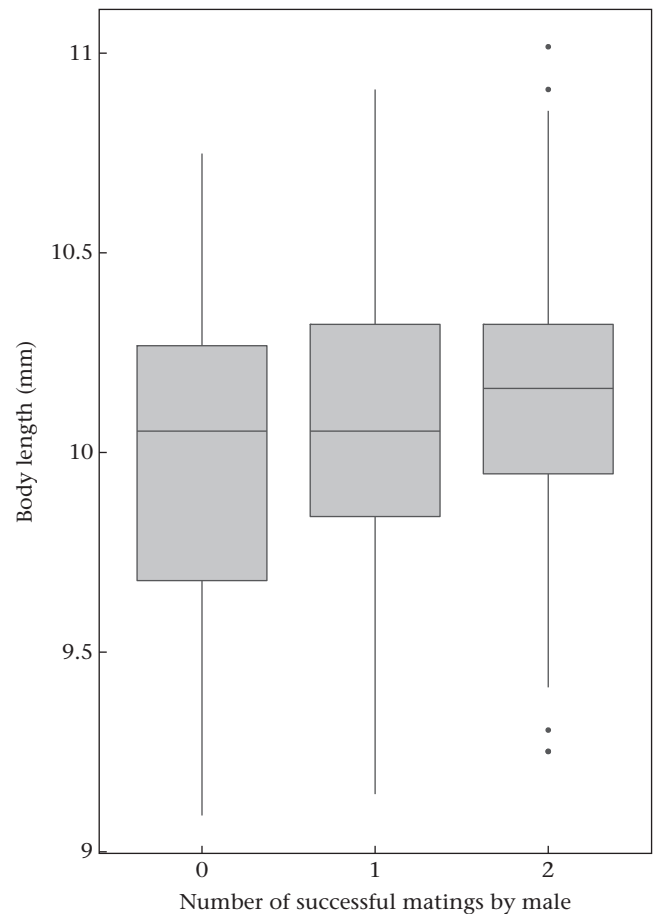


**Figure 2.** The distribution of observed (grey) and expected (white) successful matings from the mating failure assay ( $N = 296$ ).

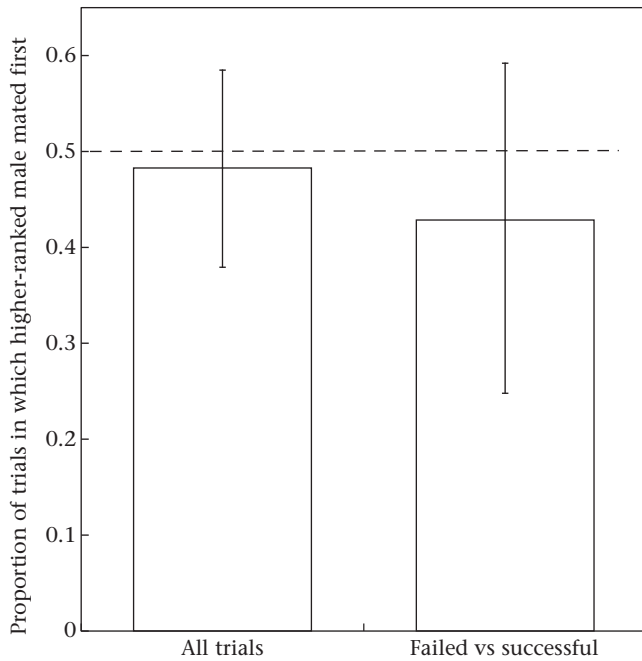
an overdispersed distribution: chi-square test:  $\chi^2_2 = 13.87$ ,  $P = 0.001$ ; see Fig. 2). Focusing on the 103 'intermediate' status males, mating failure was significantly more likely to occur in the first assay trial than in the second (66 versus 37 failures; binomial test:  $P = 0.006$ ). This is in line with Greenway and Shuker (2015), who reported a small increase in offspring production between a male's first and second matings, although there was no overall increase in male reproductive success with experience across three or four successive matings; i.e. the number of previous matings that a male had did not influence mating failure (see Greenway & Shuker, 2015, and their Figure SI.1 for further details). There was also a significant effect of male body length on mating outcome, with larger males having greater success (GLM with quasi-binomial distribution and logit link function:  $\chi_{1,235} = 6.54$ ,  $P = 0.025$ ; Fig. 3).

### Mate Choice Trial

In the subsequent mate choice phase, the focal female mated with one of the two assayed males she was presented with in 118 of the 148 trials mate choice trials conducted. However, females mated randomly with respect to male failure status, pairing with the more successful male in 48.3% of choice trials (binomial test:  $P = 0.83$ ; see Fig. 4). Similarly, in the subset of trials containing only 'successful' and 'failed' males, females again exhibited no preference (binomial test:  $P = 0.57$ ; Fig. 4). There was also no effect of male failure status relative to their rival on the latency to mate in



**Figure 3.** Effect of male body length on mating outcome in the mating failure assay. Upper and lower box bounds correspond to the first and third quartiles, with the horizontal midline denoting the median; whiskers represent  $1.5 \times$  interquartile range. Points represent outliers.



**Figure 4.** Effect of male failure status on the likelihood of mating in the choice trials. 'All trials' refers to all outcomes from the 88 trials in which the focal female mated when presented with a pair of males of unequal mating failure status (i.e. 'failed' versus 'intermediate'; 'failed' versus 'successful'; 'intermediate' versus 'successful'; see main text for definitions). 'Failed vs successful' represents a subset of 28 choice trial outcomes in which 'failed' males were paired with 'successful' males. The dashed line represents random mating with respect to male failure status. Error bars are 95% binomial confidence intervals.

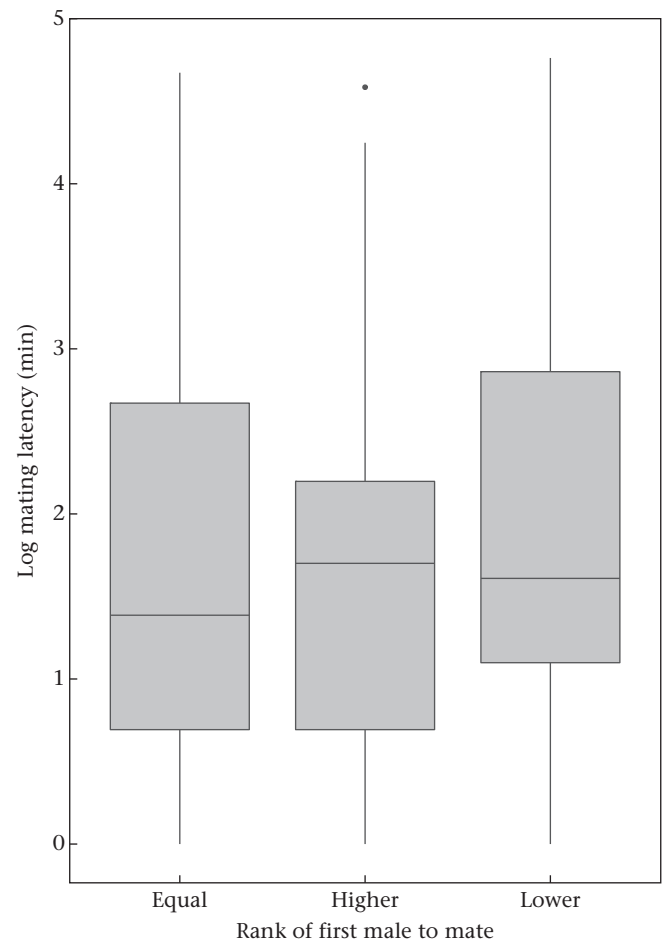
these choice trials (log-transformed latency, ANOVA:  $F_{2,80} = 0.273$ ,  $P = 0.76$ ; Fig. 5).

Although male body length had a significant effect on mating outcome in the assay phase, females showed no significant preference for the larger male in the choice trial, mating in 57 out of 116 trials with the larger male (binomial test:  $P = 0.93$ ; Fig. 6). Using a subset of 37 trials with a minimum body length difference of 0.5 mm between the two males (which represents  $1.3\times$  the standard deviation of sampled population body length), we again found no significant female preference for larger males (binomial test:  $P = 0.51$ ; Fig. 6). While females mated with one of the males in the majority of trials, no matings occurred in 26 cases. This is despite one or both males attempting to mate with the focal female in 50% of these particular trials, and suggests that females were able to avoid mating with both males in some or all circumstances if they preferred to.

In terms of male–male interactions observed during choice trials, males directed no apparent aggressive or contest behaviour towards one another. Occasionally (in 24 out of 118 trials), both males attempted to mate with the focal female simultaneously prior to one successfully coupling. Furthermore, in 26 of 118 trials, the 'unsuccessful' male attempted to mate with the focal female once she was in copula with the 'successful' male. However, such attempts did not interrupt and terminate mating, except potentially in two instances in which copulation ceased immediately following a mating attempt by the 'unsuccessful' male.

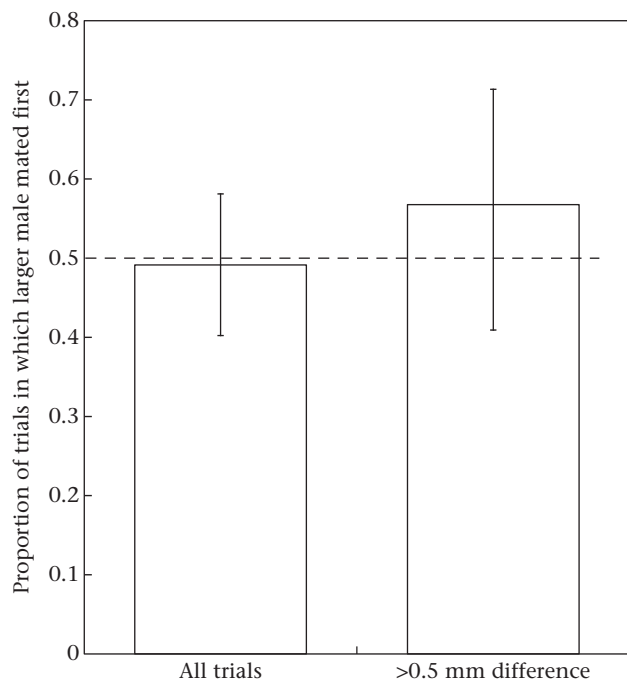
## DISCUSSION

In spite of high variation in mating failure, caused predominantly by a lack of sperm transfer, *L. simulans* females showed no preference for previously successful males over previously less



**Figure 5.** Choice trial mating latency ( $N = 118$ ) in relation to relative mating failure status of the paired choice trial competitors. Upper and lower box bounds correspond to the first and third quartiles, with the horizontal midline denoting the median; whiskers represent  $1.5\times$  the interquartile range. Points represent outliers.

successful males. Even when examining the subset of trials in which females were given a choice between males that had never fathered offspring and those that always did across two assay trials, we observed no pattern of nonrandom mating. This is despite a significant effect of male body length on mating outcome, which could potentially be used as an indicator of male reproductive quality. Females showed no preference for larger males in pairwise trials, suggesting they either do not or are unable to use this potentially informative phenotypic variation to increase their chances of being inseminated. Although the experimental design used does not preclude potential male–male competition from occurring before mating, the mate choice paradigm used (i.e. all combinations of one or two males with one or two females) has previously been shown to have no significant effect on the patterns of precopulatory sexual selection detected, at least in the sister species *L. equestris* (Dougherty & Shuker, 2014). In the assay phase, around a half of matings failed to produce offspring, with more males experiencing either multiple successes or multiple mating failures than expected by chance, reinforcing previous interpretations of mating failure as a repeatable male-associated phenotype (Greenway & Shuker, 2015). Before addressing the potential role of postcopulatory processes in mate choice and mating failure (see Eberhard, 1996), we will first consider the apparent absence of precopulatory sexual selection.



**Figure 6.** Effect of male body length on likelihood of mating in choice trial. ‘All trials’ refers to all trials in which the focal female mated when presented with a pairwise choice between males of unequal body length ( $N = 116$  trials). ‘>0.5 mm difference’ represents a subset of choice trial outcomes in which the pair of males differed in size by more than 0.5 mm ( $N = 37$  trials). The dashed line represents random mating with respect to male body length. Error bars are 95% binomial confidence intervals.

The absence of precopulatory choice in this instance is surprising for multiple reasons. First, and contrary to previous assumptions (see Sillén-Tullberg, 1981, on *L. equestris*), *L. simulans* females frequently rejected male mating attempts in choice trials, demonstrating at least the requisite ability to carry out precopulatory mate choice. Second, virgin females were used in both the assay and choice trial phases, and consequently we assumed they would be under strong selection on their first mating to acquire sperm to ensure at least some reproductive success (Kokko & Mappes, 2005). Enhanced virgin choosiness for fertility occurs in female crayfish for example, in which unmated females discriminate against potentially sperm-depleted mates based on their sexual history, whereas mated females do not (Mellan, Warren, Buckholt, & Mathews, 2014). Third, we know that matings in both *L. simulans* and its sibling species *L. equestris* are prolonged and can be very costly in terms of longevity and fecundity (Evans, 2011; Shuker et al., 2006; see Introduction). So, if matings are costly to females and can be avoided, and if the males encountered have a high variance in mating failure status, why might females not be utilizing precopulatory mate choice to maximize sperm acquisition?

#### No Precopulatory Choice for Insemination Success

To begin with, if we initially assume males are solely responsible for mating failures, there may be insufficient salient cues available to allow female assessment of male primary sexual function before mating is underway. The benefits of precopulatory choosiness, as outlined by Parker (1983), cannot be accessed if females cannot reliably discriminate in favour of previously successful males, thus shifting the exertion of choice into the peri- or postcopulatory phase of mating.

Intriguingly, Dougherty and Shuker (2016) have recently found evidence for significant precopulatory selection on male genitalia,

after controlling for body size, in the sister species *L. equestris*. This is presumably mediated by selection on an unmeasured precopulatory trait, considering that females do not come into contact with the male genitalia until mating is underway. In contrast though, no significant precopulatory selection on genitalia was reported in *L. simulans*, perhaps because a similar (unmeasured) trait is not available to *L. simulans* females, thus contributing to the observed lack of precopulatory choice for males with higher previous reproductive success (Dougherty & Shuker, 2016). However, male body length is significantly correlated with intromittent organ length in *L. simulans*, with the process length itself under stabilizing postcopulatory selection (Dougherty & Shuker, 2016; Dougherty, Rahman, Burdfield-Steel, Greenway, & Shuker, 2015). While male body size has previously been shown to influence mating success in a linear fashion (and in a nonlinear fashion in *L. equestris*), it has not previously been shown to effect fertilization success (Dougherty & Shuker, 2014, 2016). Therefore, although male body length represents a potential cue on which females could base mate choice for fertility benefits, the relationship between body length and fertilization success is not always straightforward.

This picture is further complicated by the existence of ‘intermediate’ phenotype males, which successfully transfer sperm on some occasions but not others. Although females have the potential to mate successfully with encountered males that have previously failed, choosiness should increase the likelihood of successfully acquiring sperm. How levels of choosiness map to fitness depends on the costs associated with failed matings as well as the efficacy of such choice in discriminating between high- and low-fertility males. From the opposing perspective, we might imagine that males with low fertility would be under strong selection to conceal this from potential mates in order to secure matings (dishonest signalling; e.g. Dakin & Montgomerie, 2014). Females may therefore have few informative phenotypic traits on which to base precopulatory choice for fertility, thus accounting for the observed lack of preference for larger or previously successful males. It is important to consider that we cannot rule out the occurrence of precopulatory female mate choice on unmeasured male traits in this study. However, if such choice exists, it is not functioning in this case to minimize copulations with previously unsuccessful males and mating failure.

Surprisingly few studies have in fact explicitly examined whether precopulatory mate choice can function as a fertility assurance strategy (i.e. tracked the pre- and postcopulatory success of the same individuals), instead using an indirect correlative approach. Such experiments may help to resolve the relationship between male precopulatory traits under female choice and postcopulatory fertility traits (for meta-analyses showing varying and occasionally opposing patterns, see Ferrandiz-Rovira, Lemaître, Lardy, López, & Cohas, 2014; Lüpold, Tomkins, Simmons, & Fitzpatrick, 2014; Mautz, Møller, & Jennions, 2013; Morrow, Arnqvist, & Pitcher, 2002; Reding, 2015) and clarify how widely the phenotype-linked hypothesis applies and thus mate choice for fertility benefits.

It must be noted that individuals in this experiment were derived from a population maintained under continuous laboratory culture. Although population density and associated mate encounter rate is undeniably artificially inflated under such conditions, it is potentially not unprecedented with respect to the natural history of this species (see Methods). Estimates of mating failure rates are not available for wild individuals, but nontrivial rates of sperm transfer failure (36.9%) were recorded by Tadler (1999) after only two generations (i.e. in experimental individuals with wild-caught grandparents), suggesting the phenotype is unlikely to be merely a laboratory artefact, and is of broader

evolutionary significance. Considering that females from a long-term (and presumably high-density adapted) laboratory population of *L. equestris* appear to suffer even higher costs of mating than females from a recently established population (Shuker et al., 2006), if anything, laboratory-reared females would be predicted to show heightened levels of precopulatory choosiness than their wild counterparts, although also subject to higher levels of male mating attempts. The challenge remains to quantify the costs of mating and the rates of remating in wild populations of *L. simulans* in order to test these assumptions.

#### Maladaptive Postcopulatory Male Failure

Turning to postcopulatory processes, male-associated failure may only occur once mating is underway (as a potential maladaptive mechanical side-effect of possessing complex genitalia for example), thus explaining the nonexistence of precopulatory female avoidance of ‘failure’ males. *Lygaeus simulans* males possess an elongate intromittent organ (the processus), which must pass through a valve-like structure and convoluted corkscrew section of the spermathecal duct inside the female, before reaching the spermatheca itself, where successful insemination can occur (Dougherty et al., 2015; Gschwenter & Tadler, 2000; see Fig. 6). As such, any issues with processus deployment or damage may result in repeated insemination failure. Typically, copulations of an hour or more are necessary for the processus to traverse sufficiently far along the duct to be near the spermatheca. However, Micholitsch et al. (2000) reported multiple instances of incomplete processus intromission despite prolonged copulation, which may potentially be ascribable to male mechanical incompetence. In addition, Dougherty and Shuker (2015) described high frequencies of male processus breakages after 2 weeks of repeated mating, potentially as a result of ‘wear and tear’. Subsequent dissection of multiply mated females (taken from stock culture) has revealed that a small number of females (2/30) have fragments of male genitalia present in their spermathecal ducts (see Fig. 1). Such breakages represent an occasional cost of mating to both males and females, assuming females cannot utilize sperm if the spermathecal duct is blocked by a processus fragment, but are uncommon and thus probably not a major cause of mating failure. However, although mechanical issues may occasionally arise, it seems highly unlikely that male genital deployment problems are wholly responsible for such high observed rates of mating failure, given the extremely high selection pressure acting on primary sexual function.

#### Postcopulatory Female Choice

Up to this point we have assumed that mating failure represents a male-isolated phenotype. However, it may in fact represent an ‘interacting phenotype’ between male and female mating partners, if females are capable of exerting postcopulatory choice and preventing insemination. As highlighted by Eberhard (1996), postcopulatory cryptic female choice may be enabled by a number of mechanisms once mating is underway or over, which can prevent offspring production. Importantly, postcopulatory sexual selection does not explicitly require the overlap of ejaculates or multiple mating (Dougherty, Simmons, & Shuker, 2016). Consequently, mating failures may represent a mode of adaptive female choice in some species, allowing additional opportunities to reject males upon further assessment during copulation (Candolin, 2003; Eberhard, 1996). Given the apparent lack of female precopulatory choice, this may be particularly relevant in *L. simulans*, where female consensus on male ‘quality’ could generate the individually repeatable male mating failure we observe. Of course the reverse may also be the case, with

variation in levels of individual male choosiness causing some males to withhold ejaculates when mating as a mechanism of cryptic male choice (Arnqvist, 2014). However, the same costs that may restrict the role of mating failure as a mechanism of cryptic female choice and promote precopulatory choice apply, with the addition that males typically increase their reproductive success with each mating to a far greater extent than females (Bonduriansky, 2001). High rates of mating failure potentially induced by cryptic female choice mechanisms have been documented in the flour beetle *Tribolium castaneum* and sweet potato weevil *Euscepes postfasciatus* (Kumano, Kuriwada, Shiromoto, Haraguchi, & Kohama, 2010; Tyler & Tregenza, 2013), although the extent to which females across these species can influence the movement of the intromittent organ or spermatophore transfer and prevent insemination is not yet known. Further investigation into the function of reproductive tract musculature and spermathecal duct valve structure in *L. simulans* (Fig. 1) is necessary to confirm this interpretation of mating failure outcomes.

#### Mating Failure and Mating System Evolution

However, the possibility for postcopulatory sexual selection, both in terms of the observations of widespread mating failure and possible morphological characteristics that could bring it about, does not solve the broader puzzle of mating failure in this or any other species. Rather, it challenges us again to ask why a female would go through a costly mating process before discriminating between males via postcopulatory processes and echoes the broader longstanding question of why the majority of females mate multiply. Does mating failure and low fertility drive a reliance on postcopulatory choice and polyandry or does polyandry facilitate female-postcopulatory choosiness and subsequent mating failure? While there is accumulating empirical support for the former, through the widespread occurrence of sperm depletion and limitation (Jones, 2001; Preston, Stevenson, Pemberton, & Wilson, 2001; Smith, Pateman-Jones, Zięba, Przybylski, & Reichard, 2009; Wedell, Gage, & Parker, 2002) leading to multiple mating for fertility assurance (Alonzo & Pizzari, 2013; Bocedi & Reid, 2016; Forbes, 2014; Garcia-Gonzalez, Yasui, & Evans, 2014; Wilson & Tomkins, 2015), the extent to which mating failure may be an adaptive female-induced strategy has received little attention since it was highlighted as a possibility by Eberhard (1996).

Although the strength of selection acting on successive phases of reproduction is beginning to be quantified (e.g. Collet, Dean, Worley, Richardson, & Pizzari, 2014; Droge-Young, Manier, Lüpold, Belote, & Pitnick, 2012; Kvarnemo & Simmons, 2013; Lüpold et al., 2014; Péliissié, Jarne, Sarda, & David, 2014; Pischedda & Rice, 2012; Rose, Paczolt, & Jones, 2013), we are still some way away from predicting when pre- and postcopulatory selection should come either into conflict or into alignment (in terms of favoured phenotypes or mates) or indeed why one or other episode may be absent. Framing this in terms of choice, while the general costs and benefits of being choosy and its subsequent presence or absence of mate choice in either sex have been thoroughly explored both empirically and theoretically (Andersson & Simmons, 2006; Bonduriansky, 2001; Halliday, 1983; Hunt & Sakaluk, 2014), at what point during the course of a mating this choice should be exerted has not been explicitly considered. This is likely to be dictated by the point at which the benefit:cost ratio of choice is greatest, which in turn will depend on the information available and the costs involved to each individual at each subsequent stage of mating. Although choice is typically subdivided into precopulatory mate choice and postcopulatory paternity choice, viewing these in isolation as discrete episodes is likely to hamper our

understanding of how and why individuals assess potential reproductive partners (Andersson & Simmons, 2006). Including mating failures in a more holistic approach to mate choice, viewed in the context of the continuum of a mating event, should provide insight both into the costs of choosiness and the costs and benefits of polyandry.

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