Males increase their fitness by choosing large females in the common bedbug *Cimex lectularius*

Elena Kaufmann and Oliver Otti

Animal Population Ecology, Animal Ecology I, University of Bayreuth, Bayreuth, Germany

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Abstract
Mate choice is often a role assigned to females. Already Darwin realised that males are eager to copulate, and females are choosy. However, male mate choice is not as rare as assumed. Males should choose females if females vary in quality, i.e., fecundity. Indeed, males often choose larger mates and through this preference increase fitness benefits. In addition, if mating costs reduce the number of copulations a male can potentially perform, he should be choosy. Bedbug females vary in their fecundity and female size is positively related to fecundity. Male bedbugs are limited in seminal fluid availability and, hence, the number of consecutive matings they can perform. Traumatic insemination gives males full control over mating, therefore low female mating resistance could further allow males to be choosy. Here, using mate choice arenas, we investigated if male bedbugs prefer to mate with large females. We observed mating behaviour and measured female fecundity to investigate potential male fitness benefits. Males chose to mate with large females 1.8 times more often than small females and large females laid significantly more eggs than small females. Our study provides first evidence for male mate choice based on female body size in bedbugs and males can increase their fitness by mating large females. It has to be further established if male mate choice is driven by mating costs in terms of ejaculate investment and if such male mate choice based on female size could be a driver of sexual size dimorphism in bedbugs.

Keywords
Ejaculate investment; hypodermic impregnation; male mating behaviour; motivation; reproduction; sexual selection; sperm allocation

Introduction
A central theme in biology is the quest to understand behavioural, morphological and physiological differences between the sexes (Andersson, 1994). Darwin’s re-
alisation of the sex roles, i.e., that males are generally more eager to mate, and females are the choosier sex (Darwin, 1871), together with the principles postulated by Bateman on variation in reproductive success between the sexes (1948) and Trivers on parental investment (1972) form the basis for the concept of sexual selection. Female mate preferences and their consequences for sexual selection have been extensively studied (Andersson, 1994). However, male mate choice is not as rare as assumed (Bonduriansky, 2001; Edward & Chapman, 2011) and has been established as a relatively common feature of male mating behaviour in a variety of taxa (e.g., MacLeod & Andrade, 2014; Assis et al., 2017; Belliure et al., 2018; Guevara-Fiore & Endler, 2018), even in systems with conventional sex roles. Investigating the occurrence of male mate choice in different mating systems and its association to male mating effort and fitness benefits provide a better understanding of the relative importance of male mate choice and its potential consequences for sexual selection (Edward & Chapman, 2011).

High variance in female mate quality, high male mating investment and low constraints on male choosiness can select for the evolution of male mate choice (Bonduriansky, 2001). In promiscuous species, males should choose between females if females vary in quality, e.g., fecundity. If a female phenotypic indicator, such as body size, is positively correlated with fecundity then males could base their choice on female body size and gain direct fitness benefits (Bonduriansky, 2001). In many insect species, female fecundity is reflected by female body size, because the number of offspring produced, as well as their size and survival, increase with female body size (Honěk, 1993). Also, in fishes such as guppies (Poecilia reticulata) and white-spotted chars (Salvelinus leucomaenis) female fecundity increases with body size (Morita & Takashima, 1998; Herdman et al., 2004).

Indeed, males often prefer larger mates and through this choice increase fitness benefits (Bonduriansky, 2001; Dosen & Montgomery, 2004; Reading & Backwell, 2007). For example, male Atlantic mollies (Poecilia mexicana) prefer large females over small ones (Plath et al., 2006) and males of the snail species Littorina subrotundata prefer large and virgin females (Zahradnik et al., 2008). If mating costs reduce the number of copulations a male can potentially perform, mating investment can affect choosiness (Bonduriansky, 2001). For example, in Drosophila melanogaster the evolution of male mate choice might be explained by high costs of ejaculate production or limits to courtship, which can reduce the male ability to mate with multiple females (Byrne & Rice, 2006). In addition, males from several species are known to adjust their ejaculate expenditure according to the mating status of the female they mate with (Simmons, 2001; Sirot et al., 2011). A similar adjustment might be expected if females vary in their quality. In such a scenario, male mate choice would not only involve choosing higher quality females but also investing more in terms of ejaculate in such females, i.e., males would either mate longer with high-quality females than low-quality females or increase the rate of ejaculate transfer.
In the common bedbug *Cimex lectularius*, female fecundity is relatively variable and positively correlated with body size, i.e., larger females lay more fertile eggs than smaller ones (Kaldun & Otti, 2016; Supplementary fig. S1). Similar to male *Drosophila*, male bedbugs are limited in the amount of seminal fluid available to them and, hence, the number of consecutive matings a male can perform is limited too (Reinhardt et al., 2011; Kaldun & Otti, 2016). Male bedbugs traumatically inseminate females and recently fed females cannot resist male mating attempts (Reinhardt et al., 2009). Even though males are in control of mating, i.e., in control of most pre-copulatory processes of sexual selection (Reinhardt et al., 2009), the limited availability of seminal fluid reducing the capacity of a male to mate with multiple females could build the basis for the evolution of male mate choice in bedbugs (Edward & Chapman, 2011). Low female mating resistance and a high number of potential female mates present in large aggregations (Reinhardt & Siva-Jothy, 2007) could allow males to be choosy.

Here we investigated if male bedbugs show mate choice based on female body size and whether male choice affects male fitness. First, we established in two-way choice trials if males preferred large females. Then, we allowed males to mate with the preferred female to measure fecundity. Once this preferred mating was completed, we removed the preferred female. Then we recorded if the male would mate with the non-preferred female to test a male’s inclination towards a second mating with the previously rejected female. In case the male performed this non-preferred mating, we also measured female fecundity for the non-preferred females. As copulation duration in bedbugs is positively correlated with the size of the transferred ejaculate (Siva-Jothy & Stutt, 2003), we also assessed male mating behaviour in both preferred and non-preferred matings.

**Material and methods**

_Culture and reproductive biology of C. lectularius_

All bedbugs (see origin below) were maintained in rearing pots in a CT room at 26 ± 1°C with 70% relative humidity and a light-dark-cycle of 12 hours. All individuals used in the present study were virgins. For this, we reared 5th instar nymphs to adulthood by placing them individually into wells of a 96-well microtiter plate. Each well was provided with a filter paper disk. We used individuals from three large stock populations (>1000 individuals) for our experiment (arbitrarily called A, B, C). The feeding and maintenance protocol followed Reinhardt et al. (2003). The bedbug populations originated from laboratory populations established at the University of Sheffield and have been maintained at the University of Bayreuth since 2012. Population A was collected from an infestation in London in 2008. Population B originated from Nairobi, Kenya and was collected in 2010, and population C is a lab population from the London School of Hygiene and Tropical Medicine maintained in the lab for over 50 years.
Male bedbugs mate with females via traumatic insemination using a dagger-shaped intromittent organ. During mating the female abdominal wall is punctured with the intromittent organ (Stutt & Siva-Jothy, 2001), leaving an integumentary wound in the female abdomen (Michels et al., 2015). Sperm is injected into a special immune organ in their body cavity, the mesospermalege. This organ is thought to have evolved to reduce the effect of wounding and potential transmission of microbes (Reinhart et al., 2003). Most matings occur shortly after the female’s bloodmeal because fully fed females cannot resist mating (Reinhardt et al., 2009). Males control the mating duration and therefore the ejaculate supply (Siva-Jothy & Stutt, 2003; Reinhardt et al., 2009).

Male mate choice experiment

After reaching adulthood, all adult bedbugs were fully fed once and individually transferred to Drosophila vials equipped with a piece of filter paper. We photographed eighty females and eighty males per population using a digital imaging system (Leica Application Suite, Leica, Wetzlar, Germany) attached to a dissecting microscope (M165 C, Leica) for measuring pronotum width of each bedbug with ImageJ (version 1.50i; Schneider et al., 2012). The pronotum width was used as a measure of body size because it is a parameter of skeletal size and does not change with the feeding status (Otti et al., 2013). For the mate choice experiment, we used the sixteen largest (L females) and sixteen smallest female bedbugs (S females) from a total of eighty individuals per population (N = 96). From the males, we selected sixteen average-sized individuals from each population (N = 48) and calculated the median of male pronotum width for each population and then used eight individuals above and below the median (N = 16 per population). Individuals were either three weeks or four weeks old. Only same-aged individuals were used in the same mate choice trial (A: 14 trials with three-week-old and two trials with four-week-old individuals, B: all trials with three-week-old individuals, C: 12 trials with three-week-old and four trials with four-week-old individuals). All females were fully fed immediately before a mate choice trial.

Mate choice trials

A male was presented with a small and a large female at the same time in a mating arena, consisting of a glass petri dish with a 70 mm diameter and a fresh, round filter paper. Before a two-way choice trial each individual was put under an Eppendorf tube (with the lid cut off and a string attached at the end of the tube) in a defined location in the mating arena (Supplementary fig. S2). After one minute, the tubes were lifted simultaneously to standardise the initiation of all mate choice trials. To avoid an effect of the side, the starting position of large and small females in the petri dish was alternated for every trial. In addition to recording a male’s preference for a female, we measured time to mount and copulation duration using a stopwatch (adapted from Reinhardt et al., 2009). Time to mount was recorded as
the period of time between initializing the mating trial with lifting the tubes and the male mounting the preferred female bedbug. Copulation duration we defined as the time between the successful introduction of the male’s copulatory organ into the female’s abdominal wall and the male dismounting the female. Both time periods were measured to the nearest second. This first mating was defined as the preferred mating. After a male had finished mating the female of choice, we removed her from the petri dish and transferred her to a *Drosophila* vial (see below). Then we observed for 30 minutes whether or not the male would mount the second female to test if the male would mate with the non-preferred female at all. If a male did mate the second female, we measured copulation duration and reproductive outcome of such a second ‘non-preferred mating’. After the trials, all mated females were put individually in *Drosophila* vials, provided with a filter paper and were fed weekly. Then we counted the number of viable and inviable eggs over a complete reproductive cycle, i.e., 10 weeks, similar to Otti et al. (2013).

**Statistical analysis**

All data were analysed using the statistical platform R 3.4.1 (R Core Team, 2017) with the packages *car* (Fox & Weisberg, 2011), *multcomp* (Hothorn et al., 2008), *MASS* (Venables and Ripley, 2002) and lattice (Sarkar, 2008). Using a *G* test with female size as a categorical factor and population as a grouping factor we analysed if male mate preference differed between populations. We also ran the *G* test only for the three-week-old individuals. Fisher’s exact tests were used to analyse if males differed in the likelihood to mate a small female after a large female in comparison to mating a large female after a small one. Second, we fitted generalized linear models (GLM) with Gaussian distributions with time to mount, copulation duration and viable eggs as response variables and fitted female size category and population as factors and their interaction term for both the choice and non-choice matings. For all GLMs individual age was fitted as a covariate and removed if not significant. For the analysis of the number of viable eggs, we also fitted copulation duration as a covariate, because copulation duration is positively related to ejaculate size in bedbugs (Siva-Jothy & Stutt, 2003). For the analysis of the proportion of inviable eggs, we used the *cbind()* function in R to combine inviable and viable eggs as a response variable. With this response variable, we then fitted GLM with binomial distribution on population and females size category as factors and their interaction term as well as individual age as a covariate. If populations differed significantly in a given response variable and the interaction term was non-significant, we performed *post hoc* tests to identify pairwise differences between populations using the *multcomp* package (Hothorn et al., 2008). For those tests, the *P* values were adjusted to the method by Westfall (Bretz et al., 2010). To check for normality and homogeneity, we visually inspected residual versus fitted plots. If transformation was needed we fitted GLMs and used the *boxcox()* function for finding the appropriate transformation parameter lambda, i.e., λ. We only reported significant interaction terms. In the choice matings, two males mated a large female for 510
and 814 s, respectively, which was two and three times longer than the mean copulation duration. Therefore, we present the results with and without these outliers for copulation duration. In population A, one female laid no eggs and one female laid 168 viable eggs, which was 60% above the mean. In the non-choice matings, two females laid no eggs and one small female in population B laid 120 viable eggs, which was 60% above the mean number of viable eggs for this group. For the number of viable eggs, we also present results with and without these outliers. Finally, we used paired t-tests to compare within males, whether copulation duration and egg production differed between preferred and non-preferred matings both not accounting and accounting for male mate preference.

Results

All males chose to copulate with at least one female. Male mate preference for large females was consistent over the three populations (G test: \( G = 0.184, \text{df} = 2, P = 0.91 \), only three-week-old individuals: \( G = 0.524, \text{df} = 1, p = 0.77 \)). Large females were preferred 1.8 times more often than small females (G test: \( G = 4.143, \text{df} = 1, P = 0.04 \), only three-week-old individuals: \( G = 2.404, \text{df} = 1, P = 0.12 \)) (fig. 1). Of males, 58% (18 out of 31) preferred to mate with a small female after mating a large female and 76% (13 out of 17) preferred to mate with a large female after mating a small female (Fisher’s exact test: \( P = 0.34 \)).

Preferred matings

Time to mount and copulation duration

Individual age had no effect on the time to mount (GLM with Box-Cox-transformed data, \( \lambda = 0.05: F_{1,41} = 0.016, P = 0.90 \)). Time to mount differed between female size depending on population (GLM with Box-Cox-transformed data, \( \lambda = 0.05: \).
female size category × population: $F_{2,42} = 3.279$, $P = 0.05$) (fig. 2a). In population A, males took longer to mount small females than large females. In population B, the opposite was the case and in population C no difference was found (fig. 2a). However, neither female size (GLM with Box-Cox-transformed data, $\lambda = 0.05$: $F_{1,44} = 0.229$, $P = 0.63$), nor population significantly affected the time to mount after removing the interaction term (GLM with Box-Cox-transformed data, $\lambda = 0.05$: $F_{2,44} = 1.925$, $P = 0.16$).

Older individuals copulated significantly longer than individuals that were a week younger (GLM with Gaussian distributed data: $F_{1,43} = 5.473$, $P = 0.02$; without outliers: $F_{1,41} = 12.064$, $P = 0.001$) (fig. 2b). Copulation duration did neither differ between female size (GLM with Gaussian distributed data: $F_{1,43} =$
Egg production

Individual age had no effect on the amount of viable eggs laid (GLM with Gaussian distributed data: $F_{1,42} = 0.683$, $P = 0.41$; without outliers: $F_{1,40} = 1.875$, $P = 0.18$). Copulation duration was non-significantly positively related to the number of viable eggs laid (GLM with Gaussian distributed data: $F_{1,43} = 3.646$, $P = 0.06$; without outliers: $F_{1,41} = 3.604$, $P = 0.06$). Large females laid significantly more viable eggs than small females (GLM with Gaussian distributed data: $F_{1,42} = 5.527$, $P = 0.02$; without outliers: $F_{1,41} = 27.831$, $P < 0.001$) (fig. 2c). The number of viable eggs varied significantly between the populations (GLM with Gaussian distributed data: $F_{2,43} = 4.047$, $P = 0.02$; without outliers: $F_{2,41} = 11.128$, $P < 0.001$). Females from population A and B laid significantly more viable eggs than females from population C (Tukey comparison: population A vs. C: $P = 0.03$; without outliers: $P < 0.001$; population B vs. C: $P = 0.03$; without outliers: $P < 0.001$) (fig. 2c). Four-week-old females laid significantly more inviable eggs than three-week-old females (GLM with binomially distributed data: $\chi^2 = 7.928$, df = 1, $P < 0.01$). The proportions of inviable eggs were similar for large and small females (GLM with binomially distributed data: $\chi^2 = 1.418$, df = 1, $P = 0.23$) (fig. 2d). The populations differed significantly in the proportion of inviable eggs (GLM with binomially distributed data: $\chi^2 = 24.688$, df = 2, $P < 0.001$). The different proportions of inviable eggs were caused by significant differences between population A and B (Tukey comparison: $P < 0.001$) and between population B and C (Tukey comparison: $P < 0.01$) (fig. 2d).

Non-preferred matings

Proportion of matings and copulation duration

All males were allowed a second mating after their preferred matings. In population A and C, more than half of the males copulated for a second time (non-preferred mating) (table 1). In population B, only 44% of the males engaged in a non-choice mating. Irrespective of female size, copulation duration of the choice matings, i.e., the first mating, were significantly longer than the non-preferred matings, i.e., the second mating (paired t-test: not accounting for male preference: $t = 12.411$, df = 30, $P < 0.001$; accounting for male preference: large females: $t = 9.954$, df = 17, $P < 0.001$; small females: $t = 8.210$, df = 12, $P < 0.001$). Individual age had no effect on copulation duration of the non-preferred matings (GLM with Gaussian distributed data: $F_{1,26} = 1.955$, $P = 0.17$). However, males copulated significantly longer with large females than small females in the non-preferred matings (GLM
Table 1.
Proportion of non-choice matings for large and small females separated for the populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of non-choice matings</td>
<td>0.69</td>
<td>0.44</td>
<td>0.81</td>
</tr>
<tr>
<td>Small females</td>
<td>0.60</td>
<td>0.36</td>
<td>0.80</td>
</tr>
<tr>
<td>Large females</td>
<td>0.83</td>
<td>0.60</td>
<td>0.83</td>
</tr>
</tbody>
</table>

with Gaussian distributed data: \( F_{1,26} = 8.223, \ P < 0.01 \) (fig. 3a). Across the three populations copulation duration was similar (GLM with Gaussian distributed data: \( F_{2,26} = 0.670, \ P = 0.52 \)).

**Egg production**

Neither individual age (GLM with Gaussian distributed data: \( F_{1,25} = 1.283, \ P = 0.27 \); without outliers: \( F_{1,22} = 0.228, \ P = 0.64 \), nor copulation duration (GLM with Gaussian distributed data: \( F_{1,25} = 0.390, \ P = 0.54 \); without outliers: \( F_{1,22} = 0.270, \ P = 0.61 \)) had an effect on the number of viable eggs laid in the non-preferred matings. Non-preferred matings yielded in significantly fewer viable eggs than preferred matings (paired \( t \)-test: not accounting for male preference: \( t = 2.495, \ df = 30, \ P = 0.02 \) accounting for male preference: large females: \( t = 4.709, \ df = 17, \ P < 0.001 \); small females: \( t = -1.060, \ df = 12, \ P = 31 \)). Similar to the choice matings, large females laid significantly more viable eggs than small females after a non-preferred mating (GLM with Gaussian distributed data: \( F_{1,24} = 39.804, \ P < 0.001 \) (fig. 3b). The number of viable eggs laid by females differed significantly between the three populations (GLM with Gaussian distributed data: \( F_{2,24} = 3.679, \ P = 0.04 \)). Females from population A laid significantly more viable eggs than females from population C (Tukey comparison: \( P = 0.01 \); without outliers: \( P = 0.02 \)). Females from population B laid significantly more eggs than females from population C when including the outliers (Tukey comparison: \( P = 0.02 \)). However, once we removed the outliers this comparison was not significant anymore (Tukey comparison without outliers: \( P = 0.30 \) (fig. 3b).

Four-week-old females laid a significantly higher proportion of inviable eggs than three-week-old females (GLM with binomially distributed data: \( \chi^2 = 4.619, \ df = 1, \ P = 0.03 \)). Female size did not affect the proportion of inviable eggs laid (GLM with binomially distributed data: \( \chi^2 = 0.507, \ df = 1, \ P = 0.48 \) (fig. 3c). However, the proportion of inviable eggs differed significantly between the three populations (GLM with binomially distributed data: \( \chi^2 = 16.863, \ df = 2, \ P < 0.001 \) (fig. 3c). Females from population A laid a significantly lower proportion of inviable eggs than females from the other two populations (Tukey comparison: A vs. B \( P < 0.001 \); A vs. C \( P < 0.01 \) (fig. 3c). Removing the three outliers resulted
in a significant interaction term of female size vs. population (GLM with binomially distributed data: $\chi^2 = 9.483$, df = 2, $P < 0.01$) and the effect of individual age was no longer significant (GLM with binomially distributed data: $\chi^2 = 3.030$, df = 1, $P = 0.08$).

**Discussion**

Here we show for the first time that bedbug males prefer to mate with large females rather than small females. Males who chose large females had higher fitness than males who chose small females, as large females laid significantly more viable eggs than small females (fig. 2c, Supplementary fig. S3). While most males preferring a large female chose not to mate with a small female in the non-preferred mating, all...
but one male mated the large female, after choosing a small female. This further supports the notion that male bedbugs are indeed choosy. The time to mount large and small females depended on the population. However, we found no main effect of female size and population on time to mount. We assume that males from different populations might vary in their eagerness to mate, their perception of females or their ability to mount a female. Copulation duration was not affected by female size indicating that once a copulation is initiated male ejaculate expenditure is not dependent on female body size.

Males of many species prefer larger females as their mates (Bonduriansky, 2001). For example, in the curculionid beetle *Diaprepes abbreviates* small and large males preferred larger females (Harari et al., 1999). Because limited seminal fluid availability reduces the number of subsequent copulations a male can potentially perform (Reinhardt et al., 2011; Kaldun & Otti, 2016), bedbug males might benefit from mate choice even though they are in full control of the mating process. Male mate preference for larger females might affect selective pressure on female body size and thus fecundity. On the one hand, male selection for large females might be at the basis of the observed sexual size dimorphism in bedbugs (Usinger, 1966). However, the mechanisms leading to sexual size dimorphism in *C. lectularius* are not well understood. On the other hand, if large females have to endure higher mating rates, i.e., more copulatory wounding, this might represent a cost to high-fitness females and reduce the selective advantage of larger highly fecund females over smaller females (Long et al., 2009). Therefore, the role of male mate preference in connection to selection for female body size and in sexual conflict needs further investigation. Although all males mated with one of the females presented to them, some chose not to mate a second time with the non-preferred female. Fewer males mated with a small female after mating with a large female than the other way around, indicating that bedbug males might adjust their mating effort. For the future, it might be interesting to investigate if male mate preference in bedbugs is condition-dependent (Tigreros et al., 2014) or if over a series of matings males become choosier with increasing ejaculate production costs (Byrne & Rice, 2006) or increasing mating effort (Guevara-Fiore & Endler, 2018).

**Time to mount, copulation duration, viable and inviable egg numbers for the preferred matings**

A similar time to mount a female with respect to female size confirms that males are in control of pre-mating processes, at least if females are fully fed (Reinhardt et al., 2009). In bedbugs, large females seem not generally more resistant to mating than small females, what could have been assumed from findings in other species (Pitnick & Garcia-González, 2002; Hotzy & Arnqvist, 2009). Therefore, we assume the significant variation in the time to mount large and small females between populations might be based on variation in male eagerness to mate or on variation in males to perceive and mount females. In all populations, males were very quick at choosing as most matings started within 30 s and none of the males took longer
than a minute to choose a female (fig. 2a). However, on which signal male mate preference is based remains to be shown.

In several insect species including bedbugs, copulation duration is positively related to female egg laying. Large females might not only lay more viable eggs due to their size, males might be transferring more ejaculate during such a copulation. For example, males might transfer more seminal fluid to stimulate the fertilization process (Wigby et al., 2009) or they might invest more sperm to increase fertilization success (Cook & Wedell, 1996; Lüpold et al., 2011). In *Drosophila melanogaster*, copulation duration and fecundity both strongly depend on female body size (Lefranc & Bundgaard, 2000). The copulation duration of small *Drosophila* females was shorter than that of large females and the fecundity of small females is lower than the fecundity of large females. However, it is not entirely clear to what extent size and copulation duration define female fecundity in *Drosophila*. Also in the bruchid beetle *Callosobruchus maculatus*, females copulating for longer laid more eggs than females copulating for a short amount of time (Edvardsson & Canal, 2006). In bedbugs, the male fitness benefit due to mating large females seems to be based on these females laying more eggs because of their size rather than males prolonging the copulation duration with large females and thereby transferring more sperm (Siva-Jothy & Stutt, 2003).

Overall, large females laid significantly more viable eggs than small ones and the number of viable eggs laid differed between populations. In two out of three populations large females laid more viable eggs than small females. Removing the potential outliers even resulted in a higher reproductive output for large females than small ones in all three populations. Similar to other species (Honěk, 1993; Morita & Takashima, 1998; Bonduriansky, 2001; Herdman et al., 2004) we can cautiously assume that the relation between female fecundity and body size in bedbugs is a general one.

The proportion of inviable eggs did not differ between female size categories. Therefore, we assume that the quality of eggs is not affected by female size. Rather female size defines the resource availability to produce eggs and the space to store them before laying. Although, egg storage in bedbugs is predicted to be rather short as females produce and lay eggs continuously when mated and fed regularly (Usinger, 1966). Females from one population laid a higher proportion of inviable eggs than in the two other populations. Probably this is to some degree due to variation in female or male fertility between the populations.

**Copulation duration, viable and inviable egg numbers for the non-preferred matings**

All males were allowed to mate a second time with the female they did not prefer. Only half of the males mated with the non-preferred female within 30 minutes after the preferred mating. More males mated with a large female, than with a small non-preferred female. This further indicates that bedbug males can be choosy and probably males forgo a second mating to save on seminal fluid (Reinhardt et al.,...
Generally, copulation duration of non-preferred matings was about half as long as the copulation duration of the preferred matings. This reduction was more pronounced in males that chose a large female first than males choosing a small female first (Supplementary fig. S4). In addition, in two out of three populations, the copulation durations of large females were longer than the copulation durations of small females. This shows that males put more effort in the copulations with large females. We take this as an indication that males seem to have a lower interest in engaging with a small female after having copulated with a large female. Again, this might be related to a limitation in seminal fluid (Reinhardt et al., 2011; Kaldun & Otti, 2016). Males might be less inclined to invest in mating with a female of lower quality after a mating with a high-quality female to manage their ejaculate investment (Lüpold et al., 2011; Gress & Pitnick, 2017; Guevara-Fiore & Endler, 2018).

Again, the number of viable eggs produced by large females was higher than the number of viable eggs laid by small females. The copulation duration of the preferred matings was about twice as long as the copulation duration of non-preferred matings. We assume that relatively short re-mating intervals might serve as a signal of high female density to males, which would allow them to be choosy (Edward & Chapman, 2011) and/or reduce ejaculate investment per female to maximise mating rate (Lüpold et al., 2011; Gress & Pitnick, 2017). Large females had a higher reproductive output by laying more eggs than small females, independent of preferred or non-preferred mating. This suggests that males should always prefer large females for copulation, as mate choice increases male fitness.

**Conclusion**

Although males are generally thought to be eager to mate (Darwin, 1871), we could add further evidence that males can be choosy. Even in a system where males have gained the upper hand in the pre-copulatory sexual conflict and can mate with females almost at will, we found that males do choose large and more fecund females to increase their own reproductive fitness. Therefore, male mate choice seems adaptive in male bedbugs, but we do not know the complete story yet. It has to be further established if male mate choice is driven by mating costs in terms of ejaculate investment and if such male mate choice based on female size could be a driver of sexual size dimorphism in bedbugs.

**Author contributions**

E.K. and O.O. conceived the idea and designed the experiment. E.K. carried out the experiment. E.K. and O.O. performed the statistical analyses. E.K. and O.O. interpreted the results and wrote the manuscript. Both authors read and approved of the final manuscript.
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