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

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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.

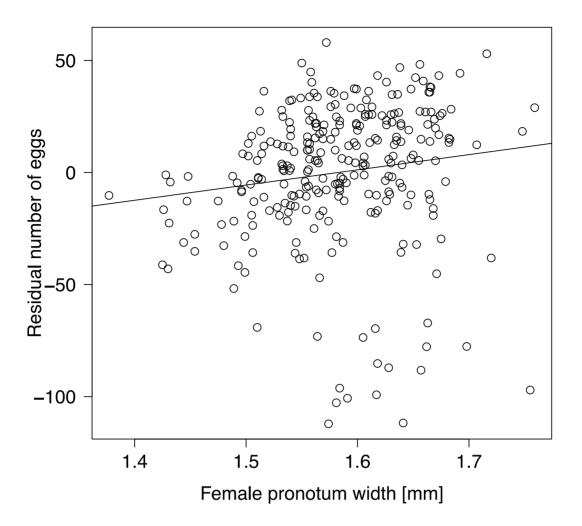
#### Keywords

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

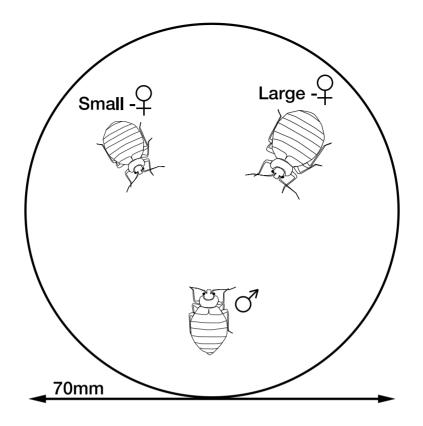
# **Supplementary material**

#### Re-analysis of fecundity data from a previous experiment

We re-analysed egg production data from a large experiment on the effect of male food restriction on female fecundity (Kaldun & Otti, 2016) to investigate a potential positive relationship of female fecundity and body size in bedbugs. We found that the number of eggs laid over a period of eight weeks increased significantly with increasing female pronotum width (ANOVA:  $F_{1,268} = 5.529$ , p =0.02) after accounting for population origin (four different populations), feeding regime (fully fed, half fed and unfed) and mating duration (60 s and full mating) in a generalised linear model. The relationship is relatively weak, probably due the considerable variation in fecundity between females (mean ± s.d. 113 ± 32). Nevertheless, this finding formed the basic idea for the present study on male mate choice.



**Figure S1.** Relationship between residual female fecundity and female pronotum width in mm across all females from the experiment on the effect of food restriction on egg production in Kaldun & Otti (2016). Here we plotted the residual number of eggs from a GLM with egg number fitted as a response variable and population origin, feeding regime and mating duration as factors against female pronotum width in mm. Larger females laid significantly more eggs than smaller females (ANOVA:  $F_{1,268} = 5.529$ , P = 0.02).



**Figure S2.** The mating arena (glass petri dish lined with a filter paper disc) used in the mate choice experiment. A male is presented with two females (small and large) in a glass petri dish with a large filter paper as base.

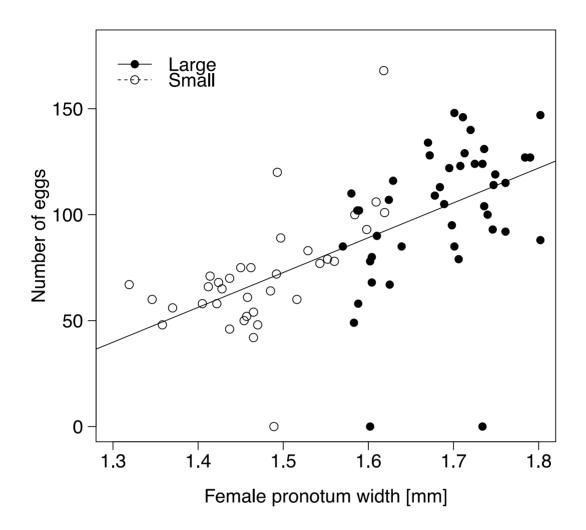


Figure S3. Relationship between number of eggs laid over the experimental period and female body size. Larger females laid significantly more eggs than smaller females (ANOVA:  $F_{1,77} = 46.910$ , P < 0.001,  $R^2 = 0.37$ ).

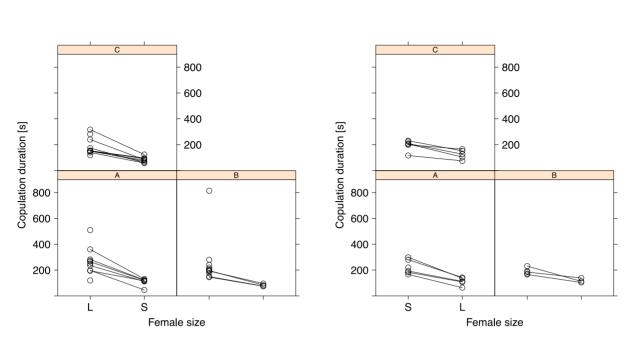
#### Within-male comparisons of copulation duration and viable egg numbers

Whilst 13 males choosing a large female did not want to mate with a small female in the following non-choice mating, all but four males choosing a small female mated with a large male in the following non-choice mating (Supplementary Fig. S4a, b). With one exception, all males had a higher reproductive output copulating with a large female first (Supplementary Fig. S5a). All but two males that mated a large female after choosing a small one had a higher reproductive output with the large female than the small female (Supplementary Fig. S5b).

#### Copulation duration

Using the packages *lme4* (Bates et al., 2015) and *lmerTest* (Kuznetsova et al., 2016) we fitted generalised linear mixed-effects models to compare the effect of population, male mate preference and mating sequence, i.e., first mating vs. second mating, and their interaction terms on copulation duration and egg production within males. We fitted population, male mate preference and mating sequence as fixed effects, individual age as a covariate and male as random effect. For egg production, copulation duration was fitted as a covariate. Covariates remained in the model if they improved the model significantly, otherwise they were removed.

The second mating was always significantly shorter than the first mating irrespective of male mate preference (linear mixed-effects model [LME] with male as random effect:  $F_{1,25.85} = 176.056$ , p < 0.0001) (Supplementary Fig. S4a, b). The change in copulation duration between the first and second mating depended on population (LME with male as random effect:  $F_{2,25.89} = 4.479$ , p = 0.02) and male mate preferences (LME with male as random effect:  $F_{1,25.77} = 4.916$ , p = 0.04) (Supplementary Fig. S4a, b). The reduction in copulation duration from the first to the second mating was much higher in males with a preference for large females than small females.



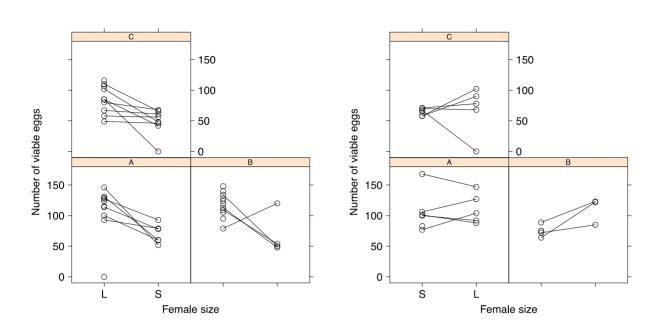
**Figure S4.** Within male comparison of copulation durations for preferred and non-preferred matings separated for population and a) for males choosing a large female first and b) for males choosing a small female first.

# Egg production

a)

Copulation duration was positively correlated to the viable egg number (LME with male as random effect:  $F_{1,69.72} = 3.732$ , P = 0.06). The number of viable eggs laid differed significantly between populations (LME with male as random effect:  $F_{2,34.25} = 8.562$ , P < 0.001). The number of viable eggs laid by the first and second mated female depended on male mate preferences (LME with male as random effect:  $F_{1,32.93} = 16.237$ , P < 0.0001) (Supplementary Fig. S5a, b). The number of eggs laid by the non-preferred female was reduced for males choosing a large female, whereas the egg number did not change in the non-preferred matings for males preferring small females.

b)



**Figure S5.** Within male comparison of viable egg numbers laid by L and S females over a 10-week egg laying period after preferred and non-preferred matings separated for population a) for males choosing a large female first and b) for males choosing a small female first.

# References

a)

- Bates, D., Maechler, M., Bolker, B., Walker, S. (2015) Fitting linear mixed-effects models using lme4. J. Stat. Softw., 67, 1–48.
- Kaldun, B. & Otti, O. (2016) Condition-dependent ejaculate production affects male mating behaviour in the common bedbug *Cimex lectularius. Ecol. Evol.*, 6, 2548–2558.
- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B. (2016) *lmerTest: Tests in linear mixed effects models*. R package version 2.0-32.