Peer Community In
Evolutionary Biology

Elevated sperm production and faster transfer: plastic responses to the risk of sperm competition in males of the black sodier fly *Hermetia illuce*

Trine Bilde *based on peer reviews by* ***Rebecca Boulton***, ***Isabel Smallegange*** *and 1 anonymous reviewer*

Frédéric Manas, Carole Labrousse, Christophe Bressac (2024) Sperm production and allocation in response to risk of sperm competition in the black soldier fly Hermetia illucens. bioRxiv, ver. 5, peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/2023.06.20.544772

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In this paper (Manas et al., 2023), the authors investigate male responses to risk of sperm competition in the black soldier fly *Hermetia illuce,* a widespread insect that has gained recent attention for its potential to be farmed for sustainable food production (Tomberlin & van Huis, 2020).

Using an experimental approach that simulated low-risk (males were kept individually) and high-risk (males were kept in groups of 10) of sperm competition, they found that males reared in groups showed a significant increase in sperm production compared with males reared individually. This shows a response to the rearing environment in sperm production that is consistent with an increase in the perceived risk of sperm competition.

These males were then used in mating experiments to determine whether sperm allocation to females during mating was influenced by the perceived risk of sperm competition. Mating experiments were initiated in groups, since mating only occurs when more than one male and one female are present, indicating strong sexual selection in the wild. Once a copulation began, the pair was moved to a new environment with no competition, with male competitors, or with other females, to test how social environment and potentially the sex of surrounding individuals influenced sperm allocation during mating. Copulation duration and the number of sperm transferred were subsequently counted.

In these mating experiments, the number of sperm stored in the female spermathecae increased under immediate risk of sperm competition. Interestingly, this was not because males copulated for longer depending

on the risk of sperm competition, indicating that males respond plastically to the risk of competition by elevating their investment in sperm production and speed of sperm transfer. There was no difference between competitive environments consisting of males or females respectively, suggesting that it is the presence of other flies per se that influence sperm allocation.

The study provides an interesting new example of how males alter reproductive investment in response to social context and sexual competition in their environment. In addition, it provides new insights into the reproductive biology of the black soldier fly *Hermetia illucens*, which may be relevant for optimizing farming conditions.

References:

Manas F, Labrousse C, Bressac C (2023) Sperm production and allocation in response to risks of sperm competition in the black soldier fly *Hermetia illucens*. bioRxiv, 2023.06.20.544772, ver. 5 peer-reviewed and recommended by Peer Community in Evolutionary Biology.
https://doi.org/10.1101/2023.06.20.544772

Tomberlin JK, Van Huis A (2020) Black soldier fly from pest to 'crown jewel' of the insects as feed industry: an historical perspective. Journal of Insects as Food and Feed, 6, 1–4.
https://doi.org/10.3920/JIFF2020.0003

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2023.06.20.544772
Version of the preprint: 3

Authors' reply, 16 January 2024

Dear Dr Blide

We really thank you for your decision on our manuscript "Sperm production and allocation in response to risk of sperm competition in the black soldier fly Hermetia illucens".

As you suggested, we corrected typos and edited the language to clarify the text. We have also reduced the length of the abstract to less than 250 words.

You can find a list of the changes, as well as a tracked-changes version of our manuscript attached to this submission.

Sincerely,

Frédéric Manas, Carole Labrousse, Christophe Bressac

Download author's reply
Download tracked changes file

Decision by Trine Bilde, posted 21 December 2023, validated 21 December 2023

Sexual selection in the Black Soldier Fly

Dear Authors

I would like to recommend your preprint and I thank you for the thorough revision.

I have chosen 'revision' becasue the preprint would benefit from language editing, and from checking carefully from typos. Unfortunately I did not find the time to list these mainly editorial changes. If it is possible for you to read the text very carefully for typos, and to find a person that could help with language editing for clarifying the text, mainly in the abstract and discussion, this would improve the impact of your paper.

Best wishes

Trine Bilde

Reviewed by Isabel Smallegange, 01 December 2023

I'd like to thank the authors for revising their manuscript so thoroughly. My queries have been answered satisfactorily. Most of my comments were on the methods, but the extra text and figure explain the methods well now. I have no further comments.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.06.20.544772

Version of the preprint: 1

Authors' reply, 09 November 2023

Dear Dr Blide

Thanks for your decision on our manuscript formerly entitled "Sperm production and allocation respond to perceived risk of sperm competition in the black soldier fly Hermetia illucens" and now entitled "Sperm production and allocation in response to risks of sperm competition in the black soldier fly Hermetia illucens".

We have followed most of the suggestions and addressed all comments. We put a particular effort into revising the materials and methods. In this section, we have addressed the confusions about the treatments by adding a figure explaining the treatments and sample sizes of the mating experiments. We added some nuances in our conclusion by mentioning the male mating rate hypothesis explaining our first result about sperm production. You will find that we've also developed the conceptual context a little further by discussing models of intensity of sperm competition. Finally, because we had a poor sample size for a combination of the two treatment, we increased our sample size for the second experiment to test an interaction suggested by the reviewers.

Overall, we would like to thank the three reviewers for the time they spent reviewing our manuscript and providing such insightful inputs. Thanks to them, our manuscript is now of much better clarity.

You can find a point-by-point reply to the comments of each reviewers below, as well as a track-change version of our manuscript attached to this submission.

Sincerely,

Frédéric Manas, Carole Labrousse, Christophe Bressac

Download author's reply

Download tracked changes file

Decision by Trine Bilde, posted 12 September 2023, validated 13 September 2023

major revision

Dear Frédéric

Your pre-print has now been reveiwed by 3 reviewers, who have provided a large number of comments and recommendations. Their comments suggest a need for improving the theoretical framework, and the reviewers have also stated many questions on the methods and analyses in particular. The subject and the findings of the study are very interesting, but there is clearly a need for revision and clarification of many aspects of the text.

Based on the comments from the reviewers, and my own reading, I recommend a thorough revision of the manuscript addressing the points raised by reviewers. Please see all the comments from reviewers attached.

Best regards
Trine Bilde

Reviewed by Isabel Smallegange, 01 September 2023

Hi,

Please find attached my comments on the manuscript annotated on the pdf. I like the idea that is tested in this manuscript but a lot of the experimental methods are not clear, which hampers understanding the results and their implications. I hope my comments help clarify the manuscript.

Best wishes,
Isabel Smallegange

Download the review

Reviewed by anonymous reviewer 1, 30 August 2023

This ms reports on a study of male reproductive traits in the economically importnat Black Soldier Fly (BSF). The paper studies male sperm priduction and sperm transference under different treatments to simulate 'sperm competition risks'. Clear responses were found for several traits as a reponse to the manipulation.

The study is interestingand relevant, but could be improved on a number of parameters.

The introduction introduces sperm competition as a novel aspect of sexual selection. This can easily be toned down as sperm competition is a very well reconised and established in evolutionary biology (so a bit much to claim a paradigm shift at this point). The referee is not convinced that the responses detected necessarily is in response to sperm competition risk and the the treatments necessarily confer sperm competition. They likely could but there might be other explanations. An increased sperm production in the presence of multiple conspecifics could be interpreted as an increased male investment in response to the potential for more maitings. This nuance could be better represented.

The test of responses in male reproductive investment is partly validated by the statement that it is known from many species but not investigated in BSF (yet). I hope there are much specific learning and knowledge to achieve and suggest that a stronger rationale and a stronger set of hypotheses would strengthen the whole paper. This would also allow strengthen the conclusion / perspectives of the reserach. It is currently claimed that the species and its fertility is important, but not discussed exactly why and how this will advance the field (as far as I known, BSF breeders do have plenty of eggs if they have enough flies). The final sentence states that the results should be integrated (where?) to control genetics (what does it mean to control genetics?).

The matrials and methods are somewhat hard to follow as several experiment with several co-variables are studied. A better description of the methods section and a figure on the experimental design will help the readers understand what is studied where - and align the experiments to the questions with the answers/results.

The text should be proof read to improve clarity and remove odd phrasing. The terminology of the two main treatments coul be developed to become even more clear and logic. ”Immediate competition risk” makes sense (I left out ”sperm, as I am not convinced that it can be assumed that it truely is sperm competition risk).

However, ”Mean risk” is not similarly logic. Would ”Historic risk” / ”Prior risk” or similar be better to logically distinguish between the two?

Apart from methodologically details, some facts about BSF reproduction are stated and in cases a foundation of assumptions. These might to some extent be anecdotal facts. One is whether the species rely on leks (which the paper did not observem and which could mean that the available area was nadequate or that no leks are required). Another is whether remaiting can be ruled out due to small space, as some personal observations suggest that mating can take place in ver small room. Please consider being clear (and provide references) for established facts, and feel free to share speculations, ideas and personal observations (but be clear about this as well).

Reviewed by Rebecca Boulton, 24 August 2023

Sperm production and allocation respond to perceived risk of sperm competition in the black soldier fly Hermetia illucens

General comments

The experiments seem appropriate to test the question of interest and are appropriately replicated. I did find it hard to follow what was done at times and some of the methods and results don't match up with each other or with the code. There is also a lot of important detail missing about theory (i.e. sperm predence/intensity vs risk models of sperm comp) as well as life history/ecology of the BSF.

Title – suggest changing to 'Sperm production and allocation in response to perceived risk of sperm competition in the black soldier fly Hermetia illucens' for clarity

Abstract - In the abstract (and elsewhere) clarify when both manipulations were applied (sperm allocation) or just one was applied (sperm production).

Introduction – provide a more detailed and nuanced description of Parkers risk and intensity models of sperm competition, including some discussion about strategic ejaculate allocation when competition is almost guaranteed (i.e. when it's better to partition out lots of small ejaculates to many females).

Methods – the main issue I had with the methods was following when the treatments were applied together, or just one (i.e. depending on whether sperm production or allocation was measured). I also had some questions about how single vs grouped males were differentiated in the sperm allocation test. It would also be very useful either in the methods or introduction to provide a more comprehensive description of ecology of the BSF (i.e. longevity, fecundity, oviposition resources, are high population densities common in mating aggregations, differences between mass culture and field conditions etc).

I have some more specific comments about the methods below:

L89-90 – did they have ad-lib access to food/water before experiments started?

L92-97 – how long were males kept like this? Did they have access to food? This is relevant when thinking about possible life-history trade-offs (i.e. was there competition over food or other resources in the grouped treatment).

L92 – 97 – so in total you had 240 grouped males but only 19 single males? Or was 19 a group of 10 but each male was kept separately?

L93-94 – were these males age matched? If not, how did you

L98-109 – this appears to be a factorial design, where mated females could either mate with a grouped male or a single male and then either alone or with 10 males or 10 females (i.e. 6 combinations of the 2 treatments in total) but it's hard to follow. A schematic showing the treatments and how they were set up (including sample sizes) would be helpful, as would a more comprehensive description of the factorial nature of the experimental design.

L102 – were they watched constantly to ensure that only first matings for each individual were recorded?

L102 – was this long enough for all pairs to copulate (report that it wasn't, L153 here). Did you record time they started copulation – this could be important if some individuals were kept in these mating groups for longer than others and this should be included in the statistical model.

L103 – how many mating pairs per replicate were used and how can you be sure females hadn't already mated with another male? And for pairs put in with 10 males did you make sure females didn't mate again before sampling?

L113 – for the grouped males were males age matched? If not, how did you distinguish the age of each male? Was age balanced across the 2 treatments?

L118 – was this section a measured grid on the slide or was it arbitrarily determined and then measured using a graticule? Did you take photographs of the preparations? If possible these measures should be validated by counting all the sperm in some of the replicates (for instance using imageJ) and comparing them to estimates.

L123 – why the day after copulation? When do they start laying/using sperm?

L124/134 – say that the head measured and taken into account for the sperm production measures of males as well as the sperm allocation.

L129-151 – state which independent variables were fixed effects/factors and covariates (continuous).

L133-134 – why wasn't the interaction effect between mean and immediate sperm competition treatment included in the model?

L133 – define treatments more clearly in methods (repeat where necessary what the treatment is) – i.e. mean risk of sperm competition (males alone or groups of 10).

L134-137 – include copulation start time in models as this affects how long pairs were in high density conditions prior to mating (for sperm allocation and mating duration), this might influence allocation strategy. It would also be worth testing whether males from the grouped or isolated treatments started copulating earlier.

Results

L153-156 – it's still not clear how these males were differentiated when they were put in a cage together with females.

L154-155 – seems likely that grouped males do copulate earlier so it would be good to take into account in models.

L163-164 – report model coefficients to support this statement.

L170-179 – in the code and in the methods it

L170 – again – why was the interaction effect between mean and immediate sperm competition intensity not fitted? The main effect of mean sperm competition is included in the model but not mentioned in the results.

L178 – report coefficients to support this statement

L196-201 – for age say that these stats are for the model with all individuals included (and this pattern was more pronounced when the long duration pair was removed).

Figure 3 – this is confusing because there were actually 3 levels in the 'immediate sperm competition' treatment, split up 'with conspecifics' into 'with males' and 'with females' to show that there is no difference and to make it clearer what this result refers to.

Figure 4 – where in the results does it say that you categorised the males and females as 'large' and 'small' or how you chose these categories? In the model that these results represent size is numeric so instead of surv plots scatter plots might be a more appropriate way of presenting the data (with one plot, points representing males and females coloured differently; similar for age and duration plot).

Discussion

A better overview of BSF mating system and life history would help to contextualise the results here, i.e. why it same-sex sexual behaviour makes sense (high densities of individuals make it hard to distinguish).

L222-223 – the results don't actually say this– for the sperm allocation models only the effect of immediate sperm comp is reported, not the null effect of mean sperm comp or the interaction effect (which isn't tested in the models).

L224-230 – this explanation could be clearer – males use social environment as a general cue to produce more sperm perhaps because they anticipate higher population densities which means both more matings and a higher risk of sperm competition, but it doesn't influence their sperm allocation strategy – only the immediate environment affects that.

L233-234 – what are these 'complex physiological mechanisms'? Males might transfer sperm in one go and then stay mounted while the female is processing sperm or as a mate guarding strategy.

L260 – could also be adaptive explanation – males are primed to produce more sperm when around females, this could be a general response to population density, it may be worth repeating the 'mean sperm competition' experiment with females which can be sensed but not mated with (i.e. enclosed in a mesh container) to see if the presence of female conspecifics also influences sperm production. Sperm production might reflect a life history trade-off between likely sperm use and longevity possible. Again it would be useful to have some

more information about BSF life history (i.e. do males produce sperm throughout life or just as larvae, do they emerge with their full complement of sperm – pro vs synspermatogeny).

L266-267 – this should come earlier and more detail about mating system needed.

L276-278 – consider the possibility of life-history trade-offs (plasticity in investment)

L279 – this is the first time this complexity is mentioned, more detail needed to understand how the spermatheca is complex and compare it to other species where more is known about the dynamics of sperm competition – for instance there is nothing on sperm precedence in this species, but this can be predicted from the shape of the spermatheca (i.e. https://doi.org/10.1016/j.zool.2016.12.001)

L281 and more generally – rearing conditions are important for productivity – do mass rearing conditions differ a lot in nature vs mass rearing and how might this influence results and efficacy of mass rearing (i.e. if not well adapted to higher sperm comp/pop density in nature – or not a problem?)

References

L344-345/L355-358 /L368-370/L397-399– formatting not consistent with other references

Code

No code to calculate descriptive statistics (L153-154; L176-179; 193-194; 198-201)

Can't find code to get the R squared value for with fig 2 (L181)

No code for producing figures

Read.Me would be useful to explain the codes for all the variable and what they refer to or at least some annotation in the code.

I get different values for anova of mod_Production and mod_Allocation – are there any data processing steps missing from the code? I.e. outlier removal etc or are any arguments missing from the anova function (to specify sums of squares etc).

It seems like age is being treated as numeric in the mod_Allocation code but in your results it looks like it's been input as a factor.

Consider using DHARMa to check model specifications