

This paper is concerned with sib-mating avoidance in natural populations of a parasitic wasp. The authors collected males (dead) and females (live) from the field at two different locations and analyzed microsatellites of daughters to determine the level of relatedness between the parents. The estimated rate of sib-mating was then compared to a threshold that was calculated based on encounter rates under random mating. The main finding is that this parasitic wasp species does not avoid sib-mating in the field, despite costs associated with sib-mating. The authors further performed an experiment to determine whether sex-biased dispersal takes place and found that males were dispersing more compared to females. In a laboratory experiment the authors then looked at the effect of male density and proportion of related males on sib-mating avoidance. Overall, density or a higher proportion of sibs did not lead to sib-mating avoidance. Several hypotheses to explain this finding are discussed.

I think this is a very interesting study. The authors have performed experiments both in the laboratory and in the field and have further designed an experiment (i.e. concerned with sex-specific dispersal) aimed to support their findings that sib-mating is not avoided in this species. The study of sib-mating avoidance in the field really is rather unique. One issue that I have with the MS, however, is that the authors claim that virtually no research has been done on sib-mating avoidance in insects. I do not think this is true, because in the laboratory several studies have been done on sib-mating (see my comment below for relevant references). Moreover, I find that the authors are not clearly describing their own previous findings on this topic (Metzger et al 2010 PLoS one). This is important, because in the current version of the MS the order of experiments does not make sense to me. If I would have been in your position I would first have performed laboratory experiments to confirm/extend previous findings on sib-mating avoidance in *V. canescens*. I would then have gone into the field to collect samples, followed by the experiment on sex-specific dispersal. I think the latter is indeed a nice addition that offers a potential explanation, but it would have been better if this experiment had been repeated at the different locations. Overall, I suggest you include a more detailed description of the work that has been done on sib-mating (and avoidance thereof) in insects, including your own work. Otherwise, very well done.

I did not have access to the supplementary files for this MS, so I have not been able to review those.

Minor comments:

I personally like the title, but it is not very informative. I suggest keeping the first part (i.e. Insects and incest), but making the second part of the title more informative (i.e. no avoidance of sib-mating in natural populations of a parasitoid wasp).

Sib-mating should be written with a hyphen in between sib and mating throughout the MS.

Abstract:

You are not mentioning your results on sex-biased dispersal in the abstract. I think that should be included somewhere.

bullet point 1:

... likely evolves in species that are... (remove the s before are).

bullet point 2:

Remove the comma after species

Replace 'congruently' by ', and as a consequence'.

bullet point 3:

Rewrite to: 'Our study consisted of genotyping...'

Rewrite to: 'With these data we were able to reconstruct the genotypic of all females' mates and estimate the relatedness of each mating pair'.

bullet point 5:

Rewrite to: 'These results suggest that *V. canescens* tolerates sib-mating in the field...'

It is either 'the common belief' or 'common beliefs'.

Rewrite to: 'This inbreeding tolerance also opens up the question as to why kin discrimination is maintained in this species'.

Does this really call into question whether inbreeding depression affects species with SI-CSD? You know that inbreeding has negative effects in this species (and others), so the real question is why sib-mating is not avoided in nature despite those costs (and indeed why discriminatory behaviors are maintained).

I would remove parasitoid wasp from your key words (it is already in the title and abstract of your paper). You could replace it with parasitic wasp to cover more ground.

Introduction:

paragraph 1:

Rewrite to: 'Various strategies to avoid inbreeding have been described in animals,'

Put a comma in front of 'such as'

Rewrite to: ' strategies are associated with...'

Rewrite to: 'gene associations'

You write that 'selection on behaviors underlying inbreeding avoidance should depend on inbreeding load, which scales the advantage of inbreeding avoidance and the costs of implementing adapted behavioural responses', but to what does selection scale up to? I suggest rewriting this sentence for clarification.

For the final sentence I suggest including a sub-sentence to explain what you mean with inclusive benefits. For instance, ', i.e. advantages for an individual's inclusive fitness'.

paragraph 2:

It is not population density itself that constrains mate availability, it is a *low* population density that constrains mate availability. This should be written clearly in the MS.

This paragraph needs a concluding statement. For instance you could summarize by saying that when mates are scarce, it might be beneficial to mate despite high relatedness.

paragraphs 3 and 4:

Both of these paragraphs also end a bit abruptly. I would suggest including a concluding sentence to each of these paragraphs to clarify the point that you are trying to make.

paragraph 4:

It is either ‘an inbreeding avoidance pattern’ or ‘inbreeding avoidance patterns’.

You mention here that inbreeding avoidance has rarely been documented in insects, especially in the wild. I agree that not much work has been done in the wild, but in the laboratory plenty of studies have by now been done on inbreeding avoidance in insects, particularly in parasitoids (e.g. Bourdais & Hance 2009 Behav Proces; Lihoreau et al 2007 Behav Ecol; Ode et al 1995 Anim Behav; Visser et al 2014 Behav Ecol Sociobiol). Not all of these studies may actually find that there is sib-mating avoidance, but the absence of positive findings does not mean that no work has been done on the topic. Moreover, your own previous work (Metzger et al 2010 PLoS one) directly addresses sib-mating avoidance, but this aspect of the paper (i.e. choice assays) is only mentioned in the discussion. These findings should already be put forth in the introduction, because it is a critical finding for your species and it puts the work into context. I suggest that you discuss more thoroughly the work that has been done so far on kin avoidance (or the absence thereof) in haplodiploids/parasitoids, including your own work. I think that this would actually strengthen your argument that only little research has been done on inbreeding avoidance in the field.

paragraph 5:

This is an excellent explanation of haplodiploidy and sl-CSD.

paragraph 6: ... ‘monandrous/polygenous’

paragraph 7:

At the end of this paragraph you refer to earlier findings and prior expectations, but what were these earlier findings and prior expectations? Please briefly repeat here.

Materials and methods

paragraph 1:

Could you include what kind of insect *Ectomyelois ceratoniae*? For instance, the moth....

I think it is a bit misleading to only focus on *E. ceratoniae* as a host. As you know *V. canescens* is a generalist (it has been documented to parasitize more than 20 hosts) and some host species may not be solitary like *E. ceratoniae* (for instance, *E. kueniella* or *P. interpunctella*). The fact that Driessen and Bernstein found about one *E. ceratoniae* per fruit is not really a good argument that patches for all hosts are small. Their result that *V. canescens* cannot really distinguish between the presence of 4 or more (up to 20) *Ephestia* larvae (compared to 1 or 2) is, however, supporting the idea that *V. canescens* does not often encounter locally aggregated hosts. If this is indeed the case in nature, it is not likely that this species experiences local mate competition (as sib-mating is not expected at the natal patch).

‘Males search for females...’

...’with a flight velocity estimated...’

paragraph 3:

'Female traps constituted of an...'

'Saliva secreted by host larvae when...'

'Traps were hung...'

'Traps consisted of a 125mm...'

paragraph 4:

'... where they were handled in a climatic chamber...'

Should DL be LD here? In other words 16 hours light and 8 hours dark?

I think you need to include a definition here for arrhenotokous and thelytokous (say sexual and asexual).

paragraph 5:

'..., hereafter referred to as wild females)'

Fewer than 3 daughters per female seems very little to me and suggests that the mothers did not have enough time to lay eggs, that the mothers did not have enough hosts or that mothers produced an extremely male-biased sex ratio. Otherwise it might be because the females caught in the field are already quite old? Why are these numbers so low? You need to explain this in the MS.

'... at each microsatellite locus...'

Paragraph 12:

I think you should clearly state in the sub-title of this paragraph that these experiments were performed in the laboratory.

Actually, it is not clear to me which individuals are used for these experiments? Is this a standardized laboratory rearing? If so, then information needs to be added about this rearing earlier in the m&m. If this is indeed another rearing, you should also pay some attention to this in the discussion as results might have been different for wild-caught rather than laboratory individuals.

Results

paragraph 2:

'Effective population sizes were estimated to be 79 for...'

Paragraph 5:

You say that random mating is suggested by your 'previous results'. I think it would be clearer if you mention here again briefly what that result was.

Paragraph 6:

Should read 'referred to as ...' throughout this paragraph.

I think paragraph 6 and 7 should be only one paragraph.

Discussion

Paragraph 1:

‘Sib-mating avoidance in *Venturia canescens* has been observed in behavioural experiments performed in the laboratory...’

Remove comma between field populations and the observed...

‘In the remainder of the discussion...’

‘...patterns observed in the field...’

Paragraph 2:

‘... assuming random encounters between...’

‘... the proportion of sibs present.’

You mention here again that Metzger et al 2010a only performed no-choice tests, but this is not the case. These authors did perform choice tests and that should be stated throughout the MS. I don't think that leaving out these details adds more novelty to your work, it only obscures the reasoning behind investigating sib-avoidance in the field. It makes more sense to me that first laboratory experiments were done to see whether sib-mating avoidance takes place, in line (or not) with previous findings of Metzger et al 2010a and then to argue that lab experiments may not reflect what is actually happening in nature, thus calling for an investigation of sib-avoidance under natural conditions. I would propose to more openly discuss the findings of Metzger et al 2010a and to introduce your own lab experiments before presenting the results on field individuals.

Paragraph 3:

The first sentence of this paragraph refers to ‘these results’. State here briefly what results you are referring to.

Paragraph 4:

‘The second hypothesis is that female choosiness in *V. canescens* is density-dependent, ...’

‘A similar reasoning may hold true for...’

Paragraph 7:

‘, the probability of matched mating increases to 50%’

Paragraph 8:

‘threatened’ should read ‘threatened’

Paragraph 9:

‘In *Venturia canescens* the evolution of kin recognition in a superparasitism context...’

‘... has a high cost for female fitness’

The sentence ‘Under the hypothesis of a primary evolution of kin recognition in the parasitism context and a secondary use in mate choice, the molecular pathways of sib recognition in the two ecological contexts should therefore be close’ is not correct grammatically, nor do I understand what you mean here. This needs clarification (or removal).

‘... unique pathway for kin recognition...’

Remove ‘The’ before superparasitism avoidance.

‘that sib-mating avoidance...’

References:

Is the reference of Chuine A. 2014 a PhD thesis or a MSc thesis? This should be mentioned here.