Peer Community In
Evolutionary Biology

Incestuous insects in nature despite occasional fitness costs

Caroline Nieberding and **Bertanne Visser** *based on peer reviews by 2 anonymous reviewers*

Marie Collet, Isabelle Amat, Sandrine Sauzet, Alexandra Auguste, Xavier Fauvergue, Laurence Mouton, Emmanuel Desouhant (2018) Insects and incest: sib-mating tolerance in natural populations of a parasitoid wasp. bioRxiv, ver. 1, peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/169268

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Inbreeding, or mating between relatives, generally lowers fitness [1]. Mating between genetically similar individuals can result in higher levels of homozygosity and consequently a higher frequency with which recessive disease alleles may be expressed within a population. Reduced fitness as a consequence of inbreeding, or inbreeding depression, can vary between individuals, sexes, populations and species [2], but remains a pervasive challenge for many organisms with small local population sizes, including humans [3]. But all is not lost for individuals within small populations, because an array of mechanisms can be employed to evade the negative effects of inbreeding [4], including sib-mating avoidance and dispersal [5, 6]. Despite thorough investigation of inbreeding and sib-mating avoidance in the laboratory, only very few studies have ventured into the field besides studies on vertebrates and eusocial insects. The study of Collet *et al.* [7] is a surprising exception, where the effect of male density and frequency of relatives on inbreeding avoidance was tested in the laboratory, after which robust field collections and microsatellite genotyping were used to infer relatedness and dispersal in natural populations. The parasitic wasp *Venturia canescens* is an excellent model system to study inbreeding, because mating success was previously found to decrease with increasing relatedness between mates in the laboratory [8] and this species thus suffers from inbreeding depression [9–11]. The authors used an elegant design combining population genetics and model simulations to estimate relatedness of mating partners in the field and compared that with a theoretical distribution of potential mate encounters when random mating is assumed. One of the most important findings of this study is that mating between siblings is not avoided in this species in the wild, despite negative fitness effects when inbreeding does occur. Similar findings were obtained for another insect species, the field cricket *Gryllus campestris* [12], which leaves us to wonder whether inbreeding tolerance could be more common in nature than currently appreciated.

The authors further looked into sex-specific dispersal patterns between two patches located a few hundred meters apart. Females were indeed shown to be more related within a patch, but no genetic differences were observed between males, suggesting that *V. canescens* males more readily disperse. Moreover, microsatellite data at 18 different loci did not reveal genetic differentiation between populations approximately 300 kilometers apart. Gene flow is thus occurring over considerable distances, which could play an important role in the ability of this species to avoid negative fitness consequences of inbreeding in nature. Another interesting aspect of this work is that discrepancies were found between laboratory- and field-based data. What is the relevance of laboratory-based experiments if they cannot predict what is happening in the wild? Many, if not most, biologists (including us) bring our model system into the laboratory to control, at least to some extent, the plethora of environmental factors that could potentially affect our system (in ways that we do not want). Most behavioral studies on mating patterns and sexual selection are conducted in standardized laboratory conditions, but sexual selection is in essence social selection, because an individual's fitness is partly determined by the phenotype of its social partners (i.e. the social environment) [13]. The social environment may actually dictate the expression of female mate choice and it is unclear how potential laboratory-induced social biases affect mating outcome. In *V. canescens*, findings using field-caught individuals paint a completely opposite picture of what was previously shown in the laboratory, i.e. sib-avoidance is not taking place in the field. It is likely that density, level of relatedness, sex ratio in the field, and/or the size of experimental arenas in the lab are all factors affecting mate selectivity, as we have previously shown in a butterfly [14–16]. If females, for example, typically only encounter a few males in sequence in the wild, it may be problematic for them to express choosiness when confronted simultaneously with two or more males in the laboratory. A recent study showed that, in the wild, female moths take advantage of staying in groups to blur male choosiness [17]. It is becoming more and more clear that what we observe in the laboratory may not actually reflect what is happening in nature [18]. Instead of ignoring the species-specific life history and ecological features of our favorite species when conducting lab experiments, we suggest that it is time to accept that we now have the theoretical foundations to tease apart what in this "environmental noise" actually shapes sexual selection in nature. Explicitly including ecology in studies on sexual selection will allow us to make more meaningful conclusions, i.e. rather than "this is what may happen in the wild", we would be able to state "this is what often happens in nature".

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Reviews

Evaluation round #2

DOI or URL of the preprint: **10.1101/169268**
Version of the preprint: 2

Authors' reply, 26 February 2018

Download author's reply

Decision by Caroline Nieberding, posted 26 February 2018

This preprint is worth a recommendation

Second round of review for PCI Evolutionary Biology of "Insects and incest: sib-mating tolerance in natural populations of a parasitoid wasp" by Collet et al

Dear Dr Collet, We are pleased to inform you that your manuscript "Insects and incest: sib-mating tolerance in natural populations of a parasitoid wasp" is suitable for recommendation by our PCI Evolutionary Biology panel. We appreciate mostly the novelty of this work, based on extensive field work for assessing the existence and extent of sib-mating mating avoidance in an insect, as well as the integrative nature of your experimental approach combining laboratory mate choice experiments and simulation work to the field data. We also appreciated the significant improvement of the introduction in your revised ms, which now places your data in the most appropriate literature survey. In case you wish to publish this work in another peer reviewed journal, we would advice to further integrate the discussion: 8 discussion points in a row is hard to digest and it is not yet 100% clear what are your most likely explanations to the data? For this, one referee has edited your .doc file including the discussion. We also think that your microsatellite data strongly suggest that dispersal is likely the main mechanism driving genetic homogeneity at the scale your quantified it, and thus high dispersal could explain why sib mating avoidance is not observed in natural conditions. Finally, one referee also found a study by Bretman et al in Molecular Ecology 2011 (by the team of Tom Tregenza) that show similar results to yours based on a four-year field study on crickets. Such work would be useful to incorporate to your own manuscript. Below you will find more detailed comments on your manuscript, and the .doc edited file is attached to this file.

Reviewed by anonymous reviewer 2, 19 January 2018

This is a revision of a paper in which sib-mating of the parasitoid Venturia canescens was investigated both in the laboratory and in the field. In the laboratory courtship behavior and mate choice were observed at different male densities and frequencies of related males. Contrary to results of a previous laboratory study, V. cancescens did not avoid sib-mating in the lab. The authors then addressed whether sib-mating occurs under natural conditions. To do so, wasps were collected from the field at two different locations. Collected females and their daughters were then genotyped, after which genotypes of fathers were inferred. The relatedness between mates observed in the field was then compared to a theoretical distribution describing the chance of sib-mating under the assumption of random mating. Similar to findings in the lab, field observations suggested that sib-mating was not avoided in nature. Genotype data were further used to determine genetic relatedness and sex-specific dispersal between two patches ~200m apart. Mean relatedness did not differ for males, but within patches relatedness between females was higher, suggesting that males of this species more readily disperse than females. This paper contributes important knowledge on the lack of inbreeding avoidance in insects under natural conditions. It further highlights that experiments under artificial laboratory conditions may not accurately reflect what is happening in nature, something that is not often recognized or addressed by the scientific community. I've included my comments directly in the MS (in red), see the attached PDF file.

Reviewed by anonymous reviewer 1, 12 February 2018

General comments

This manuscript quantifies whether one wasp species, Venturia canescens, avoid sib-matings to avoid the risk of inbreeding. This manuscript combines: i) careful field work with a robust sample size of 467 individuals (caught either in the wild or their direct F1 descendants) that were sequenced for 18 microsatellites in two populations, ii) simulations run in R to assess the expected level of relatedness and other factors from these datasets, iii) as well as lab-designed experiments in which the role of density and of relatedness in mate selection are tested. This study appear as very novel in that the study of inbreeding avoidance has been so far rarely tested in the wild except for large vertebrate taxa. This study also reveals the limitations of laboratory-based work to highlight natural patterns as they evolved in the wild and this is particularly true for sexual selection in which the social component of the environment is central to assess the adaptive value of selection but is largely and usually ignored in lab studies (see West-Eberhard in Neuroscience and Biobehavioral Reviews 46 (2014) 501–508 for example). I see this study as a example of the importance to get back to field data for getting rid of lab biases when it comes to characterize natural mate choice selection to identify the evolutionary mechanisms on action in nature. The discussion lacks some integration as there are 8 different proposed points to explain the data, some of which could be better integrated to each other, and their relative importance be assessed. In particular, the role of dispersal which has been quantified by the absence of genetic differentiation for 18 microsatellites between 2 populations separated by 300 kms, seems to be underestimated while it may well be one main mechanism explaining the absence of sib mating avoidance in the wild.

Detailed comments:

Introduction: overall very nice introduction, a pleasure to read.

- l97: the study by Robinson et al 2012 about inbreeding avoidance in the wild in insect in missing from the list of references. Please also add other relevant field work studies such as the one by Bretman et al in Molecular Ecology 2011 (by the team of Tom Tregenza). - l114-122: inbreeding depression is indeed particularly bad for species like yours that have such a particular genetic mating system, but inbreeding depression arises in any species when homozygote deleterious alleles are expressed (se early work on inbreeding in Drosophila flies by Morgan for example). Perhaps these sentences could be modified to make sure that we do not forget that your results may apply to any species.
- L118: is it known that Venuria canescens females mate only once (as written l129)? Best to remind it here then otherwise these numbers would be a maximum.

Material and methods: My general comment regarding the MM is about how different the experimental social environment in the lab is from natural conditions (as known): sexual selection is by essence a social type of selection, which means that the individuals surrounding the focus individuals are both agents and targets of selection: changing the density, the sex ratio is know to effect the expression of sexual selection (for example a review by Miller and Svensson 2014 in Annu. Rev. Entomol. 2014. 59:427–45; and West-Eberhard in Neuroscience and Biobehavioral Reviews 46 (2014) 501–508). Hence it is likely that differences between lab experiments (previous ones with no-choice experiments and current ones with a choice experiments) and field data are due to biases in the social environment of the organism compared to natural environments.

- l161: another nice reference demonstrating the effect of LMC on sex ratio: Macque et al Science 2012 (Isabelle Olivieri's team).
- L165: such evidence of dispersal in the wild for this species could be used in the discussion to highlight the role of dispersal limiting risk of sib-mating.

Results:

- L350: Compared to the lab mating experiments (choice between brothers and unrelated males), these values of relatedness in the wild are very low. Hence perhaps sib mating avoidance has evolved in the wild at much lower absolute value of kinship than those tested in your lab trials. Or sib-mating avoidance is not necessary in the wild to avoid inbreeding. - l384: did males and females have a similar level of relatedness, within patches (curiosity)?

Discussion

Overall this discussion is strange because there are 8 different points that could be better integrated and it is not clear which mechanism/hypothesis is/are more likely to the authors, given the data and their extensive knowledge about the organism life history. - l395: behavioural output of mating experiments is always a bit tricky to interpret : for example, latency to mate could decrease either because there is increased mate selectivity (to avoid brothers) as you write, or because mate choosiness decreased too -too much stress by male courtship activity, if there is no possible escape from the small cages by females ? - l408: discrepancies between different lab-based experiments on mate choice suggest that you face the same problem as others that has been reviewed recently by Miller and Svensson 2014 and by us: lab biases the social env under which mate choice is expressed. Perhaps I Metzger et al 2010a paper, the experimental conditions were less biased compared to natural conditions and allowed females to express choosiness: mating partners may be encountered sequentially in nature (and not in groups), the average level of relatedness that females may have to perceive is perhaps overall much lower (than brothers as in the lab experiments), the density may be lower, the females may reject males by escaping by flight (which is not possible in lab cages), and so on... All this may lead to frustrating estimates of mate selectivity expression in lab studies which most authors on sexual selection have avoided to think of for a long time. We have reviewed the lab biases on the expression of mate choice in a butterfly species recently and have become aware of that problem (Holveck et al, 2015; Holveck and and Nieberding, 2017, in press in Frontiers Zoology).

- L446: “proportion”: do you mean “density”, or “overall level of kinship”?
- L448: Given that the field data is what really happened, the lab data may be more or less biased to explain the field data. I would consider that your field data is much stronger / robust than your lab experiments and discuss the limits of the lab experiments on that basis. - L475: Hypothesis 6 relates to cost of outbreeding and hyp 7 (in fact hyp 6b) is related to cost of inbreeding depression. Put them together?
- L497-498: not clear: it is always bad to have more than one egg in the host, right? So it is not the cue about who laid the egg that matters, but rather the cue indicating that another egg is already present in the host - no effect of kinship in this -? What proportions of host were parasitized in the wild - is there a pressure for multiple eggs in hosts-?

Evaluation round #1

DOI or URL of the preprint: **10.1101/169268**
Version of the preprint: 1

Authors’ reply, 17 January 2018

Download author’s reply

Decision by Caroline Nieberding, posted 17 January 2018

Revise

Dear Authors, overall your work is very original, integrative and experimentally well designed. Both reviewers had trouble in the presentation of the ideas and experiments, which could be improved (see proposals). The biology of the species and previous work done on the topic also deserved better presentation. If you have the energy to present your work in a more integrated framework and to highlight its novely (assessment of inbreeding avoidance mechanisms in the wild, for non vertebrates) with more arguments including a thorough review of the existing literature, we believe that this contribution could become a first-rate quality one. Hoping that this is useful for you,

Reviewed by anonymous reviewer 1, 28 November 2017

Review of ms by Collet et al, about inbreeding avoidance in Venturia canescens; review for the PCI Evol Biol, August 2017.

General comments

This manuscript intends to assess whether matings of one wasp species (the model Venturia canescens) in the wild avoid the risk of inbreeding, and to examine several mechanisms that may have led to the evolution of inbreeding avoidance such as kin recognition and dispersal. Overall this manuscript appears to me as very original and carefully thought and designed. The main strengths include: 1) that this work combines careful field work -from which a robust sample size of 467 individuals (caught either in the wild or their direct F1 descendants) were sequenced for a total of 18 microsatellites for inferring inbreeding level, in two populations-, simulations run in R to assess the expected level of relatedness and other factors from these datasets, as well as lab-designed experiments in which some mechanisms leading to inbreeding avoidance are tested (the role of density and of relatedness in mate selection).

However, I have the problem that I have a hard time understanding: 1) how exactly the experiments were conducted, and this holds true mostly for the simulation work on the expected coefficient of relatedness and on the statistical treatment of the mate choice experiment (I detailed my concerns for the mate choice experiment below), and 2) the conceptual reasoning that put all experiments together. Regarding the first point, the simulation work could most likely be super straightforward to understand if there was simply more space dedicated to transmitting the procedure in the methods section (in the SOM or in the main text as authors prefer). This should be ok to implement as there is at this stage no length limitation (on the PCI website) for manuscripts. Regarding the latter concern, I think that this could be solved by reorganizing the ideas such that what pops up to the mind of the reader in the first place is not brought in the manuscript in the last paragraph of the discussion (for example). I propose below a new “structure” to the presentation of the ideas in the introduction that would help me, and hopefully others, to better understand everything at once.

My second main concern is the novelty of this work: The study seems very novel in that the authors claim that the study of inbreeding avoidance in the wild has been so far rarely conducted except in large vertebrate taxa, but this would be much more convincing if it was stated as such more strongly and with a more detailed review of existing studies. If done, I believe that this study has the material for publication in a firs rate quality journal. This is also why I wrote below a lengthy review trying to help revising the ms.

Specific comments

Here I list some of the places in the manuscript that justify the general comments written above. It is not exhaustive but it aims to help the authors understand where I had trouble and how, perhaps, this could be solved.

Abstract and introduction

- The authors write: “Our results suggest that V. canescens tolerate sib mating in the field despite kin discrimination, and therefore call into question the common beliefs on inbreeding depression in species with single-locus complementary sex determination. This inbreeding tolerance also opens up the question of the maintenance of the kin discrimination in this species” Comment: The fact is when reading this section of the abstract and the introduction, the reader will immediately think that :
  1) the biology of the species may very well explain this apparent discrepancy (see specific questions below) but the lack of information on these aspects of the life history of V. canescens gives the feeling that one does not have all information we would need to be able to judge;
  2) there are other options that are not discussed until very late in the manuscript (end of the discussion): the existence of kin recognition despite low risk in the wild for kin matings, may be explained also as 1) a ghost from past adaptation (when effective sizes were smaller?) for which it would be important to have evidence of past demography for the species (using coalescent methods - the genome of the species has been sequenced)? 2) and/or kin recognition is a mechanisms under selection for another reason that avoidance of sibmating; for example, it may be advantageous to localize in space related individuals

as they may bear similar genetic adaptations to similar environments (same host species, same food source, ...) such that kin recognition may be actually used for other reasons that the one mechanism under testing here, inbreeding avoidance. Third, there may also be no avoidance of kin mating because dispersal has already ensured that the average relatedness coefficient in the wild is below any sufficient inbreeding cost: both the dispersal and the cost of inbreeding for this species has been tested in the manuscript with simulations / analyses using the field data (very smartly)- but it does not come as an obvious alternative in the right place in the introduction; the cost of inbreeding (number of diploid males is provided at the end of the discussion rather than in the result section) and the dispersal effect to inbreeding avoidance is not brought upfront of the start of the introduction as it should be.
Hence, all major concepts and mechanisms related to inbreeding avoidance and the evolution of kin recognition as one of the potential mechanisms explaining inbreeding avoidance (but also other patterns) are in the manuscript, but one needs some reorganization to show how smart this study was. I propose that instead of bringing all the ideas and results one by one, the introduction does a better job by showing that the authors have thought of all alternatives, and will test quite a few of them in the results. I hereunder propose such a skeleton for the intro if this may be useful:
1) Inbreeding avoidance has evolved in many organisms because it has costs (list them), yet it also entails benefits so its evolution is the results of the balance between costs and benefits.
2) Environmental factors affects the relative costs and benefits of inbreeding avoidance (list which ones: density, population structure, social structure of the species,...), hence a) we need field data to quantify better the relative cost to benefit of inbreeding avoidance, b) what we see now is the product of past selection and the original factors may well be no longer effective (such as recent increase in demographic size would make inbreeding voidance mechanisms unnecessary).
3) Kin recognition (by means of chemical communication in many insects) is one major mechanism used for inbreeding avoidance, yet it is not the only one and kin recognition may also have been selected for other reasons (which ones).
4) Altogether, this is one of the very first study bringing these questions into the wild with robust field data and combined molecular and lab experimental work. The justification of Venturia as a model species could be further improved by providing in the intro more information on the biology of the species that justify the relevance of testing all the above points on inbreeding avoidance, cost, benefits and knowledge on kin recognition. The questions that need to be answered about the biology of the species (in the introduction of the MM): What are the chances that related individuals encounter each other for mating : does developmental time differ between sexes? Do mothers lay a clutch of eggs in the same place (group of host) or not? How long do mothers lay eggs ? Do they lay first male next female eggs? How many times mothers do mate (this info is provided later in the introduction)? All these pieces of information could allow predict the likelihood that in nature mating with kin is more or less likely, explaining that the effective rate of sib mating is obtained by random mating, or not, and that specific mechanisms should have evolved, or not.

- We are waiting too long to know (when reading) What is the evidence for inbreeding depression in Venturia? The authors write : “Inbreeding depression arises for diploids that are homozygous at the sex locus: instead of developing normally into females, they turn into abnormal diploid males that are generally unviable or sterile “, and also “the low fitness of diploid males”...But the fitness of such males is 0 right- They can’t fertilize any eggs?

- The introduction shows that a very large diversity of behavioural strategies related to inbreeding risk exists and that the explanation may be that there are different associated costs and benefits for each species. At the moment, paragraphs 2 and 3 of the introduction show case studies of varying strategies limiting (or not) inbreeding risk in various species, but the actual associated cost/benefit of inbreeding in each system is not provided. It would be nice to better link what is known in each investigated species regarding a) the patterns observed in inbreeding avoidance (or not) and, b) the associated known costs/benefits to inbreeding; that is, to know if, indeed, inbreeding is avoided when it leads to larger costs relative to benefits, and vice-versa, for each investigated species. In practise I would like to know whether , for example, the fitness advantage of related matings in the Marmosa flavipentris (if known?) or the observed costs of related mating in the ring-tailed lemurs and mole rats?

Material and methods

- The authors write the methods in a way that gives a bit the wrong feeling that they have not thought that inbreeding avoidance may be obtained by dispersal from natal habitat before actual matings occur. In fact the authors test this option as well (and validate it), and the authors also explore the cost of mating with related individuals given the current population structure (and show it is very low). But these pieces of information come at odd places in the manuscript.
- "Inference of mate's genotype : When alternative genotypes were proposed for a given female's mate, we selected the only compatible one on a case-by-case basis" How exactly was this done (there is information in the suppl data but it is not 100% clear to me).
- The whole section "Estimation of relatedness between potential versus mated partners in the field" is not clear to me and in particular the sentences ""Simulations allowed to keep track of pedigrees, yielding a perfect knowledge of kinship for all potential mates. These were classified in three categories: full-sibs, half-sibs, or unrelated. Besides, we calculated the relatedness coefficient (r) for all female-male pairs from these simulated populations (software COANCESTRY;(Wang, 2011). This allowed to estimate the optimal relatedness threshold minimizing the number of wrong assignations (i.e., unrelated pairs assigned related based on the threshold, or vice-versa). We found rcrit = 0.16 (SEM-D, Fig. S2). Logically, this threshold is lower than the theoretical relatedness between a brother and a sister (r sister-brother = 0.25; r brother-sister = 0.5) and in the interval of relatedness for 1st degree cousins (r = 0.125 or r = 0.1875 depending on the cross). With this threshold, we expect to wrongly assign 11.4% of the relationships". In fact, this notion of "relatedness threshold" that the authors say is the threshold « above which individuals were considered related. »: would it be better to define it as the "threshold at which matings occurs among related individuals more (often?) than at random" ? Also, perhaps because I did not understand the procedure, I wonder whether this threshold of r=0.16 is related to any detrimental documented effect of inbreeding in these populations/species? – I suppose it is not, that is why I propose another definition of threshold above. And what does the " the optimal relatedness threshold" is ? And how can you have « wrong assignations (i.e., unrelated pairs assigned related based on the threshold, or vice-versa) » if the full pedigrees of the simulated populations are known ? More questions: how balanced is the sex ratio in nature - As this affects the simulations of threshold it may be worth investigating how variation in sex ratio (often biased towards more males than females in terms of effective population available for mating), affects estimate of random mating in the wild- ?
- The authors write: "We also estimated the probability that a female encountered a related male in the field the day of her capture." How did they do exactly – Aa far as I understand, you use for this estimation the date of recapture (only males captures the same day as the female had a chance to mate with her, according to you) and not, for example, some distance between individuals above which encounter as a mate is no longer likely (which I would assume to be more relevant if individuals are fertile more than one day).
- The authors write: "We recorded mating latency, copulation duration, status (colour) of the successful male, number and timing of male rejections by females and status (colour) of rejected males. " I do not follow fully the statistical treatment of these data: mating outcome is the results of both male and female behaviours, and in other insects it is clear that male courtship activity is a prerequisite for females to accept or refuse the mating, that is, without male courtship there is no chance of mating. I think that the authors do correct mating success of related versus unrelated males by their relative courtship activity ,

at least in the treatment of the "latency to mate" but what for the actual number of matings? It would be good to provide the exact GEE-GLM best model structures.

- Why no males captured in Valencia? (table 1)
- I do not understand the sentence "As sexual and asexual strains coexist in V. canescens ((Beukeboom et al., 1999; Schneider et al., 2002), we used the presence of males among offspring as evidence for arrhenotoky. Mothers and their offspring were killed and preserved individually in 96% ethanol. Thelytokous individuals were discarded. » : can individuals produce only daughters, or only sons, in this species (I thought that the sex of the offspring was dependent on the fertilization of the eggs, hence all mated individuals can produce both sexes) and why discard some individuals?

Results

- The authors write: "We nonetheless treated the two populations separately because the probability that a male captured in one population had inseminated a female captured in the other is negligible. ": why is that? Dispersal distance is much shorter than 200 m for this species the distance between your two populations)?
- In the section about "diploid males: Please start by explaining the relevance of this part, that you aim to assess the cost of inbreeding in this species from your field data. And why work with the Nice population only – evidence of random mating is not correlated to genetic diversity -?
- In the section about "dispersal": there is male-biased dispersal in the species, thus how do you take this into account to your quantify the "randomness" of the field matings patterns in your microsatellite data? I would start by showing sex-specific dispersal and next estimate of the resulting mixed populations may still gain, or not, to increase inbreeding avoidance by other mechanisms.
- Section on the « effect of density and relatedness on mating success » :
  What is used to quantify mate choice? Mate choice is a complex concept that includes both the expression of mate selectivity (females for example usually become less selective when ageing or when density of partners is low - such as in beetles see Moore and Moore PNAS 2001 "Reproductive aging and mating: The ticking of the biological clock in female cockroaches") and of mate preference (females exert sexual selection on certain traits, but not on others hence they have preferences for some but not all aspects of males). As explained above, mate choice may also, depending on what trait is used to quantify it, include both male and female behaviours as there is mate choice (assumed to be the fact of females) only if male actually court the female in many insects. Mate choice is a complex trait and it is unclear whether male or / and female sexual behaviours are involved in the variation of this traits in your treatments. Your results suggest that there is no mate choice going on, such that females would not avoid related individuals. However your results also suggest that female can recognize kin from non kin males because latency to mate is reduced after females have rejected a kin male.

  To try and improve clarity it would be good that:

  1) The statistical models are more clearly described, including what is the Y response variables and what are the X variables for all models used.

  2) The biology of the individuals in the experiments is provided: what was the age of the females/males ? ...

  3) the authors write « confirmed this ability of kin recognition in our choice test, the rejection of brothers (but not that of unrelated males) decreasing the female copulation latency.". Please clarify how you expect latency to mate to reflect female mate choice (female preference, or female selectivity) for this species ? We may well expect that latency decreases because female selectivity decreases and/or because female preference increases.... It is hard to exclude, again, male willingness to mate (through variation in courtship activity) from this pattern and shorter latency to mate may well highlight variation in male courtship effort rather than variation in aspects of female mate choice.

Discussion

- The authors write « Sib mating avoidance in Venturia canescens had been evidenced from behavioural experiments in laboratory conditions (Metzger et al., 2010a). In contrast, the genetic approach developed in this study shows that in field populations, the observed frequency of matings between genetically related males and females is similar to the probability of sib mating expected under an assumption of completely random encounters. Our study thus suggests that sib mating tolerance occurs in the wild. »
  I do not agree with the conclusions because it may well be that there is no tolerance for sibmating in the species but that this has not been detected in this study as:
  1) other mechanisms are in action to avoid sibmating in the wild, such as dispersal, so that there is no need for having in addition mate choice against related individuals to evolve;
  2) there is no information of the potential detrimental effects of a threshold of 0.16 which was fixed by simulations or of having 10 (?)% chance of producing diploid males. We lack here evidence of the cost potentially associated or not to the actual level of relatedness experienced in the wild.

- The authors write "in the rest of the discussion, we propose an evolutionary scenario to explain the apparent discrepancy between patterns observed in field and in the laboratory. »
  As far as I understand, there is no discrepancy between your field and lab datasets, as you write below "Mating rates between relatives, be they inferred from genetic analyses or from a simulation model assuming random encounter between males and females, were both equal to 11%. Consistently, in laboratory cages, the proportion of sib mating perfectly matched the proportion of sib present. Both results are congruent with an absence of sib mating avoidance in Venturia canescens ". There is rather, if any, a discrepancy is between your datasets and other published data o inbreeding avoidance based on kin recognition in the lab. Such discrepancies may be explained by differences in sex ratio density, effective size, population structure,.... under which those other populations used in other publications, evolved. So it is more important to show how your multi-level approach make sense of the pattern rather than try and highlight a so called discrepancy.

- The authors write « This was shown in a small isolated population of moose, with females accepting higher levels of inbreeding in years where males are less available (Herfindal et al., 2014). A similar reasoning may also hold for highly inbred populations ». Do you mean with the word « inbreeding « in fact « inbreeding for their offspring », or ' higher relatedness with their mate »? Or "inbreeding level in their mate" : the fact is that in social wasps males cannot be inbred as they are haploid so it is a comparison that is a bit confusing.

- in the section « Tolerance to sib mating, as observed in Venturia canescens, is often explained by low inbreeding depression or high cost of outbreeding » : this section would be better placed in the introduction: what is known in Venturia regarding cost and benfit of mating with related individuals?

Orthograph

- Abstract « that sare ... »

- Introduction « habitat selection following natal dispersal is negatively correlated..." rather use the words " habitat selectivity"?

- Introduction" Responses to inbreeding... » add « response to inbreeding risk »... » ?

Reviewed by anonymous reviewer 2, 28 November 2017

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