

Marie Collet  
Université Claude Bernard Lyon 1  
43 Bd du 11 Novembre 1918  
Bat G. Mendel  
UMR CNRS 5558 Biométrie et Biologie Evolutive  
69622 Villeurbanne Cedex  
France

**The Recommender**  
*Dr. Caroline Nieberding*  
PCI Evolutionary Biology

Lyon, France, February 2018

Dear Dr. Caroline Nieberding,

Thank you very much for giving us the last comments before the recommendation of our manuscript entitled “Insect and incest: field evidence for dangerous liaisons in a parasitoid wasp” by M. Collet, I. Amat, S. Sauzet, A. Auguste, X. Fauvergue, L. Mouton & E. Desouhant (<https://doi.org/10.1101/169268>) to *PCI Evolutionary Biology*. We have taken into account your comments and the reviewers’ remarks in the revised version of our manuscript and changes are listed below.

On behalf of all the authors,

Marie Collet

## Author responses to the comments given on manuscript

### Insects and incest: field evidence for dangerous liaisons in a parasitoid wasp

<https://doi.org/10.1101/169268>

**New submission:** <https://doi.org/10.1101/169268> (revision on BioRxiv with the same doi)

We are most grateful for the second round of reviewers' comments. We have edited the manuscript to account for the referees' suggestions and comments. The reviewers' comments are reproduced in blue and italics, followed by our responses in normal black font. We have also submitted our revised version in BioRxiv. (<https://www.biorxiv.org/content/early/2018/01/17/169268>)

#### Review 1 - Reviewed by anonymous reviewer, 2018-01-19 13:21

*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. canescens* 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.*

We thank the reviewer for his/her implication in the revision of our manuscript. As his/her comments were included as revision (in red) in the MS, we have accepted all his/her suggestions. Below we will only point out the modifications that needed clarifications.

**Abstract, point 5:** We have kept the sentence about sex-biased dispersal ("The weak male-biased dispersal cannot explain entirely this pattern.") because it was explicitly asked by a reviewer in the first round of revision. Moreover, we think that it is important to exclude sex-biased dispersal as an alternative hypothesis to sib-tolerance.

**Introduction, paragraph 4:**

(Szulkin et al., 2013; *This reference is not in the bibliography*)

(but see Robinson et al., 2012 *and* Bretman et al., 2009 *This former reference is not in your bibliography. I've included the reference of Bretman 2009, because they did a long-term genetic study over several years and throughout the season in a wild insect population*)

We have added the reference of Szulkin et al. 2013 and Robinson et al. 2012 in the bibliography and Bretman et al. 2011 in the main text and bibliography of the revised version.

### **Material and Methods, Biological model:**

*(include ref Hamilton on LMC theory)*

We have added the reference of Hamilton, 1967 in the main text and bibliography of the revised version.

### **Material and Methods, Insect sampling in the field:**

*Why did you only collect females in Valence? I think you need to include some explanation here, because it would have been better if males were collected also from Valence.*

We have added explanations on the absence of male capture in Valence in the SEM-A. “Both sexes were captured in Nice and Valence populations, but males caught in Valence were discarded due to an error of manipulation during the sampling period and were therefore not available for genetic analysis.”

### **Material and Methods, Effect of male density and sib frequency on sib-mating avoidance in the laboratory:**

*How were these behaviors recorded? Was a computer program used? If yes, then mention which one, if no, then explain how the observer kept track of behaviors.*

We have added that it was by visual observation.

*We also included the number and duration of courtship (from a related or unrelated male) as a time-dependent variable. In this sentence the referee would like we use the term “duration”. We have kept the word “timing”, because we included the time at which the courtship began as a time-dependent variable.*

### **Results, Genotyping:**

*What are these values? It looks like numbered references, please check.*

The values are IC 95%, we have changed in (IC 95%: [44; 82]).

### **Results, Sib-mating avoidance in the field? Relatedness of actual versus potential mates:**

*What is this values? Standard deviation? Please explain throughout the manuscript.*

The values are IC 95%, we have added the information in the revised manuscript.

#### **Discussion, paragraph 4:**

*You need to develop this paragraph further. Why not discuss some of the work that has been done on sex-biased dispersal in the field in other animals? And then conclude that sex-biased dispersal should only have a weak effect in your system.*

We have added literature on sex-biased dispersal in insects and developed the paragraph: “Sex-biased dispersal can also shape the pattern of inbreeding avoidance in the field (Gandon, 1999; Roze and Rousset, 2005). In insects, male-biased dispersal was found mostly in social (Johnstone et al., 2012; López-Urbe et al., 2014; Vitikainen et al., 2015). In non-social insects, Downey et al., (2015) found male-biased dispersal in more than 10 species of bean beetle. This dispersal pattern is consistent with the kin competition avoidance hypothesis (local mate competition hypothesis) but not with the inbreeding avoidance hypothesis. In *V. canescens* from Nice, we found a slight male-biased dispersal at a local scale (Table 4). It is unlikely that local mate competition explains the evolution of male biased dispersal in *V. canescens*, raising the question of the inbreeding avoidance hypothesis. However, the relatedness values between and within patches (between which dispersal occurs) in both sexes was low (between 0.042 and 0.065, Table2) and no structuration at larger scale (between Valence and Nice population) was found. If male-biased dispersal indeed affected the structure of this population and encounter rates between relatives, it should therefore be a very weak effect.”

#### **Reviewer 2- *Reviewed by anonymous reviewer, 2018-02-12 10:24***

##### *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.  
- 197: the study by Robinson et al 2012 about inbreeding avoidance in the wild in insect is 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).*

We have added Robinson et al., 2012 in the bibliography and the study by Bretman et al., 2011 in the main text and bibliography.

*- 1114-122: 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 (see 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.*

As we defined inbreeding depression (for any species) in the first paragraph of the introduction, we have not changed these sentences.

*- L118: is it known that Venuria canescens females mate only once (as written 1129)? Best to remind it here then otherwise these numbers would be a maximum.*  
We have added here that *V. canescens* females are monandrous.

*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 known 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.*

We agree and have added sentences in the last paragraph of the discussion to highlight the problem of laboratory biases due to social environment: “The expression of sexual selection is dependent of the social environment in which the individuals interact, and the laboratory experiments therefore add biases (Miller and Svensson, 2014; Nieberding and Holveck, 2017). The differences between previous laboratory study and our results could highlight these differences in social environments and underline the necessity to confront laboratory and natural conditions data to estimate mate selectivity.”

*- 1161: another nice reference demonstrating the effect of LMC on sex ratio: Macque et al Science 2012 (Isabelle Olivieri’s team).*

Thanks for the advice. We have added Macke et al., 2012 in the final version.

*- 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.*

The absence of structuration at larger scale has been added in the paragraph 4 (following also the suggestion of the first reviewer): “However, the relatedness values between and within patches (between which dispersal occurs) in both sexes was low (between 0.042 and 0.065, Table2) and no structuration at larger scale (between Valence and Nice population) was found.”

#### *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.*

Sib mating avoidance could have evolved at lower absolute value of kinship in the field. In this case, by using brothers in our experiment, we were conservative and should have detected sib-mating avoidance.

We tested another hypothesis than sib-mating avoidance that could explain avoidance of inbreeding costs: sex-biased dispersal. We concluded that if it had an effect, it should only be weak.

*- I384: did males and females have a similar level of relatedness, within patches (curiosity)?*

We are not sure to fully understand your question. Females' relatedness is slightly higher than males' within patch 1 and 2 (Patch 1:  $0.065 \pm 0.002$  vs  $0.042 \pm 0.002$  and Patch 2:  $0.060 \pm 0.006$  vs  $0.048 \pm 0.006$ , cf Table 4).

#### *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.*

The discussion was constructed in order to highlight the potential biases existing in our study when comparing laboratory and field data and we therefore kept this organisation.

*- I395: 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 ?*

We agree that causes of decreasing latency to mate is difficult to interpret. In our experiment, the females had the possibility to escape male courtship (the cages' size was 30cm x 30cm x 30cm and individuals are about 0.5cm long) and we observed during some trials that female flew away after a courtship and was not disturbed for more than 15min sometimes. That is why our interpretation was in favour of increased mate selectivity.

*- I408: 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 Nieberding, 2017, in press in Frontiers Zoology).*

It is clearly difficult to make the experimental design close to the social environment experimented by the individuals in the field. Compared to the study from Metzger et al. (2010), it is indeed possible that the males are encountered sequentially in nature; however, we both (we and Metzger et al.) used brothers as related males. Moreover, our design in lab cages (30cm x 30 cm x 30 cm) allowed the females to escape males' courtship, which is less the case in Metzger study where the encounter took place in small vials (7cm x 3cm). Therefore, both studies had probably different biases on the expression of mate choice. We have added, as it was suggested by the previous comment on the M&M section, sentences in the last paragraph of the discussion to highlight the importance of social environment on mate choice (see our reply above).

- L446: *“proportion”*: do you mean *“density”*, or *“overall level of kinship”*?

We mean “density” and we have changed this in the final version.

- 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.*

We feel that our discussion clearly highlights the discrepancy between our (previous and in this study) lab and field results. We have added a sentence in the last paragraph of the discussion to state that we consider that our field results are certainly more relevant than lab experiments because of the different biases related to mate choice experiment in these conditions:” Data from the field should be considered as the more relevant and should help to identify biases related to lab conditions.”

- 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?*

We have merged the two paragraphs.

- 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-?*

It is indeed always bad to have more than one egg in the host for the female fitness and therefore, the cue indicating another egg is important. However, in term of inclusive fitness, it is worst to superparasitize host containing an egg from a relative than an unrelated egg, and therefore the kinship could have an effect. This effect was already found by several studies (Hubbard et al., 1987; Marris et al., 1996; Amat et al., 2009). A sentence was added in the last paragraph of the

discussion to clarify this point: “Moreover, the female’s inclusive fitness costs increase in the case of superparasitism of a host already parasitized by relatives.”

Moreover, in 2000, Schneider and colleagues collected 2730 carob pods (at Mont Boron, east side of Nice): 9% of fruits contained a host and of those 3% had been parasitized by *V. canescens* (Schneider PhD Thesis 2003). Indeed, the hosts are concealed in fruits and are therefore difficult to reach and parasitize, which leads us to think that the pressure for multiple eggs in hosts exists in the field.