

Dear authors,

Thank you for submitting your preprint "Sensory plasticity in a socially plastic bee " to PCI Evol Biol. We are sorry for the delays, but fortunately your manuscript has now been read by three reviewers and by myself. You will find the reviewer's comments attached.

As you will see, the reviews are largely positive, and, based on these three reviews as well as my own evaluation, I would recommend your manuscript to be included in PCI Evol Biol. However, before reaching a final decision, I would ask you to revise your manuscript according to the recommendations by the reviewers. Please address the main issues underscored by the reviewers and use their more minor comments to improve the manuscript for a wide readership when warranted. If you do not agree with a comment, please explain why in your reply.

We would like to thank the reviewers and the editor for taking the time to thoroughly read and review our manuscript. We have edited the manuscript as much as possible according to the reviewers' suggestions. We have not changed the overall narrative of the paper, but we feel that the changes that we have made clarify the key messages in the paper and also highlight the limitations and how these could be overcome with future studies. We respond to the specific comments provided by the reviewers below and in the track changes word document provided. Line numbers refer to the track changes doc.

Main issues:

-The north – south characterisation of the study sites could be presented using thermal records instead of latitude, since temperature is not entirely related to latitude in these sites. Adding to the reviewers' comments, I would say that this is important since developmental plasticity is the main driver here and your discussion focuses on this.

We have altered the text to emphasize the climatic differences, we include a supplementary table (S2) which summarizes thermal records close to the populations studied and we have added more on this to the discussion (L576-582). We still name populations based on their location for ease of reading.

-Explain why there were no reciprocal transplant from south to north early in the methods and how it affects your test of predictions.

The main reason for the lack of reciprocal transplant is due to a lack of staff. This project was carried out by one researcher and so the detailed observations required to document sociality could only be carried out in one place in any given year. We chose to transplant from north to south to increase the opportunity for sociality to occur so that more comparisons (i.e. between castes) could be made. We have added words to this effect in the text (L171-184). Depending on funding and feasibility future studies which do reciprocal transplants may be fruitful for testing hypotheses about temperature sensitivity of sensory and social plasticity.

-Please address the questions concerning statistical analyses: 1) what constitute a sample: is it an individual within a site or does each site represent a $n=1$. There are already mentions in the ms that only one individual per nest was taken, and this type of information could be made clearer to the reader to answer the concern by one of the reviewers. 2) are there random effects in the mixed models and model fitting.

A sample is one individual (L303-305). We have clarified this in the manuscript.

-Please specify what happened to the remaining individuals transplanted in nature.

These individuals were taken back to their natal site in Scotland when they entered hibernation in the buckets. We have added this in the ethics section (L656-664)

-The wording to define the different treatments and phenotypes could be revised to be quickly understandable by a non-specialist (comment about “origin” versus “development” and comment about the different types of bees studied (B1 with year, etc)

We have clarified this throughout text.

Michael Greenfield:

Review of ‘Sensory plasticity in a socially plastic bee’ by Boulton and Field This manuscript describes an innovative study addressing a central questions in social behavior and evolutionary biology, and it merits further consideration. The writing is generally clear and wellorganized, and the morphological analyses are detailed and seem to be appropriate.

Below, I list some concerns which, if addressed, could strengthen the paper considerably. 1 The authors focus largely on the north-south geographical dimension, whereas the animals are most probably responding to some cumulative thermal parameter, not latitude per se. Certainly the climate in Cornwall differs markedly from the southeast corner of Britain, and, in general, places farther west (e.g. Belfast) will differ from the eastern coast of Britain. Can thermal measures (meteorological records) for the sites and year in question be provided and then evaluated in the context of the main hypothesis ?

We have attempted to address this in the methods and provide thermal records for each site in the supplementary materials (L576-582).

2 I question whether the sample sizes in the statistics are considered correctly. Many would consider that there are only three independent samples – North Scotland, Belfast, and Cornwall, with individuals within these sites being rather non-independent. For example, they may be rather closely related genetically, in addition to developing in the same exact locality. This restriction would not necessarily invalidate the study, as field data like these are hard to come by, but it should be discussed.

See L303-305 for an explanation of sample sizes. This is a common issue in studies of this nature, and we agree that it does not invalidate the study. In this study we were limited to the ability of a single researcher to sample such a large area in a given year, or over the time funding was available for (and to record the social phenotype of each population). In the current study were interested in variation within and between populations differing in climate and social structure. Obviously with unlimited time and funding many individuals over many more populations would be sampled and multiple reciprocal transplants would be carried out.

3 At a much broader evolutionary level, there is an inherent chicken vs egg issue here. The correlation between social behavior in Hymenoptera and sensory apparatus is clear, but one

often wishes to know, or at least speculate on, the sequence in which the two characters evolved. That is, were species and populations with enhanced sensory apparatus – which may have originated for various reasons – more likely to evolve sociality ? Or, were species and populations with incipient sociality – owing to climate and or resources, for examples – more likely to evolve more sophisticated sensory receptors ?

This is an important argument and one we have attempted to do justice to in the manuscript (i.e. based on the work of Wittwer and others; L510-515). The interesting result here is that while there may be a correlation between the social phenotype and sensory apparatus, it is very unlikely to be causal. Whether there is an indirect causal effect of one on the other we can only speculate on. Future studies that sample more widely (as we suggest above), together with elaborate statistical tests such as path analysis, may help to elucidate this (in this species and in the Hymenoptera more widely).

4 My final point is an ethical one, which I hope can be addressed in a satisfactory way : The transplantation experiment, from North Scotland to the southeast corner of Britain, is innovative and provided valuable results. But one wonders whether any of the transplanted bees, of either generation, escaped and led to admixture with the local population, thereby confounding population genetic matters for future scientists ?

We have amended the ethics section to reflect this (L656-664). This is a good point we do not think that is likely to be a problem. These bees hibernate underground and buckets were returned to their natal site once they entered hibernation. It is very unlikely that they nested or hibernated nearby as the clay soil in the area is not suitable for this species (the soil is too wet in winter and the area floods, so hibernating underground is not possible). Whilst it is conceivable that males could mate with native females, we have worked at the field site in the south-east for several years and have surveyed bee species in the area in detail and have never found any native *H. rubicundus* nesting or on flowers. This is probably because the native soil in the area is not appropriate for this species, for nesting or hibernation. After returning to the site the following year we did not see any *Halictus rubicundus* in the same area.

Sylvia Anton

The manuscript by Boulton and Field reports on a nice study of the plasticity of antennal morphology in a sweat bee. It shows the influence of environmental factors on the density/numbers of different classes of antennal sensilla, but does not reveal a correlation between sensilla density/numbers and degree of sociality or the social phenotype of an individual bee. The insect model is well-chosen, the experimental design is adequate and sophisticated statistical data analysis is used (but I am not competent to judge the statistical analyses). The manuscript is very well written and the authors discuss their data honestly and draw highly sound conclusions. Not being familiar with the biology of the insect, I very much appreciated figure 1 illustrating the different life cycles. However resolution of the figure is low in the pdf, but I guess a high resolution figure will be provided.

We provide a higher resolution version of figure 1 in the updated manuscript.

One slight shortcoming of the manuscript is the choice of geographical situations for the different types of experiments: geographical bee transplanting experiments to the south were not done to the same geographical zone as in the comparison of bees from different latitudes. Data of the two experimental series can thus not be directly compared. Nevertheless, the authors very honestly discuss these limits of their study. They also propose very interesting perspectives for future studies to clarify the points that they were not able to elucidate here.

I only have a minor recommendation concerning the use of the term “origin” :

Line 344 : the term ‘origin in this sentence is confusing, because you use “origin” for the place they were collected to start with. So rather use here “development site”

This has been changed (L432-433)

In table 2 legend “origin of emergence” is clearer than in the sentence above, but still “development site” as used in the table itself would be more precise

This has been changed (L437)

Lluís Socias-Martínez

We provide a track changes version of the manuscript where we respond to comments and make changes suggested by this reviewer.