Revision needed

Dear Dr Calcagno,

I have received the detailed comments of two reviewers on your manuscript entitled "Up and to the light: intra- and interspecific variability of photo- and geo-tactic oviposition preferences in genus Trichogramma". As you will see, both reviewers liked your study. I agree with them, in that your manuscript is pleasant to read and provides useful, novel and interesting data about the egg-laying behaviour of Trichogramma wasps.

Having said that, the reviewers raise numerous points that need to be addressed to improve the clarity of the text and strengthen the conclusions. Among them, I would like to stress the importance to provide more details about the lab rearing conditions of the tested populations. This would allow you to exclude that the reported results are simple by-products of standard laboratory conditions selecting for "up and to the light" egg-laying sites, thus strengthening the relevance of your results.

I look forward to reading your detailed responses to the numerous and detailed reviewers' comments, and the associated changes in the manuscript.

Best,

Joel Meunier

Thank you. In this revised version, we have made our best to address the various comments and provide the additional requested by the reviewers. In particular, we have provided more details on how the strains were reared prior to experiments and the possible selective implications. We reply to each comment in what follows.

Reviews

Review for PCI Zoology: Up and to the light: intra- and interspecific variability of photo- and geo-tactic oviposition preferences in genus Trichogramma

General comments:

I do not have many comments to make. I really appreciated reading this article. The protocol used to unravel the main effects of photo- and geotactic components is smart and allows obtaining good sample sizes.

Introduction: This part is well written but the end of the introduction would benefit from more detailed explanations. Especially in the last paragraph, it would be interesting that the authors write their hypotheses regarding expected inter-populational variations, and interspecific differences.

This comment echoes one of the second reviewer regarding the choice of study species and our expectations. We have, in particular, added an entire paragraph to this effect in the Methods Section. In addition, we also added several sentence at the end of the Introduction, as you suggest, to lay out our main questions.

Results: I don't want to be a dogmatist, but the authors should be careful not discussing too much the results in this section. Of course, anything that helps understanding the results is welcome, but some paragraphs (e.g., P10L6-16 or P12L22-33) would better fit in the discussion section.

We have slightly reduced the Discussion parts in the Results. However, the Discussion covers other grounds and is already long, and we tend to like having some results immediately interpreted, for the simplest aspects, the moment they are presented.

Discussion: In this section, I appreciated the efforts that the authors put in trying to explain interspecific differences in the observed patterns. It is a difficult task given the poor knowledge on natural history of some species. Would it be possible to discuss more about how other species- or population-dependent life history traits, such as size, mass, energetic reserves, flight capacities, longevity, egg-load, could affect the patterns observed in this study? In addition, what could be the links between microhabitat selection and microclimatic conditions found on/under the leaves? Finally, adding more details on the relevance of your work regarding inundative biocontrol strategies would be great.

We have added several sentences in the Discussion, as well as three new references, to take these suggestions into account (as visible in the track changes version of the document). Unfortunately, Trichogramma species and populations are very hard to document with respect to the traits mentioned, especially morphological and physiological ones, considering their minute size and the almost complete absence of stable morphological differences. The best overall "fecundity" estimates available for these populations are probably the data presented in Fig. S2B. Furthermore, most

traits do not relate to photo- and geo-taxies in any obvious manner, even though they would certainly correlate with overall performance or dispersal capacity. So we really cannot do much more along this line.

Specific comments:

P2L6: Please write "Trichogramma brassicae" in full here, as it is the first time you mention the species in the text. Done

P2L8: I think it's a bold statement to say that the ecology of trichograms is not well understood. Please temper this statement a little bit. However, I totally agree that the genetic, physiological, behavioral diversity of trichograms is not well understood. We have modified the sentence accordingly. We think their ecology is still little understood in some aspects, for instance their host ranges are poorly known, the hosts allowing winter diapause aren't either, …etc

P2L24-30: This paragraph is particularly well written and it gives a good idea of the importance of the work done in your study (and yet to be done). I would just suggest adding a short sentence to make clear that we also want to select particular parasitoid strains that fit the environment they will be released in. One cannot release a parasitoid strain that has poor cold resistance in Québec, for instance (see Gariepy, V., Boivin, G., & Brodeur, J. (2015). Why two species of parasitoids showed promise in the laboratory but failed to control the soybean aphid under field conditions. Biological Control, 80, 1–7. https://doi.org/10.1016/j.biocontrol.2014.09.006)

Thanks for the suggestion. We added this sentence and reference.

P3L22: Please write the genus name in full when it begins a sentence Done

P4L5: remove the "s" after "population" Done

P7L17: How closely? Please mention the R^2 of 95% here as well, before referring to Fig S1. Done

P9L12: Please remind what P and G treatments are, in the figure caption, so the figure is easier to understand. Done

P13L2: Please provide the % variance explained by each of the two axes. Moreover, please provide (as a supplementary figure) the contribution of each variable to each dimension.
In addition, would it be possible to try representing the PCA with confidence ellipses around each group (either species, or strata)? It would allow seeing more clearly how groups discriminate on the graph. Of course, I understand it's not possible for n < 3.

We have provided the percents of variance explained and the variable loadings as asked (both in Figure 5 and in the S.I., with the new Table S1). We did not add confidence ellipses because we would not want to stretch these results too much, and, as the reviewer says, we sometimes have very few sample points in a given category.

P13L20-22: Of course, but I still think it's worth of discussion; what could be the interplay, in nature, between geotactism and phototactism? Your results suggest additive effects, so maybe the neurological and sensorial mechanisms are totally dissociated between both attraction components? This is discussed a little bit in the following paragraph, but maybe some more references from the literature can be found regarding additive vs. synergic effects in other animal groups (or even in plants?).

The perception of gravity/geotaxis is not very well known in insects. Even in Drosophila, geotaxis is probably the type of taxis least understood in terms of mechanisms (compared to photo or chemo). No organ dedicated to graviperception is known, though probably antennas (and other joints throughout the body) are involved. So we found it hard to obtain information of possible neurological or sensorial mechanisms. We nonetheless added a sentence discussing these aspects, as suggested (XXXX).

P16L4-15: In this last paragraph, or elsewhere in the discussion, it could also be interesting to mention that life-history traits and behaviors often differ a lot between natural and industrial parasitoid strains. In a biological control perspective, it is, as you mention, extremely important to optimize the match between parasitoid strain and the environment (and, of course, the target pest species). Just to give you an example, but other exist, we explored this issue in Tougeron, K. et al. (2020). Trans-generational effects on diapause and life-history-traits of an aphid parasitoid. Journal of Insect Physiology, 121, 104001. https://doi.org/10.1016/j.jinsphys.2019.104001 and in Saeed, M. M. et al. (2020). Transgenerational phenotypic plasticity of diapause induction and related fitness cost in a commercial strain of the parasitoid Aphidius ervi Haliday. Insect Science, 1744-7917.12794. https://doi.org/10.1111/1744-7917.12794).

Indeed. We have added a dedicated paragraph explaining how the rearing of parasitoids might affect their phenotypes, and also a sentence in the Discussion stressing that this should be taken into account when deploying biocontrol programs. These changes echo the similar comments from the Recommender and the other reviewer on the importance of rearing conditions.

I have enjoyed reading the preprint up and to the light: intra- and interspecific variability of photo- and geotactic oviposition preference in genus Trichogramma by Burte et al. The oviposition preference of Trichogramma wasps is important for successful biocontrol applications as the biocontrol agent (Trichogramma) and pests (mostly Lepidoptera) can be matched and the mass-rearing of the agent can be made more efficient. As the Trichogramma genus is a species rich genus, and the number of cryptic species is high, it is important to study phenotypic traits on a large scale to be able to study many species/populations but reduce the time spend. The authors present a high-throughput system for recording and automatically analysing the location of oviposition of many wasps at the same time during a number of days. Using this system they tested the whether 25 populations of 5 species of Trichogramma have a preference to oviposit more to the top of the tube or whether they oviposit in the light vs in the dark.

General comments

The authors take care not to stretch their interpretations beyond the results they obtained and in general do a good job of discussing the limitations of their research. As this is partly a methodology paper, introducing the method is important and although the authors do a good job overall, I do have some questions and remarks (see below). The strains / species / populations used in the study are all established in the lab for multiple years, some longer than others. However the authors do not report how the lines were maintained in the lab for all those years? Where they horizontal or vertical in the incubators? Was the light distribution equal? In terms of adaptation to the original stratum, is it not expected that any preference based on stratum height would have been lost after so many years in the lab?

This is an important point also stressed by the Recommender. We have added a dedicated paragraph in the Methods to cover these aspects (Page 3).

In the manuscript results are often given for a certain notable population or species in terms of phenotype, but the data for all populations is not given in the supplementary. The entire raw dataset and associated information are not available online yet, as no link is given to them. It is hard to evaluate all results in this way. For example, the preference scores per day (Fig. 3) are only given for one population, and Fig. 3b is not informative for the different populations / species or treatment but only gives an overall picture. Also the distribution of parasitism rates in the separate tubes is only given for one population (Fig. S3).

The data and scripts were available on Zeonodo (as is required from PCI); we had provided the URL through Biorxiv, but had omitted to include it in the pdf, sorry for that. It is now fixed. We did not illustrate the preference scores per day for every species, since this would be too lengthy, and these data are very well summarized by the two statistics we study in the main text: the mean value on Day 3, and the slope with respect to time (Figure 3). Regarding your last sentence, we presume you meant the distribution of parasitism rates in separate *patches*, and we agree this is an interesting element that we do not illustrate a lot (by focusing on the average position). Therefore, we have added more illustrations corresponding to different species and representative of the existing variability (Figure S3)

The notion that the observed plasticity of the preference over the five days could be due to host shortage is refuted by the authors themselves as the overall parasitism rate is low. However, it is not clear to me if this overall parasitism rate is measured across the entire tube, or is measured for the prefered sites? This should be made more clear in the text, and would also be aided by additional figures in the supplementary or online (see above).

The overall parasitism rate is at tube level in Figure S1B, as stated in the legend. In Figure S1A, it is a grand average, so it can be considered at either tube or patch level). As explained above, we agree that parasitism rate at patch level is important too. As a matter of fact, we cannot fully refute the depletion hypothesis, as locally the parasitism rate may be high. This is how we have slightly modified our sentence, and as mentioned just above, have provided more Figures (see Fig. S3).

Furthermore, we now plot patch parasitism rates rather than normalized parasitism rates, so that the numbers are more directly interpretable in the context of the "depletion effect".

The authors also refer to Trichogramma wasps as trichograms throughout the text. This is a new one for me, and upon searching this name I could only find a medical examination with the same name. I would suggest the authors to change trichograms into Trichogramma wasps, unless they have a good reason not to? I also had a hard time connecting the population names to the species throughout the text, especially when refering from the text to the figures and back again. It would help to have the species names in Figure 2 as well (maybe in a 90 deg. angle, between populations label and the population names), and in the text each time a general remark is made about a Trichogramma species result to indicate the color in parenthesis.

"Trichogram" has become used among people studying these insects (mostly in French but also in English), as a convenient shortname, since it appears this group has no common name, unfortunately. "Trichogram" seems a good candidate, but we agree it is not generally adopted, and the word is already in use in a medical context (in both French and English). We therefore replaced any instance of "trichogram" with "Trichogramma wasp" or something equivalent.

We have modified Figures 2 and 4 to add species names as you suggest.

Please have continuous line numbering for the entire manuscript, that is easier.

DONE

Abstract

The English in the abstract reads a little bit ackward and could use the help of a native English reader to finetune it.

"Appropriate" in the third sentence does not clearly refer to biocontrol agent.

Line 22-25 should be rephrased as it is a too complex sentence.

We had the abstract streamlined.

Introduction

The introduction generally reads pleasantly, however a clear rationale for the study does not entirely emerge from the introduction. A bit more background on the relation between the Lepidoptera host species and Trichogramma species would be helpful, e.g. does the oviposition site of a certain Lepidoptera species have a relation with the preference of the Trichogramma species for light and geotaxis?

In addition, what was the reason for studying the 5 Trichogramma species chosen here? Availability? Or are they hypothesized to be clearly distinct in their phenotypes? The introduction does not give a clear direction for the species tested, only somewhat for the rationale of looking at oviposition.

As mentioned in the general comments above, we have added a dedicated paragraph in the Methods to explain the rationale for species and population selection.

Page 2

Paragraph starting at line 32 is a bit vague also with respect to the traits that are "adaptively important". It is a bit difficult to figure from the text exactly which traits are meant, and "adaptively important" is a strange sentence construction. Please rewrite this to make it more concrete what is meant here.

We have reformulated. We hope it is now more concrete.

Line 42-43 has an awkward sentence construction.

Line 44: to what other purposes is referred here?

We have reformulated these two sentences.

Material and methods

It is unclear how many replicates were done per population, only later in the results I could read that it was 40 tubes per population. Please add this information to the M&M.

Was the light intensity equal along the 2*20 grid of tubes? It is not indicated that this is measured.

We have added these informations.

Page 4

Table 1: Does >2014 mean that it was sampled after 2014? Or do the authors mean <2014?

It means after 2014. We have clarified.

Line 11: disposed >> distributed or glued

Done.

Line 13-14: I assume that the Trichogramma inocula were glued to the cardboard also to enable vertical positioning of the tubes. What type of glue was used? And

were the honey droplets soaked up by the cardboard? Else it would not have stayed in place in the vertical position?

Yes they were glued, we have corrected. We used gentle glue diluted in water (the eggs are so light almost nothing is required to fix them). We have added a sentence explaining this. The honey drops were so small that they did not move by the sole virtue of their own viscosity (and eventually they were soaked up by the cardboard indeed, after a few days).

Line15: is >> in

Line 16: disposed >> placed

Corrected

Page 5

Line 18: There is no "H" treatment? I guess this should be "P".

Indeed. We used to call these "Horizontal" treatments. Corrected.

Page 6

Figure 1: In the figure and caption the pictures are referred to as J1 to J5 but in the text these are D1 to D5. Please change in the Figure and caption to D as well.

Done.

Page 7

Line 7: dark

Done

Line 30-33 is a hard to read sentence. I also would advice to add the formula's used to calculate the different values plotted and used in the statistical analyses.

Line 41-42: I don't understand what is meant here.

We have reformulated. Is it clearer?

Page 8

I'm not an expert in statistics so I cannot properly evaluate the method described in lines 5-20. But I wonder why the square root of nbT is used to weigh against? Please give a rationale for this choice.

This is because the precision (standard error) of a mean estimate is inversely proportional to the square root of the number of observations, rather than the number of observations (cf the classical formula for the standard CI of a mean). We have explained.

Results

Line 32: Add in the text that the failed or male-only hatching refers to the eight parasitized Trichogramma inocula placed in the middle of the card strip.

Done

Page 9

Figure 2: The asterix and axis font could be a bit bigger, and the dark frames are very hard to see compared to the boxes without frame. Maybe a colored pattern in the bars would help.

==We have improved readibility in Figures 2 and 4 as you suggest, adding species names and increasing the size of asterisks.==

Page 10

Line 11: are the parasitism rates referred to here overall or per patch (disk of eggs?) Because even if the overall parasitism rate was low, if the preferred patch was fully parasitized it could explain the plasticity observed. However it is not clear from the text exactly what could be the case here.

==This is an important point we also addressed in a comment above. We now provide more details on the per-patch parasitism rate, and we share your interpretation.==

On page 11, paragraph starting at line 17 also refers to the depletion effect, and also here I would have appreciated to see the distribution of parasitism along the patches.

==We now reference Figure S3 there.==

Page 12

Line 4: Please give the percentage of explained variance per component.

==Done (see response to previous Reviewer)==

Line 5: It is stated that the first PC1 axis separates populations with strong preference from those with weak preference or no preference, however populations on the right hand side of PC1 could also be contrasting in their preference for P and G, as is the case for pr002.

==This interpretation is based on the variable projections on PC1 (now provided in Table S1), according to which all three variables are negatively associated with PC1. PR002 is also very low on PC1, in addition to being far to the right on PC1. And as one can see in previous figures, it indeed has relatively weak preference scores overall.==

Line 18: I don't agree with the statement entirely, it is true that T. principium is attracted to the light based on the date, but only one of the two populations of T. brassiceae, so the phrasing of this sentence should be a bit different.

==The cluster is looking along the "P" vector in the PCA, pointing to the top and to the left. Along this axis, both T. brassicae populations are higher than all other populations (except the principium ones). Consistent with this, both brassicae populations have clear positive phototactic preferences (Figure 2A). So the cluster can be delineated (though not orthogonally to the PCA axes). Does it make sense? We have added a sentence to explain more.==

In addition, the significant difference of P score across species is not something I see reflected in Fig. 5? Please explain.

==Significance tests are available in previous figures, so we did not re-presented information on Figure 5 so as not to overload it.==

Page 13

Figure 5b: indicate that grey circles are populations without stratum data.

Done

Discussion

Page 14

Line 5: away >> a way

Done

Line 8-10: The statement here may be true for isa1729, but population f5-11 does not seem to suffer from this non-additive effect because of the strength of the preference for the single-cues? So the generality of the statement here is not accurate. Please explain.

Indeed, we discuss the two populations separately, one statement does not apply to both cases. We now explicitly state the population names at the beginning of the sentences to make this clearer (btw, the two significant non additive cases are isa1729 and corpt, not isa1729 and f5-11).

Line 23: remove "numbers".

Done

Line 24: Overall low parasitism rate is not informative as it has to do with the percentage parasitism at the prefered site, but this is not given in Fig. S2.

This is now more visible in Figure S3 (see response to earlier comments as well)

Line 42: Please explain thigmotaxis

Done

Page 15

Line 9-11: Sentence construction is a bit awkward. Please change within >> when

We have reformulated.

Line 28: Why would there be a stronger association of light preference with population from the herbaceous layer , and why would this make sense from an adaptive perspective?

We have expanded the sentence to provide one (tentative) explanation.

In case of any questions or queries, please use the following e-mail: contact@zool.peercommunityin.org.

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