PCI Ecology: Decision concerning your preprint

Dear authors,

We have now received two reviews for your preprint, and would like to apologize for the long delay. It was very difficult to get reviewers during the summer. Based on the reviews and our own reading, we find this preprint mostly interesting and well written. The study is carefully designed, and its main asset lies in the fact that several factors are tested simultaneously (harsh/mild climate at the site of origin, host morph, and seasonal abiotic conditions).

As you will see however, the reviewers raise a number of points that, if addressed, can further improve the quality of your manuscript. We would therefore like you to revise your manuscript.

As suggested by one of the reviewers, we believe that you don't have real replicates in terms of populations originating from harsh/mild winters as a single population from each condition was used. As suggested by reviewer 2 this issue is not likely to invalidate your findings, but this has to be carefully discussed in your manuscript.

We would like to raise your attention on many other comments made by reviewer 2, we find them really useful, particularly those concerning the genetic variation in plasticity in diapause induction and the potential inference of reaction norms in the insects studied. Adding this aspect(s) to your study would further increase both its quality and relevance.

We also think that the title and abstract should more explicitly state that diapause levels vary mainly between harsh and mild winter areas, and that diapause induction in oviparous hosts is observed in the harsh winter area only. This would not make your abstract any weaker; on the contrary, the two findings make more sense when taken together. The abstract and some parts of the introduction sections are a bit too technical, we believe that writing some parts of the manuscript using less field-specific jargon would help your manuscript to be read by non-experts on the field.

Please also state the sample sizes more explicitly and report your statistical results in a summary table (including also some information on the random effects). You will see that the reviewers' opinions differ on Figure 3. We would suggest you to keep the PCA plots (both detailed and per metabolite category), but to move the contributions of variables to Supplementary materials, as this would allow you to increase the legend size and readability. Could you also specify somewhere in your manuscript - or supplementary material - whether the patterns found in each of the two repeated experiments were consistent with each other?

- **We would like to thank both the recommenders and the reviewers for their time and suggestions when revising our preprint. We apologize for the delay in returning our revised manuscript. Comments and advices were extremely helpful to improve our manuscript.**
- **We have removed field specific jargon as much as possible. We have added information in the abstract concerning the population-dependent effect. PCA plots have been kept in the main text and contributions of variables to PCA axes have been moved to supplementary material, as suggested. We have specified that patterns were consistent in each of the repeated experiments. Display of statistical results has been changed by presenting the results in two separate paragraphs; one for each population that was analyzed separately. Variability linked to the parasitoid females has been taken into account in our models and**

reaction norms are now displayed in the result section. We have addressed comments of Reviewer 2 on potential maternal genotype effects on diapause induction and on response to different aphid clones, although we want to remain careful on our conclusions because our experiments were not designed to answer such question on inter-female variability.

Reviews

Reviewer 1:

Reviewed by Anne Duplouy, 2018-08-22 13:20

Tougeron and colleagues provide here an interesting study on how higher trophic levels may adjust to abiotic conditions and to the phenology and phenotype or their host. Using the predator-prey interaction between the parasitoid wasp Aphidius ervi and the aphid Acyrthosiphon pisum, they show that abiotic factors (aka: temperature and photoperiod) remain the main signal for the induction of diapause in the parasitoid wasp. However, they also show that in contrast with French specimens, the Canadian specimens of A. ervi more often enter diapause when parasitizing the sexual morph of their aphid host, than when parasitizing the asexual aphids. The authors explain that in Canada, winters are harsher than in France and the sexual morph often only occurs just before the fall/winter season. Therefore the local parasitoid would have evolved to use the aphid morph as a cue for the induction of its diapause. Finally, Tougeron and colleagues provide the metabolite contents of both sexual and asexual morphs of the aphid host, and show that the sexual morph specimens are more rich in polyols and sugar, which are potential resources that the parasitoid could use for the induction of and survival during the diapause period. This is a nice read and it is well written, with also some nice figures provided to ease both the understanding of the method and the results. I just suggest some minor things that the authors might want to consider before publication.

Consider providing the reader with an idea of the endosymbionts that inhabit your insect species. Do you believe each aphid clone carries the same symbiotic load? This could have important implication as endosymbionts have been show to affect many aspect of host-parasitoid interaction, including increased host resistance to parasitoid (Vorburger et al), but also increased host susceptibility to parasitoids (van Nouhuys et al.)

- **We only used one aphid clone so each tested individual would carry the same symbiotic community as the others. The symbiotic load of the aphid clone generation we used was not assessed, but the grandparent generation was. Our clone comes from a particular crossing that is described in Jaquiéry et al 2014. Half of the grand-parent carried *Serratia symbiotica*, the other half had no secondary endosymbiont (INRA laboratory pers. comm.), so it is likely that our clone was inhabited by *S. symbiotica*. This is now stated in the material and method section next to the description of the clone (L140-144).**

I may have missed it but why did you not provide the metabolite content of the aphids from the 17C temperature treatment? Do you think they would have differ?

- **We only provided metabolite contents of aphids from the 17°C treatment, but not from the 20°C treatment. We wanted to make comparisons between morphs produced under the same conditions, and sexual morphs could only be produced at 17°C. In addition, there**

were no differences between "control viviparous" aphids produced at 20°C and "viviparous" aphids produced at 17°C on diapause incidence in parasitoids.

- **We could expect a slight difference in concentration of some metabolites sensitive to temperature such as polyols between parthenogenetic aphids produced at 17°C and those produced at 20°C, but this difference would likely be lower than the difference in metabolite contents between viviparous and oviparous morphs produced at 17°C.**

L39: I think it is not clear from your abstract that the pattern described is only observed in Canada, where the wasp species has evolved under harsher conditions, and not in France.

- **Sentence modified to: "This pattern was only observed in parasitoids from the harsh winter area since low diapause levels were observed in the other population, suggesting local adaptations to overwintering cues." L38**

L155: 'the parasitoid does not parasitize the male aphids': Any idea why? Could this be added to the study? do the males show a different metabolite profile? are they too small to support the full development of a wasp?

- **We couldn't make parasitoids develop in male aphids, and because this article focuses on parasitoid diapause, we decided to remove analyses on male physiology from the manuscript. Data on parasitoid behavioral response to different aphid morphs and on male metabolic content will be presented in another article.**
- **We have added "probably because they are too small and have lower energetic reserves than female morphs (Tougeron et al., unpublished data)." L172.**

L164: I would provide the total number of aphid offered for parasitization per female wasp (Ntotal=X) I would think 48 right?

- **This is right. This information has been added to the text L183**

Fig1: I really think this figure is well-done.

- **Thanks!**

L218: Does this include the control viviparous too? If not why?

- **This does not include the control viviparous morphs because our objective was to compare metabolite contents between morphs produced at a given temperature. See also above our response to one of your previous comments on this point.**

Table1: Do you mean 'Figure 3'? instead of 'Figure 1'

- **Yes. Thanks for pointing that mistake, it has been modified accordingly**

Also: did you identify each of those metabolite in both viviparous and oviparous aphids? if not I would suggest to provide a table with the content of each morph separated. If yes: Do nothing.

- **Yes. We have added/clarified that "All measured compounds were found in both aphid morphs." (L327).**

L.262: I would actually start this results section by bringing forward that the origin of the parasitoid had a major effect, with Canadian ones parasitizing much more than French specimens, because it is really the first thing that shows from your figure2.

- **We have added: "As expected, parasitoids from the harsh winter environment expressed higher diapause levels than parasitoids from the mild winter environment." In the end of the 1st paragraph of the discussion (L381).**
- **We think the novelty of this paper is to show an effect of the aphid morph on parasitoid diapause, and a population-dependent response, which we want to bring forward in the result section and in the hypotheses.**
- **The fact that insect populations from harsh winter climates enter diapause at higher incidence than population from mild winter areas has already been published for *Aphidius* species. We state that diapause incidence differed between parasitoid populations (GLMM, χ^2=216, df=1, p<0.001) (L266). This is also the reason why we chose to separate both populations in our analyses and in the figures, as stated in the "statistical analyses" section.**

L.270: Provide the range for the low level diapause in Canadian population at 20C, as you have done for previous results.

- **The range has been added in the first paragraph of the result section (9.0 ± 1.5%) (L282). The entire paragraph has been revised.**

Figure2: I would provide a better description of the graph, with the meaning of the box and whiskers. 95% confidence intervals??

- **There was a mistake in the caption. It actually represents a % with a 95% CI. This has been modified in the figure and in the legend.**

Also in the legend: add 'Naturally' experiencing harsh/mild winter, so the reader does understand these are the natural conditions, not the lab conditions that you are mentioning.

- **Done**

L:276: remove the extra bracket

- **Done**

L.289: Do you mean variation in metabolite content between specimens of the 2 morphs?

- **We mean the variation within each morph. We have added "inter-individual" (L335) to make it clearer.**

Figure 3: Add the % value for your confidence ellipse in the legend. Add 'Metabilites' under the lower panels, and define the Dim-1 and Dim-2 in the legend.

- **Ellipses were constructed with a 95% CI. This has been added to the legend.**
- **As suggested by reviewer 2 and the recommender, the lower panels has been modified accordingly and moved to supplementary material.**

Reviewer 2:

Review of Tougeron et al 2018 for PCI Ecology

The major aim of this paper is to understand how diapause induction in a parasitoid wasp is affected by the host morph on which it developed (asexually vs sexually reproducing). The authors focus on two distinct wasp populations originating either from a population that experiences harsh winters (Canada) or mild winters (France). Using a split-brood design, the authors further assess the impact of temperature and photoperiod conditions on diapause induction. Differences between host morphs are also investigated in terms of various metabolites and lipid reserves. The authors thus do not focus on a single factor that could affect diapause induction, but rather focus on several biotic and abiotic factors (host morph, geographic origin and temperature in conjunction with photoperiod). Moreover, studying trait expression as a result of bottom-up effects (i.e. trait variation in lower trophic levels) is not often done. The investigation of multiple environmental factors, as well as bottom-up effects, make this an interesting study. A deeper understanding of factors affecting diapause induction is important, particularly in light of (rapid) environmental change.

General comments:

This paper is well-written and uses a thorough experimental design to disentangle how various environmental factors could affect diapause induction in a parasitoid wasp. Please find more general comments below:

I think that your data contain much more interesting information than is currently presented. For example, you actually use a split-brood family design (i.e. parasitoid offspring of 1 mother are allowed to oviposit on three different host morphs, after which they are split to continue development at 2 different temperatures). This means that aside from looking at the overall effect of host morph on diapause induction, you can also investigate the effect of a mother's phenotype on diapause induction within morphs between temperatures, as well as diapause induction between morphs within temperature. Looking at Figure 2, the right panel, you can see that there is no diapause induction at 20 degrees (i.e. no variation), but based on the variation at 17 degrees, it could be that there are differences in slopes (of reaction norms) both within morphs at different temperatures, and between different morphs at 17 degrees. I wonder what happens if you plot the % diapause induction of offspring of each female for each morph and compare it between morphs and between temperatures. If you find differences between slopes, even only some of them, it shows that there is genetic variation for plasticity in diapause induction depending on temperature and/or morph. The same holds for the left panel, but only the oviparous morph shows overlap between the minimum and maximum at each temperature. For viviparous and control viviparous, the reaction norms could, however, still differ between morphs of the same temperature (but this could be true for both temperatures). I find this extremely interesting, because it would enable you to say something about the ability of certain parasitoid genotypes to more easily overcome variation in host morphs and temperature (see a review by Sgro et al 2016 Ann Rev Entomol that largely focuses on plasticity for diapause induction). From a population persistence perspective, this means that in the face of climate change, for example, there is still genetic variation for plasticity to allow the population to move to a new optimum. If you can find this in your data, I think that including those results would make your paper more impactful. Even if you find that there is no genetic variation for

plasticity in diapause induction that would still be interesting and subsequently justifies analyzing your data as group means.

- **As suggested, we have compared reaction norms within temperature conditions between morphs, and within morphs between temperature conditions. We have excluded "control" morphs as their effect on diapause induction does not differ from viviparous morphs.**
- **Comparison of reaction norms of diapause levels within the offspring of each female showed that the offspring of some female had stronger responses to changes in temperature or in host morph than others. There were more differences in reaction norms among females for the response to host morphs than for the response to abiotic conditions. In some rare cases, the response is even reversed (i.e., positive slopes and negative slopes). There is variation for plasticity in diapause induction among female genotypes, depending on temperature and morph. As examples, we chose here five parasitoid females that showed contrasted reaction norms in each population (see figures below).**

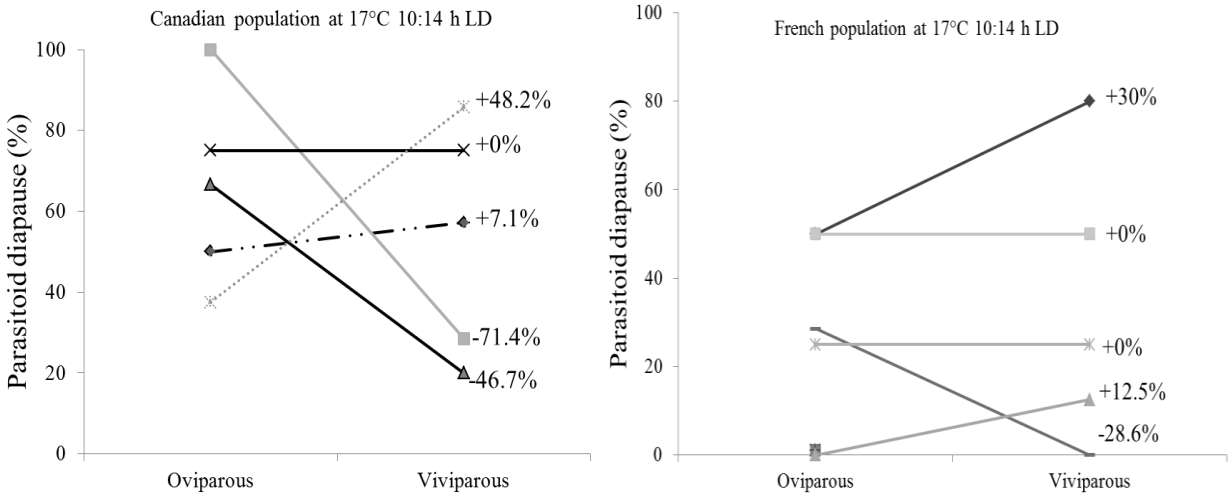

Figure: Comparison of reaction norm slopes among the offspring of five parasitoid females from the Canadian (left) and French (right) populations exposed to both oviparous and viviparous pea aphids, at 17°C 10:14 h LD.

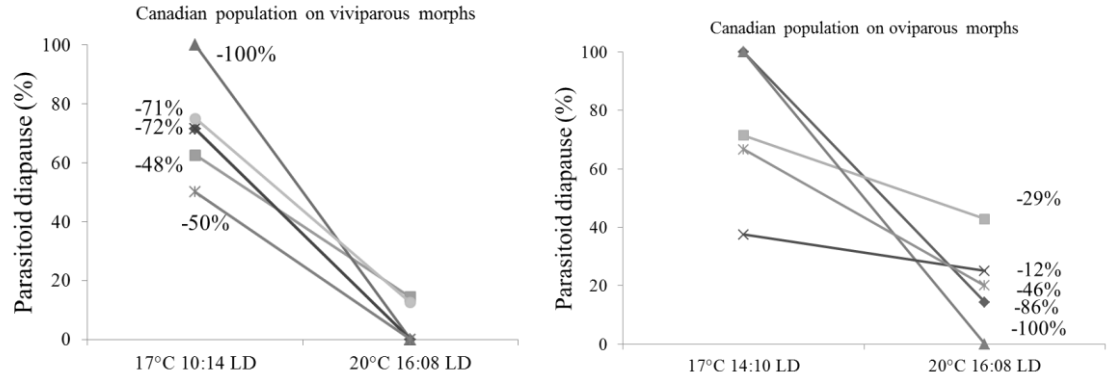

Figure: Comparison of reaction norm slopes among the offspring of five parasitoid females from the Canadian population exposed to viviparous aphid morphs (left) or oviparous aphid morphs (right) at either 17°C 10:14 h LD or 20°C 16:08 h LD.

- **In the manuscript, we have added similar figures representing the RN for each treatment. For clarity issues, we chose to represent all 24 reaction norms (corresponding to the 24 tested females) but to not display the values of RN slopes for each female, as these slopes are synthetized in the main text and can be easily retrieved from our supplementary data sheet.**
- **We agree with the reviewer on the interest of such genotypic effects in diapause induction in an evolutionary perspective. "*Polymorphism in the response of diapause induction cues (i.e., in plasticity) is known to be responsible for variability in diapause levels within populations experiencing different environmental conditions, but is still to be more deeply explored.*" (L382-384). We have added some information about RN comparisons in the material and methods section: "*Our split-brood family design also allowed comparing reaction norms of diapause levels in the offspring of each parasitoid female, both within morphs at different abiotic conditions, and within abiotic conditions among morphs.*" (L203-205). We gave ranges of RN slopes for each comparison for both populations in the result section (L295-311). We talk more about these finding in the conclusion section: "*In addition, there was variation for plasticity in diapause induction among female genotypes, mostly in response to the parasitized morph but also to abiotic conditions, as determined by slopes of the reaction norms. This means that there is genetic polymorphism in diapause plasticity within populations, which may allow natural selection to act in the context of rapid environmental and climate changes (Sgrò et al. 2016).*" (L500-503)**
- **That being said, we cannot make stronger conclusions in the present study about these reaction norm slopes because diapause levels in the offspring are calculated, for each temperature condition tested, on a maximum of 8 aphids (sometimes less, due to mortality occurring from parasitism to mummy development). Therefore, it increases the likelihood that there is variability in reaction norms between genotypes.**
- **Data for each female is made available as a supplementary material sheet so anyone can use our data for further analyses focused on genotypic variation in diapause induction.**

I also wonder how development on host morph affects oviposition preferences of the mother. You have included the order in which hosts were offered as a random variable, but how much of the variation in your models was explained by this? The mothers developed on a parthenogenic clone able to produce both morphs, but on which morph were they reared? And did that affect her preference for a certain host morph during the experiment or affect the responses of her offspring?

- **Random effects arising from the female identity and the host order had extremely low effects on total variance, 2.10^{-2} and 5.10^{-10}, respectively. This information has been included in the result section.**
- **Mothers were all reared on viviparous parthenogenetic morphs. We designed our experiment to control for any preference of the parasitoid. Nevertheless, parasitoids were never exposed to the three host morphs at the same time. In this way, we excluded any preference/choice bias and only had to control for any "first exposure" bias.**

Figure 1 is extremely helpful for understanding your experimental design. Nice job!

- **Thank you.**

Reporting of the statistics is a bit minimal. Could you include a table containing all significant factors (fixed with potential interactions, as well as random) or at least include all stats findings in the results section? For example, you mention that diapause induction was higher when parasitoids developed in oviparous aphids compared to the other morphs, but the stats are not reported here. The same is true for other findings. We need to see those stats to be able to verify your claims.

- **All statistical findings have been included in the results section, including interaction effects and random factor effects. We have separated the text in two paragraphs; one for each population because models were ran separately for each parasitoid population.**

At first I was a bit distraught by the fact that you only used one population from each geographic location. This effectively means that your sample size is 1, which makes it hard, if not impossible, to generalize your findings (i.e. to confidently state that the geographic origin/winter conditions have an effect on diapause induction). However, it seems that Aphidius ervi populations show considerable gene flow, i.e. there is only little genetic differentiation between populations, at least when populations are sampled from the same continent (see Hufbauer et al 2004 Mol Ecol, who showed haplotypes differed by only 1 or a few nucleotide substitutions). You would, therefore, not expect that sampling multiple populations from the same geographic origin would lead to significantly different results. This is a strong argument supporting the use of only a single population from each geographic origin, which I think you should include in your manuscript.

- **This is indeed a strong argument. In addition, even if gene flow was not that strong, we would expect higher differences among Canadian and French populations than among populations of a same location. We have added: "One population per geographic origins was used as high gene flow has been reported in *A. ervi* populations which therefore present little genetic differentiation (Hufbauer et al. 2004). Even if gene flow was weak, we would expect higher differences between Canadian and French populations than among populations of a same location" to the manuscript (material and methods section L134).**

I think the title is a bit misleading, because your results show that a higher diapause induction depending on host morph is only obtained for the Canadian population at the higher temperature. You don't generally find that host morph induces diapause (because this is not the case for all the other treatment combinations). Perhaps a better title would be: Host reproductive status induces diapause at a higher temperature in a parasitoid population experiencing harsh winters. This is your key finding and you won't overstate your results.

- **Thanks for the advice. We have changed our title as suggested by the reviewer "Sex makes them sleepy: host reproductive status induces diapause in a parasitoid population experiencing harsh winters"**

You find that host morphs contain between 51 and 61% fat. That is a lot. I wonder if this high number is due to the use of both methanol (extraction of diglycerides) and chloroform (extraction of triglycerides). From a nutritional point of view, the analysis of only storage fat (triglycerides) would have been more interesting. In any case, there is no significant difference here, so there should be no difference in terms of host quality (expressed as lipid content) between morphs.

- **We indeed extracted any type of lipids with that method, but as suggested by the reviewer, it can be a proxy of total available triglyceride reserves in insects.**
- **Oviparous aphids had more fat reserves than viviparous aphids, although the difference of 0.12 in fat content ratio was only marginally significant. Fat content ratio is expressed as a % of the lean dry mass, so it can be misleading to represent it as a direct proportion of fat reserves, although this ratio is useful to make comparisons among morphs with respect to aphid mass. The fat mass represents 37% and 33% of the dry mass of oviparous and viviparous morphs, respectively. We have added this information to the result section (L345-346).**

To me it seems more intuitive to first describe the analysis of aphid-associated traits, because it is these traits that you expect to lead to potential differences in diapause induction (along with geographic origin and abiotic conditions). You already mention that there are no differences between clones in the material and methods, which is good, but if your goal is to understand how aphid physiological traits affect diapause induction, you first need to know what those differences are. For dry weight and lipids, there are no differences, but for the metabolomics analysis you do find interesting differences that could be related to diapause induction. I would also include on which species/trophic level you are working on the in the subheadings, i.e. 'Diapause induction in A. ervi parasitoids' and 'Metabolomic analyses and lipid reserves of aphid host morphs'.

- **We think we first have to show that aphid morphs have an effect on parasitoid diapause and then focus on the mechanistic understanding of such effects and thus present aphid-associated traits (metabolomics, lipid reserves).**
- **We have modified the subheadings as suggested.**

Regarding the metabolite analysis, you state in Table 1 that metabolites detected in the two morphs are listed. But were there also metabolites present only in 1 morph and not the other? If so, these compounds might actually also play a role in differential induction of diapause, and it would thus be important to include these. Please clarify whether this was verified.

- **These compounds could play a role but as shown in Figure S1, each metabolite was found in each aphid morph. We have added the following information in the legend of Table 1 "Each metabolite has been found in each morph" and L327.**

I also wonder whether you would obtain different results if you analyzed metabolites under exactly the same conditions as your diapause induction experiment (i.e. 2 temperatures and 2 photoperiods). I would expect at least temperature to have an effect on levels of certain metabolites. I think you need to make it clearer (in abstract/results/discussion) that you only tested for differences between morphs, not different temperatures/photoperiods.

- **We would indeed expect an effect of temperature on aphid metabolite content. However, our goal in this study was to test for a morph effect, not to test whether potential temperature stresses experienced by aphids could affect parasitoid diapause. It would not have made too much sense to produce sexual and asexual morphs of aphids under a 12:12 h LD / 17°C condition and then to expose them to 16:08 h LD / 20°C.**
- **We have made clearer that morphs were "produced under the same conditions" (L34-35) in the abstract. It is also stated in the material and methods section (L232).**

Moreover, were the aphids used for this experiment kept at 20 degrees and 16:8 RH (the standard rearing condition as described in the 'biological materials' section)? I think it would be good to mention that again in the respective section where you describe the procedures.

- **Tested aphids in metabolic experiments were all produced at 12:12 h LD / 17°C and kept at these conditions until imago molt. They were then frozen at -20°C. This is stated at the beginning of the "Metabolomic analyses and lipid reserves" section.**

Minor comments:

Abstract:

Page 2, line 26: explain what a holocyclic clone is, for instance in brackets.

- **In the abstract, it is written "producing two morphs with either asexual (viviparous females) or sexual (oviparous females)". In order to keep the abstract short, we have added a definition in brackets in the methods section; "i.e., alternating between sexual and sexual morphs" L212.**

Page 2, line 27: Perhaps this is true for aphids, but generally asexual reproduction does not necessarily equal viviparity, nor sexual reproduction and oviparity. This is a bit confusing in this sentence. I suggest the following: '…producing asexual and sexual morphs that are viviparous (i.e. laying embryos) and oviparous (laying eggs), respectively'….

- **This is true. We have replaced the sentence following your suggestion: "...producing asexual and sexual morphs that are viviparous females (laying embryos) and oviparous females (laying eggs), respectively, the latter being only present at the end of the growing season. L28-29**

Page 2, line 38-39: I agree that the host's physiology could play a role, but you should state here also that you only found differences in 1 population. This means that host physiological status alone does not direct diapause induction, but that this response also depends on geographic origin.

- **Sentence has been modified to: "This pattern was only observed in parasitoids from the harsh winter area since low diapause levels were observed in the other population, suggesting local adaptations to overwintering cues."**

Page 2, line 41: I would not say that this leads to phenological synchronization, because technically you do not show that (you need actual field data to show that). You could say that it is likely, but currently this statement is too strong. Moreover, I think that one of the novelties of your work is that you focus on multiple environmental parameters that could influence diapause induction, as well as bottom up effects. That should be mentioned in your abstract, because that is where the gap in knowledge lies.

- **We have modified the end of the abstract by: "Host quality thus varies across the seasons and represents one of the multiple environmental parameters affecting parasitoid diapause. Our results underline strong coevolutionary processes between hosts and parasitoids in their area of origin, likely leading to phenological synchronization, and we point out the importance of such bottom-up effects for trait-expression, and for the**

provision of ecosystem services such as biological control in the context of climate change."

Introduction:

Page 3, line 75: Again here explain what viviparity is and describe it as a character that is not the same as parthenogenetic reproduction: 'Asexual (parthenogenic) females can reproduce without mating and lay live offspring, whereas sexually reproducing females produce eggs (oviparity) after mating with males'.

- **We have modified this sentence as suggested.**

Page 3, line 84: This is not correct. A common evolutionary history refers to members of a group that share a common ancestor. I think what you mean to say is that host and parasitoid have coevolved over long periods of time.

- **This was indeed not correct. We have replaced the sentence by "Hosts and parasitoids have coevolved over long periods of time"**

Materials and methods:

Page 4, line 139: Explain what alates are.

- **We have replaced two occurrences of "alate" by "winged" in the text.**

Page 6, line 214: You state that samples were dried out at 60 degrees for 2 days in a freeze-dryer. This does not seem to be correct. The point of a freeze dryer is that it dries the material through freezing, not by heating. So you either dried your samples in an oven or you freeze-dried them. Please revise accordingly.

- **Thanks for pointing that out. This is a mistake. Samples were dried out through freezing in a freeze-drier. We have removed "60 °C" from the manuscript.**

I personally find the supplementary figures with comparisons between morphs for each metabolite much clearer than the results of the PCA that are now included in the MS. In Figure 3, the names of the compounds are difficult to read in the bottom panel and you don't mention the abbreviations here in the legend (to be able to interpret the upper panel). In fact, only 1 line in your results section is dedicated to the PCA results (p9, lines 282-283), while the rest of this section highlights the findings of Figures S1 and S2. I would just include Figures S1 and S2 in the manuscript itself, in addition to Figure 3.

- **The bottom panels with contributions to each axis have been moved to supplementary material. We have put the abbreviations back in the figure legend.**
- **We however think the best figure to illustrate our claims is the PCA (currently Fig. 4) because it shows relative values of metabolites, rather than absolute values in Figure S1 and S2. Of course Figures in supplementary material are useful to quantify each metabolite or metabolite group, but text in the result section already describe these statistical differences.**

Discussion: P13, line 417: What is GABA? Please write out in full.

- **GABA stands for gamma aminobutyric acid. We have replaced the abbreviation by the full name in the text.**