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.

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?

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

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.

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.

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.

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.

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'.

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.

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. 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.

Minor comments:

Abstract:

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

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'….

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.

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.

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'.

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.

Materials and methods:

Page 4, line 139: Explain what alates are.

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.

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.

Discussion:

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