8th January 2020

Dear Editor,

You notified us on October 28, 2019 that we were encouraged to review and resubmit a revised version of our manuscript entitled "Herbivore intraspecific variation and evolutionary divergence drive trophic cascade strength".

We thank the editor and the two reviewers for their constructive comments that significantly improved the manuscript. We believe that the revised version satisfactorily addresses most of those comments (see our detailed point-by-point explanations below) and more concisely explains our main findings, which should be of a broad interest for the *PCI Ecology* readership. In addition to addressing the points made by reviewers, we have also made changes to improve the flow and clarity of the text without any impact on our main findings. If you have any questions, please do not hesitate to contact us. We really appreciate your assistance in this matter.

We would be grateful to you for reconsidering this manuscript and we hope that you would approve this new version. We look forward to hearing from you at your earliest convenience.

With kind regards,
Arnaud Sentis on the behalf of all co-authors.

Decision by the handling editor *(Dr. Sara Magalhães)*
Dear authors, First of all, I deeply apologize for having taken so long with this reviewing process. Most of the time was spent trying to find reviewers that would agree on reviewing, although I admit that part of the blame is on me too… In any case, we have received two reviews that I find very helpful. As you will see, both reviewers are overall very positive about your paper, but they also raise some very pertinent issues. I also liked this paper very much, it is very clearly written and the experiments are elegant. However, I totally agree with the referees' comments and urge you to modify the article accordingly.
Our response: we thank the handling Editor for the positive comments on our manuscript. As recommended, we have modified the manuscript to address your concerns as well as the reviewer concerns (see below and the blue font text in the main text).

Handling editor:
I herewith state my main comments on the article (note that these are mostly the reviewers comments stated otherwise): - I agree with the second reviewer that stating "intraspecific variation drives trophic cascades" (cf. title) is misleading. But I see this from a different angle. In my opinion, to test whether intraspecific variation affect trophic cascades you would need to have treatments with more or less intraspecific variation. This was not the case. So you do show that there is intraspecific variation in the ability of aphids to modulate trophic cascades, but you don't show that the amount of variation matters. As the second reviewer, I still think your question is interesting, but it should be formulated in a less ambiguous fashion.
Our response: we agree that we did not show if the amount of intraspecific variation matters for the strength of trophic cascade. We thus modifies the title and main text to state intraspecific

differences in phenotypic traits and host-plant specialisation influence the strength of trophic cascades (see below for more details).

Handling editor:
- I also agree with the first reviewer that there is no clear reason to state that you are testing the role of "evolutionary divergence". First, I do not see evidence for the fact that alfafa lineages have diverged more from clover lineages than from each other. Indeed, there can be very distantly related lineages within the same host race. Second, I also think that lineages from different plants should be considered as a special case of different lineages in general. In particular, the statistical analysis should reflect this. That is, there should be one statistical model that includes lineage in general, and then specific planned comparisons to compare between host races in particular. I would say this is a more elegant way of analysing the data.
Our response: We agree that lineages may differ strongly. However numerous studies have repeatedly showed that genetic variation within biotype is much smaller than inter-biotype variation, which has helped to define the complex of at least 15 biotypes found in the so called pea aphid (Acyrthosiphon pisum). See for instance Peccoud et al. 2009 (PNAS), Ferrari et al. 2012 (Evolution), Nouhaud et al. 2014 (JEB), Nouhaud et al. 2018 (Mol Ecol). The clones chosen here are representatives of populations on which these studies have been conducted, so the inter-biotype differences reported here reflect well evolutionary divergence since these biotypes split.

We conducted the statistical analyses as suggested. The first step was to test for the differences among lineages in general and then we tested for differences among host races (lines 157-179).

Handling editor:
- Finally, I agree with the first reviewer that assessing the effect of each lineage on the strength of the trophic cascade requires a specific test that was not performed, if I understand the stats well. That is, it is tested whether, for each lineage, there is a trophic cascade, but not whether these cascades differ in strength.
Our response: a significant trophic cascade implies that predators have a significant positive effects on plants. In this study, we used Linear mixed model with plant biomass as an independent variable. The dependent variable were predator presence/absence, aphid lineage and the interaction between aphid lineage. We found a significant interaction between aphid lineage and predator treatment (χ^2 = 13.57, *df* = 5, *P* = 0.0185) indicating that the effect of predator on plant biomass depend on the aphid lineage. In other words, the strength of the trophic cascade depends on aphid lineage. Then specific post-hoc test were conducted to determine, for each aphid lineage, the predators had significant effects on plant biomass. We have clarified this point both in the statistical analyses (Line 163-166) and in the result section (Lines 242-244).

Handling editor:
Minor comments - I found that the introduction could be a bit streamlined. In particular, you refer to intraspecific variation in the middle of the second, then in the end of the third paragraph. I would move the former (lines 60-62) to just before the latter.
Our response: modified as suggested

Handling editor:
Also, you mention that the role of intraspecific variation for the occurrence of trophic cascades has been studied before. It would be nice to know in which way the current study adds to the available literature. From what I gathered with the references you cited (and check also Clegg

2018 Ecology and Weiss and Post 2013 Oikos) it should be relatively easy to single out your original contribution. But this should be explicitly stated.
Our response: We thank the handling editor for the additional reference. Recent studies showed that intraspecific variation in predator traits or personality can influence the strength of trophic cascade. However, there is surprisingly few studies on the importance of herbivore interspecific variation for trophic cascade strength. Additionally, the hypothesis that the strength of trophic cascade would depend on herbivore population growth rate remains largely untested. We clarified these contributions (Lines 73-78).

Handling editor:
- Line 83: change to "the faster their growth, the stronger the trophic cascade".
Our response: modified as suggested.

Handling editor:
- Lines 381-388: basically, I guess that what you're trying to say is that aphids from Clover may have a lower assimilation efficiency. That is, they eat more (thus impact more the plant) but their conversion into eggs is lower. Right?
Our response: yes it is what we meant. We added a sentence to clarify it (lines 415-417).

Handling editor:
- In the Discussion, I think you need to come up with one or more explanations for the differences found between the effects of clover vs alfalfa trophic chains. For example, the fact that ladybeetles are bigger when fed on alfalfa aphids suggest that these are of better quality, which goes in line with my previous point. Also, the fact that alfalfa aphids reach higher densities on this plant (in absence of predators) suggest that there are better adapted than clover aphids. This should be discussed.
Our response: We discussed this point as suggested (Lines 397-402 and 415-417).

Reviewer 1 (Bastien Castagneyrol)
Arnaud Sentis and colleagues addresses the effect of intraspecific variability in aphid population growth rate on the strength of tritrophic interactions. The paper is well written, well structured, and the study has been well conducted. I only have a few general comments.
Our response: we thank reviewer 1 for the positive and constructive comments on our manuscript. We have modified the manuscript accordingly (see below for more details).

Reviewer 1
I must say that I have been a bit surprised that intraspecific trait variability was actually strain-specific differences in population growth rates. Not that this questions the validity of the paper, but my feeling is that "intraspecific trait variability" is maybe too broad. "Trait" is generally defined at the individual level. Yet, here, "trait" is a "population" trait. This is a bit misleading, but maybe the individual- and population-levels are confounded when one look at aphids? I would be more comfortable if the paper was explicitly framed in terms of "population growth rate" at least in the 'methods' and 'results' sections. Or, at least, if the authors could add a couple of sentences discussing this issue in the 'introduction' and 'discussion' sections.
Our response: we have modified "intraspecific trait variability to intraspecific differences in phenotypic traits and demography. We also agree that population growth rate is not a

phenotypic traits but it is the result of phenotypic traits as population growth rate depends on life history traits (fecundity, age at maturity, mortality). We have clarified this point in the revised manuscript (lines 83-86).

Reviewer 1

The introduction reads well, but I had to read the paragraph L71-85 twice to fully understand the research question. The sentences L77-85 are central to the paper, but how the authors jump from predator and herbivore efficiency to population growth rate and adaptive divergence is not completely clear. My feeling is that it works quite well for aphids because the amount of damage to the plant is directly proportional to the number of aphids and their population growth rate, but it is not necessarily true for other herbivores (e.g., defoliators). I suggest to expand a bit this section to avoid any confusion. In particular, the effect of "adaptive divergence" (L98) on the strength of trophic cascades is not really well introduced. At least, I missed some information to fully get the point here.

Our response: as suggested, we expended this section to clarify this point (see lines 70-99).

Reviewer 1

The analyses look nice. I commend the authors for having managed to link the effect of predators on plants and on herbivores, and the other way round. Although the approach based on effect sizes is surely correct, it was not completely clear how treatment means were aggregated when calculating Rp and Ra (L172-183). I guess treatments means were aggregated at the lineage level (n = 6) and then at the biotype level (n = 3), but that was not clear. Please expand.

Our response: we thank reviewer 1 for the positive comment on our statistical analyses. Treatment means were directly aggregated at the biotype level without considering the lineage. We clarified this point (line 207).

Reviewer 1

Related: another option would have consisted in using model coefficient parameters from (G)LMM. Because of the significant Predation × Lineage or Predation × Biotype interactions, the authors could have extracted treatment specific estimates and corresponding 95%CI instead of calculating raw means and CI. The advange of this approach is that would have accounted for the effects of the three time replicates and the effect of the 6 lineages when aggregating the data at the biotype level. But, again, to the best of my understanding, the analyses are correct at present.

Our response: we agree that it would be another option to present the results and added the model output as supplementary materials (Table S1 and S2). Our results remain unchanged. However, we feel that it is less intuitive than the effect size presentation and the reader would have to do the calculus to get the estimates for a given treatment. For instance, from Table S2, if we want to know the plant biomass for the alfalfa biotype with predator, we have to do the following calculus: 12.9829+2.8710-1.3855. We agree with reviewer 1 that it is more precise but it is certainly more difficult to understand and it does not influence our conclusion. Additionally, the effect size presentation is often used in the trophic cascade literature (e.g. Schmitz et al. 2000 American Naturalist) and it allows analysing effect sizes against other independent variables such as population growth rate. Such analyse would be more difficult to interpret using a LMM as the predators also influence population growth rate. We thus decided to keep the effect size presentation while providing the treatment specific estimates as supplement information.

Reviewer 1

None of the two biotypes were reared on their host plants. I wandered whether the results (notably, strength of the tritrophic interaction between the two biotypes) would have been the same on clover and alfalfa. Maybe the could be commented?

Our response: we agree that it would be very interesting to conduct a similar experiment but on the three host plants (clover, alflfa and pea) to investigate potential trade-offs in host-plant specialisation and their consequences for trophic cascades. It also links to the last comment of the handling editor on the fact that alfalfa aphids reach higher densities on the broad bean (in absence of predators) suggesting that there are better adapted than clover aphids. We discussed this point in the Discussion (lines 397-402 and 415-417).

Reviewer 1

More specific comments

L83 - "growth" should be "grow"? Modified

L111 – Do not start the sentence with "H." Modified

L146 – fully spell "30" at the beginning of the sentence. Modified

L148 – Not sure what 1/3 of 20 replicates is ;-) We modified for "6 or 7".

L149-150 – In understand that this data was used to confirm that the abiotic environment was fairly constant, but this information is not used. Maybe the authors could simply comment on it, or drop it. We removed this sentence.

L169 – There was no previous mention of lineage colour before. We added information on lineage colour (lines 128-130).

L206 – Results: please also report the variance component for the random effects in (G)LMM (random factors and residuals) as it helps assessing the amount of variance explained by the fixed effects. We reported the variance components in the Table S1 and S2.

L229-230 – I disagree. Letters above bars indicate that in the absence of predators, only one of the lineage differed from the others. 10TV and T734 were not different from T9005, LL01 or OX683. We modified for "Without predators, lineage 10TV had a stronger impact on plant fresh biomass than T734, LL01 or OX683, whereas with predators, lineages 10TV and T734 had a weaker impact on plant fresh biomass than LSR1"

L262 - "Strongly" is a matter of appreciation. Maybe it is overstated. We removed "strongly".

L269-270 – This statement about the dashed line could go to the figure caption. We decided to keep it in the main text as the figure caption already contains information about the dashed line and the way to interpret it.

L274 – In the figure caption, here and elsewhere, it would help the reader labeling the y-axis as "Tritrophic interaction strength" or "Plant response to predation", and the x-axis as "Aphid response to predation"

L302-305 – “positively” is odd, because the strength of the predator effect increases as the value becomes more negative. We removed “positively”.

L323 – Throughout the discussion, it would be nice to refer to the appropriate figures. Because there is quite a lot of results, it will greatly help the readers. We added references to figures.

L330 - “evolutionary divergence”: with only two biotypes, it is hard to generalize that much. Maybe tone down? We modified for host-plant specialisation.

L350-363 – I would have phrased this paragraph the other way round: (1) likely effect of population growth, (2) possible effect of other unmeasured traits. We tried to
Our response: modified as suggested.

Overall, I liked the paper. I hope that my comments will help. Best regards, Bastien
Thank you for the positive comments. They are very helpful.

Reviewer 2 (*anonymous reviewer)*
The authors present some interesting experiments in which they use 3 genotypes of two different “biotypes” of aphids (specialized on different host plants) to evaluate the consequences of genetic variation for trophic cascades. They use a single host plant that both biotypes of aphids can feed on and measure how predation affects herbivore density and plant wet weight. They find that variation among aphid genotypes influences the magnitude of the effects of predation in reducing impacts of the herbivores on the plants.
Our response: we thank reviewer 2 for the positive and constructive comments on our manuscript. We have modified the manuscript accordingly (see below for more details).

Reviewer 2
The study makes a nice contribution. However, I do not find focusing on “evolutionary divergence” of the host races is not particularly useful. Really, what the authors have is intraspecific variation, period. There are presumably smaller differences among clones within host races, and larger differences among host races, but treating intraspecific variation and evolutionary divergence as two separate things is a false dichotomy. Many might argue that the host races are actually different species.
Our response: as suggested by the handling editor and reviewer 1, we changed intraspecific variation and evolutionary divergence for intraspecific difference and host-plant specialisation that are more accurate terms. Nevertheless, we still think that host races should not be considered as different species as they can successful cross-reproduce and their evolutionary divergence is very recent (Peccoud et al. 2009 PNAS). You can have intraspecific variation without evolutionary divergence. The two biotypes used here are both genetically and ecologically differentiated, likely resulting from adaptive divergence initiated by divergent selection on one or the other host plant. The fact that host races are different species is another issue, which is presented extensively in Peccoud et al. 2009 (PNAS). The clover and alfalfa biotypes used here definitely exchange genes, although partially, and cannot be considered as incipient species.

Reviewer 2
The paper would make a stronger contribution if a means of quantitatively comparing the strength of trophic cascades is presented visually in a schematic graph in the introduction.
Our response: we renamed the axes of our figures to make it cleared and added a few sentences to clarify the comparison of the strength of trophic cascades (lines 293-296, 189-195). We also refer to Schmitz et al. 2000 American Naturalist and Hedges et al. 1999 Ecology where they present in details the effect size method and the way to interpret the results. Nevertheless, we are ready to include a schematic graph at the next revision round if needed.

Reviewer 2
The authors should use "of" and "in" more to produce a paper that is easier to read. E.g. "the strength of trophic cascades" not "trophic cascade strength". And "intraspecific variation in herbivore traits" not "herbivore intraspecific trait variation."
Our response: we modified the text as suggested.

Reviewer 2
Below are comments that came up in the course of reading the manuscript, some minor some more major.

Reviewer 2
When describing the statistics, the authors don't explicitly define how they measure the strength of a trophic cascade, though they say that is what they are studying (e.g. their first "step"). Specifically in the section between line 155 and 164, what exactly would the results be that would tell you that one trophic cascade is stronger than another? the paper would be stronger if that were stated explicitly. The analyses focus plant and predator biomass and on aphid density (why not biomass?). However, it seems to me that to evaluate relative strength of a cascade, what is needed is not, for example, plant biomass alone, but first how much plant biomass changed relative to a control when predators were added, and second whether that amount change differs by aphid lineage. That is not mentioned as part of the analysis at all. In sum, it would be really helpful if the authors outlined clearly and quantitatively how the strength of a trophic cascade is measured, and how the strength of one cascade is compared to the strength of another cascade.

OK, now I see that starting on line 172 there is a definition of trophic cascade strength. So, the problem is rather that the first "step" of the statistical analysis needs to be re-written. Currently it says, "(1) investigate whether trophic cascade strength differed among aphid lineages to test for the existence of intra-specific effects" but that is not at all what they describe in their first "step" of analyses.
Our response: we have clarified the way we measured the strength of a trophic cascade with both the LMMs (Lines 157-179) and the effects size (Lines 181-195). Additionally, we added the LMMs output as supplementary tables.

Reviewer 2
I continue to hold that clearly defining, in the introduction, how the strength of a trophic cascade is measured and compared would make the paper stronger. In fact, as figure 2 is the way the

test is actually done, if I understand correctly, it would be really useful to make a mock-up of that figure showing equal strength of trophic cascades, and what stronger or weaker cascades would look like in that plot space. Better yet would be to have a response variable (Y-axis) that IS "Strength of Trophic Cascade" or "Trophic Cascade Strength". (with the latter being fine for a figure, but really for the text the former should be used)
Our response: we modified to figure axis as suggested and added a few sentences to clarify to way to interpret the graphs. However, we decided to not include a mock-up as the methods are explained in details in Schmitz et al. 2000 and Hedges et al. 1999.

Reviewer 2
Lines 190-192 – it is a stretch to call population growth of aphids without predators measured over 10 days, during which density would have grown a lot, the instantaneous population growth rate. If, during that time, plant quality drops (as clearly it does given the results) there it cannot be considered an instantaneous rate.
Our response: we removed the term "instantaneous".

Reviewer 2
line 210 –should read "two times heavier" or "twice as heavy" (not twice heavier)
Our response: modified as suggested.

Reviewer 2
Figure 1 would be better if the error bars were confidence intervals (e.g. 1.96*SE rather than just the SE)
Our response: We do not understand why it would be better if there were confidence intervals because it is just a matter of multiplying the error bars by 1.96.

Reviewer 2
Line 265 "Predator indirect effect" should read, "the indirect of predators"
Our response: modified as suggested.

Figure. 2. Relationship between the magnitude (log ratio ± 95% CI) of the predator effects on aphid density and on plant fresh biomass according to aphid lineage (a) and biotype (b) Would be more easily understandable (and would aid in interpreting the text the follows) if it read, "The relationship between the magnitude () of the direct effects of predators on herbivore density and the indirect effects of predators on fresh plant biomass by aphid lineage (a) and biotype (b)."
Our response: modified as suggested.

Reviewer 2
In general, avoid "predator effects" – instead use "effects of predators" Avoid "plant fresh biomass" and use fresh plant biomass" (or wet weight)
Our response: we modified the text as suggested but, in some instance, we decided to keep the condensed version to keep the text short and direct.

Reviewer 2
line 284 – change “predator indirect effects” to “indirect effects of predators” here and everywhere. Also, replace “lineage population growth rate” with “the population growth rate of the aphid lineage”
Our response: we modified the text but not for all of these cases as we feel that the text would not be easier to read if it is full of pronouns.

Reviewer 2
Line 302 would read better as “The effects of predators on aphid density () were [not was] positively associated with the population growth rate of the different aphid lineages.”
Our response: modified as suggested.

Reviewer 2
406 “variations” should read “variation” here and elsewhere in the paper (there were instances of it earlier than this line). Just like “information,” variation is used in the singular in almost all cases in English.
Our response: modified as suggested.

Reviewer 2
420 and 424 “cascade” should read “cascades”
Our response: modified

Reviewer 2
426 – associated with (not to)
Our response: modified