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Sophia Antipolis, December 10th, 2020

To Marta Montserrat, recommender for PCI Ecology

Subject: Manuscript Revision

Dear Marta Montserrat,

Please find attached our revised version the manuscript now entitled " Consistent variations in personality traits and their potential for genetic improvement of biocontrol agents: *Trichogramma evanescens* as a case study".

We were pleased to learn that the recommender and the reviewers found our manuscript interesting, and we would like to thank them for the constructive comments and suggestions they made. We agreed with most of the concerns raised and addressed all of them in the revised version. We provided detailed answers to all the comments on the following pages.

In expectation of your decision,

Sincerely yours,

For the authors

Silène Lartigue

I. Response of the recommender

by Marta Montserrat, 2020-10-29 08:09

Revision needed

In the preprint presented by Lartigue et al. the authors introduce a novel perspective for the genetic improvement of Biological Control Agents (BCAs) based on behavioral traits that relate to the performance of individuals as BCAs, and that define the personality of individuals. The authors propose the genetic improvement of personality traits to be used as tool to create highly performant BCAs.

The idea is novel and the manuscript has potential to contribute significantly to agricultural sciences and to the development of new strategies to improve pest control with methodologies that are respectful with the environment and the human health.

Yet, in my opinion, the potential success for using behavioral traits to breed natural enemies is likely to be severely hampered by the extremely low heritability values (<0.1) found in most of the behavioral traits and the two personality scores. This is a severe drawback that the authors need to address, not only by acknowledging the limitations of their strategy (as they do in the last paragraph of the discussion) but by offering real solutions, or convincing ways, to overcome this drawback, if only to convince the reader that introducing the genetic improvement of personalities in the biological pest control programs is realistic and feasible.

Response of the authors:

Thank you very much for your interest in our manuscript and your comments.

You are right, the low heritability values we found in this study are a weak point if we want to consider these personality traits in biocontrol, and we need to discuss it more. We therefore added a discussion about some solutions to overcome the main drawback of low heritability values (lines 574 to 582), for example genomic selection, that is already used to select traits with low heritability values in livestock animals (Hayes et al., 2009). Indeed this method is currently under development to consider it in the selection of biocontrol agents, as suggested by the first reviewer (comment number 6) and Leung et al., 2020.

Hayes, B. J., Bowman, P. J., Chamberlain, A. J., & Goddard, M. E. (2009). Invited review: Genomic selection in dairy cattle: Progress and challenges. Journal of dairy science, 92(2), 433-443.

Leung et al. 2020 Next-generation biological control: the need for integrating genetics and genomics. Biol Rev.

II. Reviews

First reviewer

Reviewed by Bart A Pannebakker, 2020-10-06 10:57

Dear Martha,

I have read the paper "Consistent variations in personality traits and their potential for genetic improvement in the biocontrol agent *Trichogramma evanescens*" by Silène Lartigue et al. In their paper, the authors explore the variation in personality traits in minute *Trichogramma* wasps, for use in biological control. Animal personality is a relatively new field, and the authors are among the first to link this to the efficiency and potential for genetic improvement of biocontrol agents (BCA). Furthermore, the authors describe a high-throughput phenotyping system that allows the determination of the personality of the tiny wasps. Finally, they correlate the personality traits to more traditional used measures of biological control agent quality. All in all, I find this a very interesting manuscript that describes a new angle in the maintenance and improvement of quality of BCAs. Furthermore, the methodology is sound and solid and the paper is well written. I only have a few minor points which could help to further improve this paper:

Thank you very much for your interest in our study and your useful comments. We provide answers to your comments on the following lines.

- (1) l. 76: The reference should be = Wajnberg, Bernstein & van Alphen, 2008
Done (l. 76).
- (2) l. 108 & l. 204: I am missing a good description of the personality traits that were investigated (boldness, activity and exploration). There is a reference to Reale et al 2007, but it would help the reader to provide a bit more definitions in the text.
Thank you for this comment. We added the definitions of the five main personality traits described in Réale et al. 2007 (l. 110 to 115).
- (3) l 184: Can you indicate why an LCD screen was used? It becomes clear in the text below, but it would help to guide the reader to explain that it was used to provide the surroundings in which to investigate the personality.
We changed the sentence to make it clearer (l. 195).
- (4) l.234-239: Can you shortly indicate what the different areas represent in the caption, so the figure can be understood without consulting the text
Thank you for this comment. We added a few lines in the caption to explain the variables we measured in each area (l. 252 to 257).
- (5) L467-4670: It might seem very obvious that bigger females are more active. Bigger females have more fat, so more energy to spend. They therefore can use more energy to explore the area and don't need to save it for reproduction (or actually have more energy left for exploration). Can you include a discussion of this explanation, or am I wrong here?
You are right, of course. It is indeed obvious (and it has already been shown) that bigger individuals are more active. We did not include it at first because it can be part of the Pace-

of-life hypothesis, but we do agree that it is better to be more specific about it, therefore we added a discussion at lines 496 to 504. However, it is important to note that our results did not show a significant phenotypic correlation between exploration and body size. The positive phenotypic correlation we found was between body size and shyness (the contrary of boldness) or activity.

- (6) 1.523-525: Indeed, a low heritability might make selection on these traits difficult. One option is to move to genomic selection, as indicated in the recent paper by Leung et al. 2020 Next-generation biological control: the need for integrating genetics and genomics. Biol Rev. doi:10.1111/brv.12641. Genomic selection is especially useful when traits display a lower heritability. While this is currently still under development, it might be useful to shortly refer to genomic selection and/or the paper by Leung et al. 2020 here. Thank you for this useful point, which is similar to the comment of the recommender. We added a discussion about it at lines 574 to 582 (also see our response to this comment in part I).

Second reviewer

Reviewed by François Dumont, 2020-09-18 18:53

The proposed article is interesting, well written, and detailed. The approach is very relevant to the emerging field of biological control agent selection. However, researchers who will be interested in this approach do not require expertise in animal personalities. I think the authors need to explain better the link between the measures taken and their interpretation in terms of personality. The manuscript is also limited to a few examples of parasitoids, while BCA's genetic improvement targets more diverse groups and situations. Therefore, the article could have a broader impact if it better integrated other organisms such as predatory mite and omnivorous bugs. Besides, it will be interesting to link to more conventional BCA improvement studies based on pesticide resistance. Links between animal personality and pesticide resistance have already been established. It would be interesting to discuss it further here. Finally, the study of animal personality is relevant to understanding the impact of pest insects. How could the methods proposed in the manuscript apply to these pests?

Thank you very much for your interest in our study, and for your comments and discussions about it. The topics you pointed out in this paragraph are also pointed out in your comments listed below, on which we provide detailed answers.

Yet, we do not think a discussion about pesticide resistance is a key point in our study, even if, as you mentioned, studies show links between personality and pesticide resistance (see Royauté et al. 2015). Indeed, we are more in favour of optimizing the utilisation of biocontrol “alone” rather than optimizing the utilisation of biocontrol combined with pesticides. The large support (governmental and other research funds) in developing efficient biocontrol methods mainly aims to the complete replacement of pesticides. Therefore, our choice is to not discuss it in this manuscript.

Royauté, R., Buddle, C. M., & Vincent, C. (2015). *Under the influence: sublethal exposure to an insecticide affects personality expression in a jumping spider. Functional Ecology, 29(7), 962-970.*

- (7) Title: You could have a title with greater span. *Trichogramma* are more of a "case study" to a useful method for a wide variety of insects.
Thank you for this useful comment. You are right: our title and our discussion, as pointed out in several of your comments, could have a greater span than the species and taxa we studied. We therefore changed the title of our manuscript to specify that *Trichogramma evanescens* is just an example.
- (8) l 84-85 : There are other studies on predatory mites (Nachappa et al. 2010; 2011) and omnivorous bugs (Dumont et al. 2016, 2017, 2018; and Chinchilla et al. 2020). Your focus is too limited to parasitoids in my opinion. The method you propose can be interesting to larger audience.
Yes indeed, you are right, this method can also be useful to other BCA species (parasitoids and predators). We added a few lines about it in the conclusion part (l. 586 to 588).
- (9) l 86-87 : However, some have measured several traits (see Nachappa et al. 2010; 2011). Thank you for these examples, we added them at lines 84 to 86.
- (10) Line 117: I do not see how personality measures are superior to measures of foraging behaviour. Differences in foraging behaviour may result from personality differences, but ultimately what is of interest to the use of BC is its foraging behaviour.

Thank you for this comment. Our aim is not to demonstrate that personality measures are superior to foraging behaviour measures. Of course, differences in foraging behaviour may result from personality differences, and if repeatable over time and contexts, individual differences in foraging behaviour may also be considered as personality traits.

If individual differences in foraging behaviour are not consistent through time and contexts (i.e. they cannot be considered as personality traits), investigating the personality traits underlying may (i) help understand how the individuals/populations vary in their foraging behaviour, and (ii) provide a basis for selection if the personality traits investigated are heritable.

If individual differences in foraging behaviour are consistent through time and contexts, studying the suggested traits (i.e. activity, exploration or boldness) may be a benefit as it can be done with more standardized protocols. Indeed, foraging strategies tests usually imply more complicated arenas or experimental sets, with a high variability in prey or host behaviour and/or life history, that does not provide an easy way to estimate repeatable individual differences and needs more human and/or material resources to provide a large enough number of replicates.

Moreover, measuring foraging behaviour (or at least testing it in the limited conditions encountered in a laboratory) may not take into account other behaviours (such as boldness) that could have an impact on biocontrol in the field. For example, studying boldness traits could help us understand how the biocontrol agents behave under a potential risky situation (presence of a predator for example) that is hardly tested in BCA foraging behaviour experiments.

Finally, when studying personality traits and even more when studying its potential application to biocontrol, an important point is to evaluate the relationship between personality traits and other behavioural, physiological and life history traits. Thereby, the study of personality traits does not exclude the study of foraging behaviour. On the contrary, studying the relationship between “classical” personality traits and “classical” foraging behaviour traits may help having a better overview of the optimal set of traits that are of importance in biocontrol.

- (11) L 124: We have observed that more zoophagous lines of mullein bugs are also more cannibalistic (Dumont et al. 2018). Therefore, voracity is linked to diet and interaction with conspecifics. Perhaps aggressiveness has something to do with that.

Yes indeed, the relationship between voracity and aggressiveness has been shown or suggested several times (see for example Wolf and Weissing (2010)). Although very interesting, we did not add this point in the text because we thought it is not a major point.

Wolf, M., & Weissing, F. J. (2010): An explanatory framework for adaptive personality differences. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1560), 3959-3968.

- (12) L 137 : among ? (instead of between)

We think “between” is more correct in this case, as we are referring to specific traits.

- (13) L 166: So how many generations during this periods?

As we reared *T. evanescens* individuals at 25.5 ± 1 °C, $70 \pm 10\%$ RH, the time between each generation was about 10 days. Thereby, as we conducted the experiment for 9 weeks, individuals from six generations were studied during this period. However, three replicates per line (and subline) were used at each experimental session to avoid “generation” being a confounding factor in the analyses. The statement “corresponding to six generations of *Trichogramma evanescens*” was added within brackets (line 173 to 174).

- (14) L 178: I like that figure.

Thank you!

- (15) L 208-209: Why? Is there an ecological explanation for this presumption?

See below (comment 16).

- (16) L 212: I don't understand the connection between measurements and boldness.

In order to estimate boldness in *Trichogramma evanescens*, we tested different design possibilities to create a “risky” situation for the individuals (as it is usually done to measure boldness in other species). We first looked for an event that could have made them freeze in order to measure the time it would have taken them to unfreeze. Most of the designs tested (short luminous flash, short vibration or short sound flash) were unsuccessful to make the individual freeze and/or were very difficult to standardize, mainly because of the size of the individuals. However, we observed that some *T. evanescens* individuals seemed to pause, or hesitate, to enter a dark zone in their breeding tubes. We developed the design presented in this paper in order to evaluate the behaviour of the individuals at the border of a dark zone. In order to estimate the “hesitation” (or latency) of the individuals at this border, we measured the time until the female first entered the dark area, and we looked also at the behavioural change (in speed and in

sinuosity) in the area near the border. Between the five main personality traits described by Réale et al. (2007), these variables seemed to be more linked to boldness. Yet, this is just a hypothesis, and the most important aim of our study is to look at consistent individual differences in behaviour that could be of interest in genetic improvement, rather than attributing the variables we measured to a personality trait in particular.

(17) L 214: Shouldn't the new paragraph start here?

Yes indeed, thank you!

(18) L 220: Being active in Zone 4 is not more of a boldness than activity? I have the impression that the line between boldness, exploration and activity is thin and somewhat arbitrary. I'm initiated to the concept of personality but I have limited knowledge on the topic. It's may be the case of other researcher interesting in your method. I think you must explain better how your measures are actual measures of personality.

Yes, the other reviewer pointed that out too (see comment number 2). We added a description of the personality traits studied at lines 110 to 115.

Moreover, as mentioned in our response to the comment number 16, the most important aim of our study is to look at consistent individual differences in behaviour that could be of interest in genetic improvement, rather than attributing the variables we measured to a personality trait in particular. We do understand that the line between the traits between boldness, exploration and activity can be thin for some variables, but we tried our best to link the variables we tested one of the five main personality traits Réale et al. (2007) described. Also, see our response to comment number 19.

(19) L 222: I dint it difficult to separate the three personality measures from the measurements you use. A shy individual could be very active in Zone 1, but not very active in zone 4. I'm not convinced.

We do understand this comment. As we measured our three personality traits in the same arena and during the same experiment (a choice we made because individuals from *T. evanescens* are small and fragile, in order to decrease the number and time of manipulations of the female and to make it possible to do the experiment over a high number of females), we decided to focus on only one area per personality trait so that the measure of each trait could be more “independent” from the measure of the other traits. The border area was for boldness, area 1 was for exploration (as it was the first area the individual discovered), and area 4 was for activity. We did check for the activity in area 1 and for the exploration in area 4, and the results were similar. We added an explanation at lines 214 and 215 to 218.

(20) L 230: You mean between 776 and 996 individuals per line?

No actually, we obtained measurements for 776 to 996 females in total per behavioural trait, from the 24 lines. We added a precision at lines 244-245.

(21) L 273: Is the "session" more about "generation"?

No actually, see our response at the comment number 13.

(22) L 395: The values are pretty low.

Yes indeed, as we mentioned in the discussion (line 483), these values are lower than the mean value for animal behaviour which around 0.24 (Dochtermann et al. 2019). However, as pointed out by the other reviewer (comment number 6), genomic selection is one option that can be considered for the selection of traits even with low heritability values. We added a discussion about it at lines 574 to 582.

Dochtermann, N. A., Schwab, T., Anderson Berdal, M., Dalos, J., & Royauté, R. (2019). The heritability of behavior: a meta-analysis. Journal of Heredity, 110(4), 403–410.

- (23) L 403: I don't understand how these lines can have the A and C at the same time, but not B.

These boxplots and the letters associated are the results of the Tukey all-pair comparisons tests we did.

The lines 3 and 18 have the letters *a* and *c* at the same time, but not *b*. This means that they are significantly different from the other lines that have the letter *b* but not *a* and *c*: the lines 13 and 10.

Lines with the same letter can be considered as a group in which no line is significantly different from another line of the group. A line with the letter *b* is not significantly different from the other lines with the letter *b*. However, the lines 3 and 18 cannot have the letter *b* as they are significantly different from two lines from the group with the letter *b* (lines 13 and 10).

- (24) L 440: I think the article is interesting for researchers not necessarily familiar with animal personality. It would be appropriate to better explain how these measures are related to personality. This is not evident in your text.

We added a sentence at lines 475 and 476 to explain that we considered only as personality traits the variables that were repeatable. Also see our response to comments number 18 and 19.

- (25) L 455-456: Okay, but ultimately what does that mean? Did the individuals captured at different sites in France come from releases? Wouldn't these individuals actually come from the same population? And that there would be less diversity in this initial population than in natural populations? Just wondering.

We supposed the individuals we captured at different sites (private gardens, fields where no *Trichogramma evanescens* releases had been done) did not come from releases. However, it is right to suggest that the different lines we worked on do not represent the whole natural diversity in this species. Yet, as the heritability of the longevity trait was not as low as the other traits, they must have been some diversity between the lines anyway; and we did check for genetic diversity with microsatellites (line 156).

- (26) L 469: Interesting.

Thank you!

- (27) L 477: In my opinion, that would be relevant to several other BCA. It would be nice to make more connections to other BCA (i.e. *Nesidiocoris tenuis*). In my opinion, you will have more impact as well.

You are right. We changed the title (l. 510-511) (see our response to comment number 7) and added a part in the conclusion (l. 586 to 588).

- (28) L 496: Read also Nachappa et al. 2010 and 2011. It measured the heritability of voracity, residency time, and conversion rate in a predatory mite. Then she went to the field to compare the effectiveness of her strains and the dynamics with the prey. Could you link it to animal personality?

Yes indeed, it is an interesting study. In order to link these traits to animal personality, the measures should be repeated at least one time for each individual in order to measure the repeatability of these traits.

- (29) L 500-501: That depends. In many systems, parasitoids are subject to pressure from other predators (intraguild predation). Shy individuals might be effective in a strategy that relies on the use of various BCA. I am thinking in particular of the zoophytophagous bug *Dicyphus hesperus* and the parasitoids of whiteflies in greenhouses (use in North America). In Europe, the bugs *Macrolophus pygmaeus* and *Nesidiocoris tenuis* could be in the same situation. We discuss it in Dumont et al. (2018; From evolutionary aspects of zoo...).

Thank you for this useful comment. We added a discussion about the fact that, if various BCA species are used and there is possibility of intraguild predation, shy individuals might be less predated as they might be less willing to take risks compared to bold individuals at lines 537 to 545.

- (30) L 504: Performance can depends on the context. For example, highly exploratory lines may be more effective in a large field context, while less exploratory lines may have qualities that make them effective in a greenhouse setting.

Yes indeed, it is a very good point, thank you for this suggestion. We added a few words to precise that at line 547.

- (31) L 509: Interesting. Several research opportunities here.

Yes indeed!

- (32) L 511: This is also interesting. And it could be applied to pests too. For example, colonization of new areas could be done by more explorative, but less fertile individuals.

Yes, you are right. Indeed, the link between personality and biological invasions is an important question when considering the application of personality in ecology and has already been the subject of several studies (see for example Wolf and Weissing 2012). This is a very interesting topic, however we think it is not directly related to our study and we decided not to discuss about it in this study, having already a large amount of points to discuss about.

Wolf, M. and Weissing, F.J. (2012) Animal personalities: consequences for ecology and evolution. Trends Ecol. Evol. 27, 452–461

- (33) L 513: The same kind of questioning is encountered when studying aggression among the Nabis (current project).

We will be interested in reading your paper and discuss about it with you!

- (34) L 515: There are certainly links to diet and voracity. We observed genetic variation in zoophagy and food specialization in the zoophagous predator *Campylomma verbasci* zoophytophageous (see articles by Dumont, Lucas and Réale). The choice of food

resources can be associated with several personality traits in my opinion. Some resources may be associated with more encounters with predators who share the same resource. Hence, "shy" individuals would choose plant resources rather than animals. In the case of an insect with ambiguous status such as *C. verbasci*, it can make the difference between being beneficial or harmful. This trade-off is also observed in the BCA marketed in Europe, *Nesidiocoris tenuis* (see Chincilla et al. 2020).

This is also very interesting topic, thank you for your comment. However, we think it is not directly related to our study and therefore decided not to discuss about it in this study.

- (35) L 517: Are there methods to measure aggressionness? Aggressiveness will certainly influence behaviours such as cannibalism. Mass breeding would be strongly influenced by this personality trait. You might also think it influences the diet.

You are right, aggressiveness, and sociability, could have an important impact on mass breeding, as well as on field efficiency (aggressiveness and dispersion have been shown to be correlated in several species, see for example Cote et al. (2010)). We are not aware of any visible sign of aggressiveness in *Trichogramma evanescens*, but we did try to develop an experimental design to measure sociability, which was unsuccessful. Of course, we do not say that it is impossible to measure sociability and aggressiveness in *Trichogramma evanescens*, only that the designs tested up to now were not successful.

Cote, J., Clobert, J., Brodin, T., Fogarty, S., & Sih, A. (2010). Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1560), 4065-4076.

- (36) L 540: Can wait to see the results of those studies.

Thank you!