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Evolutionary Biology

Selection on a single trait does not recapitulate the evolution of life-history traits seen during an invasion

Inês Fragata *and* ***Ben Phillips*** *based on peer reviews by 2 anonymous reviewers*

Julien Foucaud, Ruth A. Hufbauer, Virginie Ravigné, Laure Olazcuaga, Anne Loiseau, Aurelien Ausset, Su Wang, Lian-Sheng Zang, Nicolas Lemenager, Ashraf Tayeh, Arthur Weyna, Pauline Gneux, Elise Bonnet, Vincent Dreuilhe, Bastien Poutout, Arnaud Estoup, Benoit Facon (2020) How do invasion syndromes evolve? An experimental evolution approach using the ladybird Harmonia axyridis. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/849968

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Biological invasions are natural experiments, and often show that evolution can affect dynamics in important ways [1-3]. While we often think of invasions as a conservation problem stemming from anthropogenic introductions [4,5], biological invasions are much more commonplace than this, including phenomena as diverse as natural range shifts, the spread of novel pathogens, and the growth of tumors. A major question across all these settings is which set of traits determine the ability of a population to invade new space [6,7]. Traits such as: increased growth or reproductive rate, dispersal ability and ability to defend from predation often show large evolutionary shifts across invasion history [1,6,8]. Are such multi-trait shifts driven by selection on multiple traits, or a correlated response by multiple traits to selection on one? Resolving this question is important for both theoretical and practical reasons [9,10]. But despite the importance of this issue, it is not easy to perform the necessary manipulative experiments [9]. Foucaud et al. [11] tackled this issue by performing experimental evolution on source populations of the invasive ladybug *Harmonia axyridis*. The authors tested if selection on a single trait could generate correlated responses in other life history traits. Specifically, they used experimental evolution to impose divergent selection on female mass, and reproductive timing. After ten generations, they found that selection for weight did not affect almost any other life history trait. However, nine generations of selection for faster reproduction led to correlated phenotypic changes in developmental, reproduction and survival rate of populations, although not always in the direction we might have expected. Despite this correlated response, none of their selected lines were able to fully recapitulate the trait shifts seen in natural invasions of this species. This implies that selection during natural invasions is operating on multiple traits; a finding in agreement with our growing understanding of how selection acts during introduction and invasion [12,13]. Populations undergoing a colonization process may also be subject to a multitude of different selective pressures [14,15]. The authors expanded their work in this direction by testing whether food availability alters the observed correlations between life history traits. The pervasiveness of genotype by environment interactions observed also points to a role for multiple selective pressures in shaping the suite of life-history shifts observed in wild ladybug populations. The work from Foucaud and colleagues [11] adds to a small but growing list of important studies that use experimental evolution to investigate how life-history traits evolve, and how they evolve during invasions in particular.

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Reviews

Evaluation round #2

DOI or URL of the preprint: **https://doi.org/10.1101/849968**

Authors' reply, 22 April 2020

Our apologies for being slow to get this back to you. Coronavirus chaos. Thank you for your thorough revisions and careful responses. We think that the manuscript is improved and will be of added value to the field. We will write a recommendation, but in the meantime there are some minor points remaining that you might like to address. Foucaud et al.: We are glad that the quality of the manuscript has been improved enough to now reach unambiguously the quality standards of PCI Evol Biol.

Minor points: Page 3, lines 18, 20, and other places – typo in your reference manager, "Phillipps" Foucaud et al.: Done Page 8, line 14, do you hypothesize, or assume? Foucaud et al.: Done Page 15, line 11 – "phenotypic changes" instead of "changes in beetle phenotype" Foucaud et al.: Done Page 15, line 17 – do you have some measure of uncertainty around the narrow-sense heritabilities? It is presumably large; it usually is. Foucaud et al.: We computed standard error of the mean heritability using the recommendation of Roff (1996, p.140). We agree that measures of uncertainty around the estimated narrow-sense heritabilities should be large. However, in our case, standard errors are low, notably for the age at first reproduction selection because of the relatively low precision of our measure (in days) when approaching very fast development times. Page 17, line 14 – typo MAF. Foucaud et al.: Done Page 18, line 11 – You did not cite sup figure 4 before sup fig 5 Foucaud et al.: Done. The order of all figures and supplementary figures was checked. Page 21, lines 7 and 8. You might want to drop "highly" and "quickly". Foucaud et al.: Done Page 21, line 13 – replace G0 by initial (so that it reads initial generation) Foucaud et al.: Done Page 25, line 14 – typo "[as] a trial and error..." Foucaud et al.: Done Page 25, paragraph at line 4. [Optional] This paragraph might be simplified? It seems that all of these points could be simplified into: a) selection is longer, b) selection is more complex (multiple traits, temporal dynamism), c) more variance (allowing trial and error). Foucaud et al.: Done Page 25, line 18: 21 –

That sentence is a bit difficult to parse, maybe invert it would work better? ”It remains unknown whether the invasion and its associated syndrome were successful due to such lottery effects or because of the purging of deleterious mutations during the multiple introductions, creating individuals with overall higher fitness (Facon et al 2011) Foucaud et al.: Done Page 26, lines 1:3 – Also a bit difficult, maybe something like this would work: ”The phenotypic differences between control and selected lines were magnified in stressful conditions for the fast reproduction lines. Differences between heavy lines and light and control lines were exacerbated under stressful diet” Foucaud et al.: Done Page 26, line 5 – Should this figure be referenced in the results also? Foucaud et al.: Done Page 26, line 15 and also Figure 8 – We are not sure we follow the figure caption here. How do positive slopes indicate high variation or negative slopes lower variation? Figure 8 just shows the correlation between fecundity and female body mass. Under normal conditions, one expects that there is a positive correlation between mass and fecundity as bigger females lay more eggs and smaller ones, smaller number of eggs. In stressful environments this correlation goes the other way around, because bigger females need more food and thus have less resources to allocate to egg laying. That’s what you see on your controls, and that you mention in the discussion. Perhaps re-word, or explain the ideas in this caption more carefully. Foucaud et al.: The precedent caption was too interpretative, and is discussed in the main text, so we simply removed any interpretation from the caption. Page 28, line 16 – you have an extra “ before R.A.H. Also as it is the sentence is a bit redundant. Maybe remove “Some of the authors are PCI recommenders” and leave the rest (which already indicates that some of you are recommenders). Foucaud et al.: Done Figure Legends – In general you should indicate what is the source of the error bars, I am assuming standard error between replicate lines, but it is good to write it out. Foucaud et al.: Done Page 36, line 5 and 10 – need to update the color code :) Just a comment on figure 1: An interesting feature from this figure is the magnitude of variance between body mass lines vs the variance for age of first reproduction. Naively, this may suggest that there is highly polygenic base for size (which is seen also in the literature) and may explain the lower rate of adaptation, i.e. many genes of small effect (not to add anything, just thought it was interesting to see it). Foucaud et al.: Color code is now updated. Thank you for this interesting remark. Page 36, line 16 and 20 – you can remove the indication of the panels (A-C) and (D-F) as you are already explaining each one of them in detail afterwards Foucaud et al.: Done Figure 5 – There is no indication for C and D in the figure per se Page 37, line 15: add selection line or regime at the end of “female body mass selection” Foucaud et al.: Done Figure 8 Legend – “Treatment”. Also, this would be simpler to parse if there were three colours (one for each treatment as in earlier figures), and two symbols/linetypes (for each diet). Foucaud et al.: Color and symbols were modified accordingly.

Decision by Inês Fragata and Ben Phillips, posted 15 April 2020

Our apologies for being slow to get this back to you. Coronavirus chaos. Thank you for your thorough revisions and careful responses. We think that the manuscript is improved and will be of added value to the field. We will write a recommendation, but in the meantime there are some minor points remaining that you might like to address.

Minor points:

Page 3, lines 18, 20, and other places – typo in your reference manager, “Phillipps”

Page 8, line 14, do you hypothesize, or assume?

Page 15, line 11 – “phenotypic changes” instead of “changes in beetle phenotype”

Page 15, line 17 – do you have some measure of uncertainty around the narrow-sense heritabilities? It is presumably large; it usually is.

Page 17, line 14 – typo MAF.

Page 18, line 11 – You did not cite sup figure 4 before sup fig 5

Page 21, lines 7 and 8. You might want to drop “highly” and “quickly”.

Page 21, line 13 – replace G0 by initial (so that it reads initial generation)

Page 25, line 14 – typo “[as] a trial and error…”

Page 25, paragraph at line 4. [Optional] This paragraph might be simplified? It seems that all of these points could could be simplified into: a) selection is longer, b) selection is more complex (multiple traits, temporal dynamism), c) more variance (allowing trial and error).

Page 25, line 18: 21 – That sentence is a bit difficult to parse, maybe invert it would work better? ”It remains unknown whether the invasion and its associated syndrome were successful due to such lottery effects or because of the purging of deleterious mutations during the multiple introductions, creating individuals with overall higher fitness (Facon et al 2011)

Page 26, lines 1:3 – Also a bit difficult, maybe something like this would work: ”The phenotypic differences between control and selected lines were magnified in stressful conditions for the fast reproduction lines. Differences between heavy lines and light and control lines were exacerbated under stressful diet”

Page 26, line 5 – Should this figure be referenced in the results also?

Page 26, line 15 and also Figure 8 – We are not sure we follow the figure caption here. How do positive slopes indicate high variation or negative slopes lower variation? Figure 8 just shows the correlation between fecundity and female body mass. Under normal conditions, one expects that there is a positive correlation between mass and fecundity as bigger females lay more eggs and smaller ones, smaller number of eggs. In stressful environments this correlation goes the other way around, because bigger females need more food and thus have less resources to allocate to egg laying. That’s what you see on your controls, and that you mention in the discussion. Perhaps re-word, or explain the ideas in this caption more carefully.

Page 28, line 16 – you have an extra “ before R.A.H. Also as it is the sentence is a bit redundant. Maybe remove “Some of the authors are PCI recommenders” and leave the rest (which already indicates that some of you are recommenders).

Figure Legends – In general you should indicate what is the source of the error bars, I am assuming standard error between replicate lines, but it is good to write it out.

Page 36, line 5 and 10 – need to update the color code :) Just a comment on figure 1: An interesting feature from this figure is the magnitude of variance between body mass lines vs the variance for age of first reproduction. Naively, this may suggest that there is highly polygenic base for size (which is seen also in the literature) and may explain the lower rate of adaptation, i.e. many genes of small effect (not to add anything, just thought it was interesting to see it).

Page 36, line 16 and 20 – you can remove the indication of the panels (A-C) and (D-F) as you are already explaining each one of them in detail afterwards

Figure 5 – There is no indication for C and D in the figure per se Page 37, line 15: add selection line or regime at the end of “female body mass selection”

Figure 8 Legend – “Treatment”. Also, this would be simpler to parse if there were three colours (one for each treatment as in earlier figures), and two symbols/linetypes (for each diet).

Additional Comments of the managing board:

Mandatory modifications.

As indicated in the ’How does it work?’ section and in the code of conduct, please make sure that:

-Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data.

-Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused.

-Details on experimental procedures are available to readers in the text or as appendices.

-Authors have no financial conflict of interest relating to the article. The article must contain a ”Conflict of interest disclosure” paragraph before the reference section containing this sentence: ”The authors of this preprint declare that they have no financial conflict of interest with the content of this article.” If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: “XXX is one of the PCI XXX recommenders.”

In order to reach a better referencing and greater visibility of your recommended preprint, we suggest you to do the following modifications :
-**add the following sentence in the acknowledgements**: ”Version 3 of this preprint has been peer-reviewed and recommended by Peer Community In Evolutionary Biology (`https://doi.org/10.24072/pci.evolbiol.100096`) »

 If you use bioRxiv to post your preprint, **add this sentence also in a footnote of the 1st page of your pdf**, it will be interpreted as a specific footnote section by bioRxiv.
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-make visible this information in Google Scholar search (which is quite important).
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For word template, please be careful to correctly update all text in these templates (doi, authors’ names, address, title, date, recommender first name and family name ...). Please be careful to choose the badges “Open Code” and “Open Data” only if appropriate (in addition to the “Open Access” and “Open Peer-Review” badges). If some of the reviewers are anonymous, indicate for example “Albert Ayler and two anonymous reviewer”. Indicate in the “cite as” box the right version of your preprint. It is version 3.

For Latex template, main.tex and sample.bib should be filled. Please be careful to choose the badges “Open Code” and “Open Data” only if appropriate (in addition to the “Open Access” and “Open Peer-Review” badges). Preambule_xxx.tex should be modified (comment lines 115, 117) to select badges. If some of the reviewers are anonymous, indicate for example “Albert Ayler and two anonymous reviewer”. In sample.bib, indicate the right version of your preprint. It is version 3.

I hope this is clear. Do not hesitate to ask any help if you need.
Once you have made these modifications (plus those requested by the recommender), you should upload a new version of the article on the preprint server. Please tell us when you have done so.
Thanks.

Evaluation round #1

DOI or URL of the preprint: **https://doi.org/10.1101/849968**

Authors' reply, 30 March 2020

Decision by Inês Fragata and Ben Phillips, 2020-01-20 11:49 Manuscript: `https://doi.org/10.1101/849968` Revision round #1

Dear Authors, Your work has been assessed by two reviewers and both the recommenders of this preprint. Whereas we all agree that the work is very interesting and clearly has an added value to the study of invasive species, there is some (minor) changes that would improve the current manuscript. Below you will find the general comments from both the recommenders in addition to those of the reviewers. The comments of reviewer 1 were very positive and you should focus on them improve the clarity of the manuscript. You should focus more on reviewer's 2 recommendation of a (supplementary?) figure explaining the experimental design to make it even clearer. In general, both recommenders feel that the english and structure of the paper are good, and the comments provided below are mostly to improve the already good readability of the manuscript and the link to other areas in life-history evolution. General comments This was a really interesting piece of work. Well-conceived, well executed, and well reported. I see no major flaws, but have seen a few areas where the communication can be improved. Also, I think there are some important links to be made to broader interest in life-history evolution under strong selection; interest that reaches beyond the special case of life history evolution in invasive species. The work deserves and will gain greater attention if you link to this broader literature as well. Additionally, there is just one methodological problem that can be tackled quite easily (hopefully). Population 1 was exposed to 8 generations to the lab environment before start of the divergent selection, however population 2 only was exposed to 2 generations. Since your populations were not lab adapted in the beginning, this may mean that your population 2 may be adapting to the lab environment in addition to your selected conditions. Moreover, since they are taken from different seasons and years this might lead to some variability in your analyses (which is also referred by reviewer 1). One possible way to mitigate possible problems that may arise is for you to redo the analyses using as data the difference between selected lines and controls. This way you reduce the signal of possible lab adaptation and reduce some of the differences present due to variation between populations. Foucaud et al.: Thank you for having acted both as recommender and reviewers of this preprint, in addition to the two reviewers 1 and 2. We found the comments and criticisms helpful and cogent. Specifically, we have carefully taken into account most minor corrections. We also have run the analyses as suggested by you and reviewer 1 and found out that the results are confirmed. We have kept the previous analyses in the main text for simplicity's sake but we explicitly discuss the point in the discussion section and we provide the R code and data necessary to conduct the analyses again in the online supplementary material of the manuscript. We also added new figures in the supplementary materials to clarify the experimental design. We finally better explain the rationale for multivariate analyses in the text in response to the interrogations of reviewer 1. We hope that the quality of the manuscript has been improved enough to now reach unambiguously the quality standards of PCI Evol Biol. Below, we provide the detailed answers addressing these comments (numbers of lines and pages correspond to the new version with tracked changes).

Specific comments Introduction The abstract is very nicely written. The introduction is also well written and well argued. The value of artificial selection experiments in disentangling contributions to "invasion syndromes" is well made. The final paragraph sets out the study very nicely, and it is clearly a really interesting and worthwhile set of research questions. Below are some minor suggestions for improvement. Congruent with this, comparative studies of biological invasions have revealed that, while the ecological principles and evolutionary forces underlying invasions can be similar across invasion cases, different species respond to these constraints through a variety of strategies resulting in the apparent idiosyncratic nature of invasions Perhaps delete from "resulting in..." Foucaud et al.: We reword this part of the introduction (l.16-20 p.3).

Most importantly, several authors have pointed out that the processes underlying invasion success act at the population level within so-called "invasive" species Vague. Please clarify. Foucaud et al.: We now have modified the text accordingly (l.21-27 p.3 and l.1-4 p.4). We hope that there is no ambiguity left.

Experiments comparing native to introduced populations or distinct introduced populations to each other show that phenotypic evolution is common. There are a couple of review papers here that are relevant. Phillips et al Ecology 91:1617; and Chuang and Peterson Global Change Biology 22:494. Foucaud et al.: Thanks for these relevant references. We added both of them (l.10-11 p.4).

Here, we define the set of traits that evolve in concert and contribute to the success of invasive populations as an 'invasion syndrome'. This is fine: there is a set of trait differences that seem to consistently emerge in invasive populations of this species. In the following paragraph you talk about how such a syndrome might emerge: selection on multiple traits, genetic correlations, or both. Genetic correlations are self explanatory, but I think the introduction could be strengthened slightly by pointing out that, during invasions, we may well expect selection on multiple traits. This selection could happen either during the introduction phase (see e.g. Chapple et al TREE 27: 57; Blackburn and Duncan 2001 J Biogeog 28:927), or during the invasion phase (see the reviews mentioned previously). This point could be woven together with your point about historical selection (nearly all populations are spatially dynamic at some point, right?), and may also be a chance to touch on broader literature about other recent causes of selection on life history (e.g., fisheries, hunting, climate change, others..) Foucaud et al.: We added these references and modified the text to more clearly refer to the selection on multiple traits in the particular situation of biological invasions (l.7-18 p.6). Note that as one reviewer found the Introduction lengthy, we decided not to talk about other recent causes of selection on life history linked with human activities.

Methods Adult H. axyridis individuals were sampled in the native area of the Jilin province, China Is there any reason for this location? Is it a source location for any of the invasions? Foucaud et al.: The Jilin province is a source location of the population first introduced in Western North America, which further went invasive worldwide. Our Jilin population was sampled at the same location than the N-China 3 population used in genetic analyses by Lombaert et al. (2011). We now include this information in the manuscript (l.15-17 p.8).

Regarding collection and establishment of populations: is there any chance that selection during diapause (1500 -> 900 individuals, for example) could have influenced your results? Foucaud et al.: Diapause might have had an influence on individuals that first entered our different selection schemes. However, we wish to stress that (i) both populations underwent diapause (only the duration differed) and (ii) rate of mortality during diapause was largely similar between the 2013 and 2015 populations. We thus hypothesize that less cold-tolerant individuals failed to survive diapause in both cases. We added a sentence on the ms to clarify this point (l. 26-27 p.8 and l.1 p.9).

Is there any chance that ongoing selection in captivity through generations 1-8 influenced your results? These are issues that are unavoidable, but I flag them here as issues worth pondering in the Discussion. Foucaud et al.: An impact of lab environment selection on our comparative results would require that six additional generations in the lab broke all genetic correlations among phenotyped traits that would still be present with two generations in the lab. Theoretically, the expectation would rather be that positive genetic correlations evolve into negative genetic correlations following strong directional selection, especially in the case of life-history traits correlations (Roff, 1996). Empirical work on genetic correlations following laboratory adaptation is scarce, and usually suffer from large error estimates, but to date no obvious patterns of genetic correlation evolution is predictable following laboratory adaptation (e.g., in Drosophila subobscura; Matos et al. 2000), leaving it a possibility that in some populations genetic correlation were positive while in another they could be zero. In addition to that theoretical context, we now have tried to exploit our data to evaluate the possibility that different results in the Age at First Reproduction (AFR) vs Female Body Mass (FBM) selection experiments stems from differential duration of lab rearing prior to the experiments (2 generations for AFR vs 8 generations for FBM) We conclude that it is unlikely for the following reasons. As a first test, we compared G0 founding populations with controls lineages at the end of selection. This was not possible for the FBM selection due to lack of complete multivariate phenotyping data for G0, so we focused on the AFR selection experiment where complete data were available. Both univariate and multivariate analyses indicate that most traits evolved only slightly or not at all between G0 to G10 in the absence of selection within the AFR scheme. Lab adaptation was thus fairly undetectable for all phenotyped traits. Because only two generations took place in the lab before the start of the AFR experiment (against eight generations in the FBM scheme), it is conservative to hypothesize that lab adaptation could probably not explain the differences uncovered between our selection schemes, because it should either not influence these traits, or before the third generation of lab-reared individuals. It is however important to note that we detected a strong lab adaptation signal in the comparison between G0 and control lineages for the AFR selection, but only for survival traits. Male and female survival increased for G10 control lineages when compared to G0 individuals in the AFR selection. This indicates that we would have been able to detect a multivariate effect of lab adaptation would have it occurred. Furthermore, this is another illustration of the lability of the genetic architecture of phenotypic traits (in the case of lab adaptation, only survival evolved and no other traits), which is the main point of our ms. Although the evidence is very compelling, we feel that the fact that we do not have similar data for the FBM selection prevents us to include all these analyses in the current version of the ms. We however provide all R code and data to demonstrate this point in our online supplementary material. Second, following the Recommenders' suggestion, we did our analyses once again using the difference between selected lines and the means of our controls lines. To alleviate the burden of potentially unexpected distributions, we analyzed these differences using non-parametric bootstrapping (all code provided in our online supplementary material). Over all phenotyped traits, this analysis indicated that only body mass and male survival (only in the light lines) were influenced by FBM selection. Therefore, like in our initial analysis, analyzing the difference between selected and control lines pointed out the lack of correlated phenotypic response in FBM selection. On the contrary, this second analysis demonstrated that most phenotyped traits responded to selection on Age at First Reproduction (body mass, development time, age at first reproduction, fecundity, male and female survival, but not hatching rate nor larval survival rate). In conclusion, the analysis of the difference between selected lines and control lines yielded the same results as our previous analyses, indicating a lack of influence of different lab adaptation periods between our two selection schemes. The second analysis method, although sound to investigate this particular issue, may be more difficult to interpret and illustrate compared to our initial method. We therefore chose to simply present the results of the second method in our current answer to reviews and include it in our publicly released code, but to keep the more direct, easily understandable analysis in the main text of the manuscript. We explicitly discuss these points in the discussion section (l.21-26 p.25 and l.1-11 p.26).

Twenty-four families that mothered at least 10 females that survived to adulthood were kept for heritability estimation. Why not use all families for estimation of heritability? Foucaud et al.: We agree that this point was not explained as precisely as required. On the 43 couples established, only 30 had offspring. For these 30 families, most had between 10 to 30 offspring, with only 6 families having less than ten, and typically only 2 offspring. To our view, including these very small families would have unnecessarily inflated our heritability estimations while the remaining 24 families both enable satisfying estimations while representing a good proportion of all productive couples (24/30). We made this clearer in the present version of the ms (l.9-13 p.9) and provided the complete dataset together with the R code necessary to plot the distribution of offspring number per family and directly visualize the basis of this particular choice.

Also, given likely multiple paternity, presumably you are systematically underestimating broad sense heritability? Might be worth mentioning that here. Foucaud et al.: We agree and we now include this remark in the ms (l.24-26 p.9).

More generally, perhaps the focus should be on estimating the magnitude of heritability rather than testing against a null of zero. If you simply make interval estimates of heritability (and decline to calculate p-values), power analysis become redundant. I note that both your estimates turn out to be highly significant, so again power analysis seems redundant. Foucaud et al.: We agree that p-value calculations are rather misused in the current scientific literature and this suggestion is most welcomed. We thus now only indicate the interval estimates of heritability and removed both p-values and power analysis from the present version of the ms, as suggested (l.1-10 p.10 & l.10-15 p.16).

"chosen as follows to initiate the next generation: (i) for the control lines, 50 random females were chosen, (ii) for the light lines, the 25% lighter females were selected, and (iii) for the heavy lines, the 25% heavier females were selected. For each line, 10 days after emergence, 50 males were randomly chosen and added to the 50 chosen females". Do you mean 25% heaviest/lightest? Also, worth clarifying that the random males were chosen from within the relevant line. Also, worth pointing out earlier that selection is on female phenotype only, and explain why this is sensible. Foucaud et al.: Yes, we mean 25% heaviest/lightest. We modified the text accordingly (l.1-2 p.11). We also included a Supplementary Figure describing our selection schemes as well as our phenotyping procedures, as suggested by Reviewers (Suppl Fig 2 in the present ms).

"after emergence, >70 clutches per line were collected to establish the next generation. After hatching, a minimum of 33 boxes per line, each containing 12 larvae" Please clarify whether these clutches were stratified across females, and also what you mean by "boxes" (i.e. how boxes play into the design). Foucaud et al.: These clutches were not controlled for the identity of their mother (i.e., all females and males were kept together in a single arena for egg-laying). However, we are confident that no single/few females and/or males contributed most to the next generation of individuals before the selection step for several reasons. First, the egg-laying took place over a very dense substrate of crumpled paper (typically >20 sheets). Dense crumpled paper enabled individual females to isolate from other adults before laying their eggs (egg cannibalism is very common in this species), and we could visually identify that most females were indeed isolated next to their last clutch when collecting eggs. Second, while we indicated that >70 clutches per line were collected, most lines and generations produced over 200 clutches in four days of mating and egg-laying, a number impossible to reach by a low number of mothering females. Third, the ten days of virginity for both males and females imposed by our protocol is rather unusual in this species and adults of both sexes were willing to mate multiply as soon as put into contact, as has been checked during the course of the selection. In conclusion, we are confident that most individuals of both sexes contributed to the next generation of individuals before the selection step, and made the necessary change in the present version of the ms (l.5-9 p.11). Additionally, we have now clarified what we meant by "boxes" (l.9-15 p.11). These are actual small boxes (diameter 55mm) that were used to ensure a balanced contribution of clutches (and their mothers) to the next generation of selection, both during hatching and larval development steps. For hatching, 2 clutches were distributed per box and each of these boxes contributed to a single larval development box. For larval development boxes, only 12 larvae from a single hatching box (hence from 1-2 clutches) were reared. A mean of 40 larval development boxes were kept for each line and generation (see Suppl Fig 2 and 3 of the present version of the ms for a global view of this procedure).

Note that these lines included eggs from the earliest reproducing females as well as eggs from later reproducing females. Is it possible that these also contain eggs from offspring of the earliest reproducing females? Foucaud et al.: This is not possible because all eggs laid before those days were removed every 2 days prior to day 19-21. Generations were hence discrete and consecutive during all the experiment. We included this precision in the current version of the ms (l.3-4 p.12).

One last thought worth considering is whether you should estimate narrow sense heritability from the selection experiment. You have a lot of data there, and you have the Breeder's equation (with S known) to lean on. Foucaud et al.: Thank you for this suggestion. We added in the revised version of the ms the estimates of narrow-sense heritability drawn from the response of our experimental lines to selection (l.3-10 p.10 & l.25-27 p.16). As expected, these estimates of narrow-sense heritability were noticeably smaller than our broad-sense heritability estimates calculated from fullsib families. R code necessary to compute these estimates was also added in our released code.

Results "Both selection experiments operated on the phenotypes of individual beetles as expected from heritability estimates". This sentence does not make sense. The selection experiments were conducted on phenotypes of individual beetles and have nothing to do with heritability. Foucaud et al.: Accordingly this sentence has been modified (l.16-17 p.16).

"age at first [reproduction] of the different lines" Typo. Foucaud et al.: Done (l.22 p.16).

Table 1: why the boldface on some lines/results? Foucaud et al.: Originally, it was simply meant to underline the two traits selected in our experimental lines, but we now removed this unnecessary element of style.

Figures 1-4. Really nice figure panels. Foucaud et al.: Thanks for the comment. As with all analyses and figures, the complete code for the generation of such figures (a combination of ggplot, cowplot and custom script) is available.

For the figures maybe it would be go to change colors to be accessible to color blind people Foucaud et al.: Colors have been changed accordingly to take into account people with color vision deficiency.

Discussion In contrast, control lines displayed no evolution of age at first reproduction and only a slight increase of female body mass compared to the G0 generation, indicating no [ongoing evolutionary response] to rearing conditions Suggested re-wording. Foucaud et al.: We modified the text accordingly l.20-21 p.23.

These results confirm several studies showing that in many species body mass or size and age at first reproduction are heritable and can rapidly evolve in artificial selection experiments There is a recent resurgence of interest in the idea of selection on body size and correlated change in life history. It would be useful to link to that work. See, for example, recent papers by Malerba and Marshall. Really interesting that selection on time to reproduction (i.e. reproductive rate) had all sorts of correlated effects, whereas selection on body size appeared free of these outcomes. There are implications here beyond Harmonia and invasive species. Foucaud et al.: Thanks for these references that we did not know. We now have rephrased the text accordingly (l.20-26 p.24 and l.1-9 p.25) to add more information about the fact that selection on body mass had no strong effects on other traits.

additive genetic variance-covariance matrices (G-matrices) and therefore on future evolutionary trajectories For more recent references on the evolution of G-matrices, see work by Blows et al. Foucaud et al.: Following your advice, we found a relevant reference by Blows & Mcguigan (2015) and we added it l.21 p.26.

our results underline the importance that G × E interactions could have for the studies of biological invasions Is it worth mentioning the recent article by Williams et al in TREE about maintenance of variation on invasion fronts? GxE places constraints that might maintain variance, right? Foucaud et al.: We added this reference and indicated that eco-evolutionary dynamics of spreading populations may also be constrained due to the balance between selective and stochastic processes (l.11-13 p.29).

whole-genome scans to compare the genomic regions showing signals of selection associated with invasive natural populations to the genomic regions showing signals of selection in our experimental lines. I think this may have been done already for damselflies and trees (Therry / Rieseberg); if so, it might be worth linking to that work here. Foucaud et al.: We found some interesting references (such as Swaegers et al. (2015) Neutral and adaptive genomic signatures of rapid poleward range expansion or Hodgins et al. (2015) Comparative genomics in the Asteraceae reveals little evidence for parallel evolutionary change in invasive taxa) corresponding to genomic studies of invasive species. However none of them compares the genome of invasive populations and the genome of lines selected in the lab. So we decided not to include these references.

Additional requirements of the managing board: Please ignore this message if you already took there requirements into consideration. As indicated in the 'How does it work?' section and in the code of conduct, please make sure that: -Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad (to pay) or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data. -Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused. -Details on experimental procedures are available to readers in the text or as appendices. -Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest with the content of this article." If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: "XXX is one of the PCI XXX recommenders."

Reviews 1) Reviewed by anonymous reviewer, 2020-01-09 16:53 I really enjoyed reading this manuscript.

The motivation for the study was clear, the study system seemed very appropriate. I don't have many detailed comments, rather, I hope my more general suggestions can be used to clarify and perhaps tighten areas of the paper that are a bit harder to understand. There were no line numbers so it was not easy to comment on smaller sections of the paper. I have not run Multiple Factor Analyses before and thus refrain from commenting on those analyses. Introduction Really nicely done here. My only comment is that I would have liked to have read predictions that were specific to the study system. The authors provide an excellent summary of how syndromes can evolve in general, but the reader does not get a lot of information (any?) on why syndromes in this particular species might evolve the way they do, and across multiple populations. Having explicit predictions here may help frame the Results section and help the reader understand why certain traits might be more likely to show a correlated response with the trait under selection. Foucaud et al.: Unfortunately, we still do not have definite explanation for this suite of evolutionary shifts occurring during the Harmonia invasion. However, we added in the new version of the ms three potential explanations that could assist the reader's understanding (l.14-22 p.5).

Methods Starting populations: Population 1 was used for artificial selection on body size (after diapause), and Population 2 was used for artificial selection on time to first reproduction (with no diapause stage). It's possible (but maybe unlikely?) that using two different populations (and two different diapause conditions) contributed to the difference in outcome of selection on these two traits. I would have liked 1-2 sentences in the Methods or Discussion that convinced the reader not to worry about these differences in starting populations. Foucaud et al.: In the first version of the ms, we were not making it clear enough that (i) both populations underwent diapause (only the duration differed) and (ii) rate of mortality during diapause was largely similar between the 2013 and 2015 populations. We thus hypothesize that less cold-tolerant individuals failed to survive diapause in both cases. We added a sentence on the ms to clarify this point (l. 25-27 p.8). Most importantly, the additional analyses asked by the recommenders (cf. those using the difference between selected lines and the means of our controls lines, see below) convincingly show that the difference in outcome of selection on the two traits are unlikely to come from the fact that the two selection schemes are initiated from two different populations. See above our response to the recommenders above.

Divergent selection on female body mass: toward the bottom of pg 8: should this be the 25% lightest females & 25% heaviest females? Or did you mean 25% lighter/heavier than the average? I also didn't understand ">70 clutches per line". Did the 70 clutches come from 50 females – does that means that some females contributed multiple clutches? To the next generation Also "33 boxes": This is the first mention of a 'box' and it's unclear what a box is here. Foucaud et al.: These comments have also been made by the Recommenders and led to substantial clarification of this part of the ms (l.1-15 p.11) and two new Supplementary Figures (see below for details). Briefly, females had the opportunity to lay multiple clutches but (i) our use of dense crumpled paper, (ii) the usually large fecundities displayed during selection, (iii) the distribution of collected clutches in actual small size boxes (hatching boxes) and (iv) the collection of larvae in as many hatching boxes as possible have limited a putative unbalanced contribution of individual females in our selection lines.

Pg 11 – Large paragraph – I found this paragraph to be the most confusing of the whole paper. I don't have good suggestions for how to simplify this section, but perhaps adding a figure with the overall selection design would help. Foucaud et al.: We added a new supplementary figure to help readers go more smoothly through this inevitably large paragraph (Supplementary Figure 3 of the present version of the ms).

Results Direct responses to experimental selection – paragraph 2, last line – there's a word missing somewhere in there. Correlated responses to experimental selection – no issues here Foucaud et al.: Thanks, the word "reproduction" was missing. We added l.22 p.16.

Multivariate response to selection & Environmental context and trait expression – I read these sections but was not particularly engaged with them because I did not have a good sense for what to expect. My mundane summary was that different traits responded in different ways and sometimes it depended on the food type, but I think there's a more meaningful point of these sections that could be brought out more. Foucaud et al.: We are convinced that these sections were necessary to stress out two points that might be missed from multiple single trait analyses. Multivariate analyses basically gave us access to how variance covariance structure of the traits involved in the syndrome is affected by selection and environmental context. The expectations were set in the introduction paragraph (l.25-27 p.5 & l.1-18 p.6). In brief, in the scenarios where traits are linked by constraints and where selection on one trait results in indirect selection on the others, we expect the variance covariance structure to be robust to selection. In contrast, in the scenario where the syndrome results from multiple independent selection pressures, we expect that the variance covariance structure will strongly be affected by selection with one trait responding to selection alone. Clearly none of our experiments follows the former prediction. In both of them the relationships between traits are affected. In FBM one trait changes and the others not much. In the AFR a set of traits changes together leading to profound rearrangement of the correlations between traits. We have completely rewritten the result section on this part (l. X-X p.18-20 and p.22). About the second point (environment), we wanted to explore whether our conclusions would be robust to environmental variation, hence our stressful experimental treatment. We could demonstrate that environmental context interacts with selection resulting in particular phenotypic syndromes.

Discussion Pg 20 – end of the middle paragraph – ends with “we show selection on age at first reproduction drove the evolution of female body mass, but that the reverse was not true”. This is a really interesting result and I wanted to know more about why this might happen, and whether other studies have reported similar results. Similarly, I would have benefitted from a stronger link between the ‘syndrome’ results and other studies that have also looked at suites of traits that have evolved (or not evolved) in response to range shifts or to environmental change. The Discussion is overall a bit light on citations. I finished the paper not having a good sense of how this study compares with the large body of literature on life history trait responses to selection. Foucaud et al.: Thank you for this useful comment. The recommenders made a similar comment. We now give more information about the fact that selection on body mass had no strong effects on other traits and we also added several key-references in the Discussion (see above).

2) Reviewed by anonymous reviewer Ladybug or ladybird???? Foucaud et al.: In North America the insect is primarily referred to as a ladybug whereas in British English, the insect is called ladybird. As in our previous studies we used ladybird, we replaced ladybug by ladybird all along the preprint.

General Comment about the Introduction: It is extremely lengthy. Try to write the relevant things which can help the readers to get the idea of the invasion syndrome. Give more examples of the ladybirds in which such studies have been reported. It will be good in terms of readers if you give the biological background of the experimental model. I didn’t find much info on the experimental species. It seems more like a review than the research article. I would also recommend English editing as at lots of places grammer was wrongly used. The theme o of the paper is missing in the Intro. Foucaud et al.: Works where invasion syndromes have been reported properly are very rare because documenting such syndromes represents a huge amount of work. So we are not aware of other studies on ladybirds reporting such a syndrome. Besides we use the biological model as a good model to test a general idea not as a good model to learn more about the biology of ladybirds so we are not particularly keen on developing on that point. As the 2 recommenders do not seem to share the same feeling about the introduction (“The introduction is also well written and well argued. The value of artificial selection experiments in disentangling contributions to ”invasion syndromes” is well made. The final paragraph sets out the study very nicely, and it is clearly a really interesting and worthwhile set of research questions”), we decided not to rewrite the introduction as suggested. Finally we have made further English editing all along the ms with our American co-author.

A fruitful approach to understanding the determinants of invasion success. Suggested re-wording: ... to understand... Foucaud et al.: This sentence is now removed.

Material and Methods Is it only one month or there is some range of months when beetles are available?? Foucaud et al.: Beetles are available all year long, though not in the same places. October is a particularly convenient month to collect them in Northern China because they are migrating to their diapausing sites and usually form huge migrating flights.

You mean first time when female laid eggs?? It should be more clear to the readers. Foucaud et al.: Exact, we now clarified what the age at first reproduction mean (l.19 p.8).

The methodology should be more clear as it is creating confusion to the readers. I would suggest a flowchart for the methodology so that it could be more clear.

Foucaud et al.: We agree and we added two new supplementary figures accordingly (sup fig 2 and 3).

What do you mean by ”multi-copulating”??I Foucaud et al.: It means that males and females can have many efficient copulations with the same or different partners during their lives. We added this clarification l.16-17 p.9.

Do the mating status of the new male same as that of female or each day female was given mating with unmated males? Foucaud et al.: Males were taken at random from base population 1 and could have already mate or not. In either case, H. axyridis males are always eager to mate with females (or other males).

“Age at first reproduction of females was recorded as the number of days between hatching as an adult, and the first clutch of eggs”. Not clear Foucaud et al.: We reworded this sentence l.20-21 p.9.

Why only for 8 generations? Foucaud et al.: Eight generations represent a balance between a sufficiently long experiment to observe the studied signal and limited time and man-power resources (i.e., almost a year, with 2800 females weighted at each generation).

What does this mean??? Do the egg laying observe up to 19 days???? Foucaud et al.: As mentioned in the ms (l.1-4 p.12), 19-days old females were allowed to lay eggs for 24h.

When possible, couples were formed immediately after weighing (or the next day at the latest), by putting [keeping] one male and one female in… Suggested re-wording. Foucaud et al.: Done (l.19 p.13).

Couples [Females] were checked daily for eggs Foucaud et al.: Done (l.23 p.13).

“For both selection experiments, two MFA dimensions had eigenvalues greater than one, hence explaining more than any given phenotypic trait alone”. What does this mean? Foucaud et al.: In brief, an eigenvalue of one indicate a composite variable which as the same informative value as any given observed variable (see Le et al. 2008 for more information for the MFA method).

… “$p = 0.011$). See Figure 5D for an illustration using plots of replicate selection lines, where selected and control lines are separated on dimension 2. In both experiments, male and female survival rates were significantly and positively correlated with dimension 2 (all $\rho > 0.71$, all $p < 0.001$….”. Either give the exact P value or write P…..Don’t mix and write. Foucaud et al.: This writing is standard practice in scientific literature; we hence preferred to ignore that remark.

Try to give exact results of the study. Don’t make it unnecessarily lengthy. Foucaud et al.: In line with the remark of Reviewer 2, this paragraph includes only 2 sentences on MFA, 2 sentences on correlation matrices and 2 sentences on radar plots. All sentences had the same goal of explaining that these different types of visualization of correlations between variables all indicate that individuals from our 2 selection experiments displayed different phenotypic correlation structures.

Don’t use such terms, you can’t expect the results. It is a blind study, right? Foucaud et al.: We removed this term l.4 p.21 and l.1 p.22.

“with reproduction delayed for all lines under food stress, whether selected to be heavy or light (approx. +3 days)”. Give the+/-values or exact number of days

Foucaud et al.: Done (l.8-9 p.21).

“$|\rho| > 0.78$”. What this symbol means???? Either give exact values or >. Avoid mix writing. Foucaud et al.: ρ is the standard symbol for correlation. We do not understand the second comment as we are indeed using >.

« in radar plot illustrations and correlation matrices (Figure 6 and 7, Supplementary figures 2 and 3, top vs. bottom rows)”. ????? Foucaud et al.: We did not quite understand this question. Sorry about that. We thus ignored that comment.

I read half of the discussion and I really found it lengthy and not up to the mark which made me stop reading further. Discussion seems to be like result section written in elaboration. You have to discuss your work here. Giving only references will not work. Discuss the work in detail. Foucaud et al.: We admit that the discussion is long. The reason is that the paper synthesizes the results of two experiments each with many traits measured in two environmental conditions. So unlike simpler papers, the discussion has to make an extended integrate summary of the results. We also think that because it is one of the very first papers that addresses the question of an invasion syndrome, it is utterly important to link the results to a more general evolutionary ecology context. As the other evaluators of the manuscripts have not shared this criticism we have decided to keep the discussion as it was.

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Decision by **Inês Fragata** and **Ben Phillips**, posted 20 January 2020

Revision round #1

Dear Authors,

Your work has been assessed by two reviewers and both the recommenders of this preprint. Whereas we all agree that the work is very interesting and clearly has an added value to the study of invasive species, there is some (minor) changes that would improve the current manuscript. Below you will find the general comments from both the recommenders in addition to those of the reviewers. The comments of reviewer 1 were very positive and you should focus on them improve the clarity of the manuscript. You should focus more on reviewer's 2 recommendation of a (supplementary?) figure explaining the experimental design to make it even clearer. In general, both recommenders feel that the english and structure of the paper are good, and the comments provided below are mostly to improve the already good readability of the manuscript and the link to other areas in life-history evolution. General comments

This was a really interesting piece of work. Well-conceived, well executed, and well reported. I see no major flaws, but have seen a few areas where the communication can be improved. Also, I think there are some important links to be made to broader interest in life-history evolution under strong selection; interest that reaches beyond the special case of life history evolution in invasive species. The work deserves and will gain greater attention if you link to this broader literature as well.

Additionally, there is just one methodological problem that can be tackled quite easily (hopefully). Population 1 was exposed to 8 generations to the lab environment before start of the divergent selection, however population 2 only was exposed to 2 generations. Since your populations were not lab adapted in the beginning, this may mean that your population 2 may be adapting to the lab environment in addition to your selected conditions. Moreover, since they are taken from different seasons and years this might lead to some variability in your analyses (which is also referred by reviewer 1). One possible way to mitigate possible problems that may arise is for you to redo the analyses using as data the difference between selected lines and controls. This way you reduce the signal of possible lab adaptation and reduce some of the differences present due to variation between populations. Specific comments Introduction

The abstract is very nicely written. The introduction is also well written and well argued. The value of artificial selection experiments in disentangling contributions to "invasion syndromes" is well made. The final paragraph sets out the study very nicely, and it is clearly a really interesting and worthwhile set of research questions. Below are some minor suggestions for improvement.

> Congruent with this, comparative studies of biological invasions have revealed that, while the ecological principles and evolutionary forces underlying invasions can be similar across invasion cases, different species respond to these constraints through a variety of strategies resulting in the apparent idiosyncratic nature of invasions

Perhaps delete from "resulting in..."

> Most importantly, several authors have pointed out that the processes underlying invasion success act at the population level within so-called "invasive" species

Vague. Please clarify.

> Experiments comparing native to introduced populations or distinct introduced populations to each other show that phenotypic evolution is common.

There are a couple of review papers here that are relevant. Phillips et al *Ecology* 91:1617; and Chuang and Peterson *Global Change Biology* 22:494.

> Here, we define the set of traits that evolve in concert and contribute to the success of invasive populations as an 'invasion syndrome'.

This is fine: there is a set of trait differences that seem to consistently emerge in invasive populations of this species. In the following paragraph you talk about how such a syndrome might emerge: selection on multiple traits, genetic correlations, or both. Genetic correlations are self explanatory, but I think the introduction could be strengthened slightly by pointing out that, during invasions, we may well expect selection on multiple traits. This selection could happen either during the introduction phase (see *e.g.* Chapple et al *TREE* 27: 57; Blackburn and Duncan 2001 *J Biogeog* 28:927), or during the invasion phase (see the reviews mentioned previously). This point could be woven together with your point about historical selection (nearly all populations are spatially dynamic at some point, right?), and may also be a chance to touch on broader literature about other recent causes of selection on life history (e.g., fisheries, hunting, climate change, others..) Methods

> Adult H. axyridis individuals were sampled in the native area of the Jilin province, China

Is there any reason for this location? Is it a source location for any of the invasions?
Regarding collection and establishment of populations: is there any chance that selection during diapause (1500 -> 900 individuals, for example) could have influenced your results? Is there any chance that ongoing selection in captivity through generations 1-8 influenced your results? These are issues that are unavoidable, but I flag them here as issues worth pondering in the Discussion.

> Twenty-four families that mothered at least 10 females that survived to adulthood were kept for heritability estimation

Why not use all families for estimation of heritability? Also, given likely multiple paternity, presumably you are systematically underestimating broad sense heritability? Might be worth mentioning that here. More generally, perhaps the focus should be on estimating the magnitude of heritability rather than testing against a null of zero. If you simply make interval estimates of heritability (and decline to calculate p-values), power analysis become redundant. I note that both your estimates tunr out to be highly significant, so again power analysis seems redundant.

> chosen as follows to initiate the next generation: (i) for the control lines, 50 random females were chosen, (ii) for the light lines, the 25% lighter females were selected, and (iii) for the heavy lines, the 25% heavier females were selected. For each line, 10 days after emergence, 50 males were randomly chosen and added to the 50 chosen females.

Do you mean 25% *heaviest/lightest*? Also, worth clarifying that the random males were chosen from *within the relevant line*. Also, worth pointing out earlier that selection is on female phenotype only, and explain why this is sensible.

> after emergence, >70 clutches per line were collected to establish the next generation. After hatching, a minimum of 33 boxes per line, each containing 12 larvae

Please clarify whether these clutches were stratified across females, and also what you mean by "boxes" (i.e. how boxes play into the design).

> Note that these lines included eggs from the earliest reproducing females as well as eggs from later reproducing females.

Is it possible that these also contain eggs from *offspring* of the earliest reproducing females?

One last thought worth considering is whether you should estimate narrow sense heritability from the selection experiment. You have a lot of data there, and you have the Breeder's equation (with S known) to lean on. Results

> Both selection experiments operated on the phenotypes of individual beetles as expected from heritability estimates.

This sentence does not make sense. The selection experiments were conducted on phenotypes of individual beetles and have nothing to do with heritability.

> age at first [reproduction] of the different lines

Typo.

Table 1: why the boldface on some lines/results?

Figures 1-4. Really nice figure panels.

For the figures maybe it would be go to change colors to be accessible to color blind people Discussion

> In contrast, control lines displayed no evolution of age at first reproduction and only a slight increase of female body mass compared to the G0 generation, indicating no [ongoing evolutionary response] to rearing conditions

Suggested re-wording.

> These results confirm several studies showing that in many species body mass or size and age at first reproduction are heritable and can rapidly evolve in artificial selection experiments

There is a recent resurgence of interest in the idea of selection on body size and correlated change in life history. It would be useful to link to that work. See, for example, recent papers by Malerba and Marshall. Really interesting that selection on time to reproduction (i.e. reproductive rate) had all sorts of correlated effects, whereas selection on body size appeared free of these outcomes. There are implications here beyond *Harmonia* and invasive species.

> additive genetic variance-covariance matrices (G-matrices) and therefore on future evolutionary trajectories

For more recent references on the evolution of G-matrices, see work by Blows et al.

> our results underline the importance that G × E interactions could have for the studies of biological invasions

Is it worth mentioning the recent article by Williams et al in TREE about maintenance of variation on invasion fronts? GxE places constraints that might maintain variance, right?

> whole-genome scans to compare the genomic regions showing signals of selection associated with invasive natural populations to the genomic regions showing signals of selection in our experimental lines.

I think this may have been done already for damselflies and trees (Therry / Rieseberg); if so, it might be worth linking to that work here.

Additional requirements of the managing board:

Please ignore this message if you already took there requirements into consideration.

As indicated in the 'How does it work?' section and in the code of conduct, please make sure that:

-Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad (to pay) or some other institutional repository. Data must be reusable, thus metadata or accompanying

text must carefully describe the data.
-Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused.
-Details on experimental procedures are available to readers in the text or as appendices.
-Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest with the content of this article." If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: "XXX is one of the PCI XXX recommenders."

Reviewed by anonymous reviewer 2, 09 January 2020

I really enjoyed reading this manuscript. The motivation for the study was clear, the study system seemed very appropriate. I don't have many detailed comments, rather, I hope my more general suggestions can be used to clarify and perhaps tighten areas of the paper that are a bit harder to understand. There were no line numbers so it was not easy to comment on smaller sections of the paper. I have not run Multiple Factor Analyses before and thus refrain from commenting on those analyses.

Introduction Really nicely done here. My only comment is that I would have liked to have read predictions that were specific to the study system. The authors provide an excellent summary of how syndromes can evolve in general, but the reader does not get a lot of information (any?) on why syndromes in this particular species might evolve the way they do, and across multiple populations. Having explicit predictions here may help frame the Results section and help the reader understand why certain traits might be more likely to show a correlated response with the trait under selection.

Methods Starting populations: Population 1 was used for artificial selection on body size (after diapause), and Population 2 was used for artificial selection on time to first reproduction (with no diapause stage). It's possible (but maybe unlikely?) that using two different populations (and two different diapause conditions) contributed to the difference in outcome of selection on these two traits. I would have liked 1-2 sentences in the Methods or Discussion that convinced the reader not to worry about these differences in starting populations.

Divergent selection on female body mass: toward the bottom of pg 8: should this be the 25% lightest females & 25% heaviest females? Or did you mean 25% lighter/heavier than the average? I also didn't understand ">70 clutches per line". Did the 70 clutches come from 50 females – does that means that some females contributed multiple clutches? To the next generation Also "33 boxes": This is the first mention of a 'box' and it's unclear what a box is here.

Pg 11 – Large paragraph – I found this paragraph to be the most confusing of the whole paper. I don't have good suggestions for how to simplify this section, but perhaps adding a figure with the overall selection design would help.

Results

Direct responses to experimental selection – paragraph 2, last line – there's a word missing somewhere in there. Correlated responses to experimental selection – no issues here

Multivariate response to selection & Environmental context and trait expression – I read these sections but was not particularly engaged with them because I did not have a good sense for what to expect. My mundane summary was that different traits responded in different ways and sometimes it depended on the food type, but I think there's a more meaningful point of these sections that could be brought out more.

Discussion Pg 20 – end of the middle paragraph – ends with "we show selection on age at first reproduction drove the evolution of female body mass, but that the reverse was not true". This is a really interesting result and I wanted to know more about why this might happen, and whether other studies have reported similar results. Similarly, I would have benefitted from a stronger link between the 'syndrome' results and other studies that have also looked at suites of traits that have evolved (or not evolved) in response to range shifts or to environmental change. The Discussion is overall a bit light on citations. I finished the paper not having a good sense of how this study compares with the large body of literature on life history trait responses to selection.

Reviewed by anonymous reviewer 1, 01 January 2020

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