Dear Editor,

We thank you and the two reviewers for your time and constructive comments on our manuscript and we are pleased to submit a new version. We tried to take all the comments into account and we modified our work according to suggestions except for the phylogenetic control as we explain below.

First, we have extensively rewritten the manuscript to tone down some of our conclusions, to be more careful in our conclusions and to discuss more clearly the limit of our results. We also discuss the additional possibility of introgression of *C. bursa-pastoris* by *C. orientalis* during range expansion as we recently proposed in another work. (Kryvokhyzha D, Salcedo A, Eriksson M, Duan T, Tawari N, Chen J, Guerrina M, Kreiner JM, Kent TV, Lagercrantz U, Stinchcombe JR, Glémin S, Wright SI, Lascoux M Parental legacy, demography, and introgression influenced the evolution of the two subgenomes of the tetraploid *Capsella bursa-pastoris* (Brassicaceae) bioRxiv **doi:** https://doi.org/10.1101/234096). We have also redone a bit differently some analyses and we now present the results in a slightly different way as proposed by the reviewers. Although we understand the suggestion of including a phylogenetic control, we did not follow it for the following reasons.

- First, in a strict statistical sense we don't have enough species replicates (only four species) and a proper test of the effect of mating system and ploidy effect would require at least few tens of species with different mating systems and ploidy levels. We are aware of this limit and we discuss it more clearly. But we also think that despite this important limitation our work is still interesting and participate to the step-by-step accumulation of results on the question we address.
- Second, there is an intrinsic difficulty of phylogentic control with allopolyploids because of a non-tree like evolution. We are aware of two recent works in this direction but they cannot handle generalized mixed linear models. (https://www.biorxiv.org/content/early/2017/09/28/194050, https://www.biorxiv.org/content/early/2015/08/05/023986

We answer in detail in the following. Note that we have updated the manuscript on bioArxiv (without tracked change). The track-changed version is provided with the re-submission (note that typo have been directly corrected).

We hope that this version will now be suitable for recommendation.

Sincerely,

Xuyue Yang, Martin Lascoux and Sylvain Glémin

Dear Sylvain,

Both reviewers and I found that your study addresses an interesting topic, and that the data and analysis you present are generally sound. However, the reviewers point to a few statistical inconsistencies and that your conclusions do not follow from the observations and should be tempered. I agree with the reviewers and think that your paper would be improved if you revise it in light of their suggestions.
I single out the major concerns that were raised. First, you could try to control for phylogenetic relatedness and geographic distribution. It appears that taking the data from this study together with that of the accompanying paper (Petrone-Mendoza et al.) is possible. Although such model would be unbalanced you might have some power to dismiss these factors; and even if the results are negative or ambiguous, they would strengthen the discussion. A second related concern is that both the appropriate trait scaling and testing for an interaction between rosette surface and treatment are missing. Including them would also facilitate the interpretation of results. In general, it is highly pertinent to clearly state possible life-history differences between species that would lead to a correlation between mating system and competitive ability independently of ploidy. You need to better explain why positive results in only one out of the three fitness traits measured is sufficient to support your conclusions. Lastly, I would argue that a causal connection deleterious recessives and mating system cannot be established with the comparative data you have. It would be interesting, for example, to perform mutagenesis and check for differential inbreeding depression.

With my best wishes, Henrique

Reviewer 1

Review of "Competitive ability of a tetraploid selfing species (Capsella bursa pastoris) across its expansion range and comparison with its sister species" by Yang et al.
December 9, 2017
This paper aims at testing the hypothesis that selfing species should show reduced competitivity relative to outcrossing species. This prediction is based on two possible mechanisms: either a trade-off between colonization ability and competitivity, or an increase of the mutation load in selfing species or populations. This prediction is tested using an experiment in controlled conditions involving four sister-species of the Capsella genus: three selfers (two diploid and one tetraploid) and one diploid outcrosser. The idea of the experiment is to compare the fitness of individuals of each species in the presence or absence of individuals of another species. The fitness is estimated through the measure of one vegetative trait (rosette surface at two successive time steps) and two reproductive traits (the number of flowers and the probability of flowering). The authors predict that the tetraploid selfing species should show a lower decrease in fitness than the selfing diploid species, because of partial masking of the effect of deleterious mutations. The authors claim that the main results are in agreement with the predictions.
We think that the addressed question is interesting and is worth investigating, that the experiments are well- suited with the aim of the study, that the statistical analysis are well-conducted and the results are convincing. However, we think that the interpretation of the results needs much improvements, tuning and clarifications. Indeed, the authors make strong statements based on limited evidence, and alternative explanations for the results could be further discussed. In particular, only one of three measured traits showed significant differences in the response to competition among species. One can argue that the authors are biased in their intepretations in order to find evidence that go in the same direction as their predictions. Indeed, one could interpret the same results as evidence against the actual predictions: since two of three fitness traits do not show significant differences, selfing has no effect on competitive ability. We thus suggest that the authors be more moderate in their interpretations and conclusions.

Major comments

Our major concern is about the conclusions of the authors regarding the agreement between their results and theoretical predictions. Throughout the discussion, the authors write sentences such as (e.g. p.14) "Our main findings are in agreement with theoretical predictions." Using the same results, one can conclude the opposite: most measured fitness traits are not in agreement with theoretical predictions. It seems to us that the authors draw strong conclusions that are only weakly supported. The paper would benefit from more balanced statements, interpretations and conclusions.

We agree with this comment and we have rewritten different parts of the manuscript in a more balanced way. Especially, we have added a section in the discussion to discuss the limit of the study. We think that this new presentation is now more in line with the results.

Another important concern is about the absence of control for phylogenetic relatedness between species or within species/between area. This is important to take into account because of possible pseudo-replication in the statistical analysis. This further weakens the interpretation of the results by the authors since the significant differences that are observed on only one trait could solely be due to phylogenetic relatedness. This is true both for between and within species comparisons.
A related point is whether the three selfing species evolved self-fertilization from a single or multiple independent events. This is important because if all selfing species are derived from a single ancestral selfing species, it would not be surprising that they share common features without giving good insights about the questions addressed in the paper.

As explained in the beginning of the letter, we did not correct for phylogenetic relatedness because we have too few species if we would like to consider a proper test with a species as a point, and because we are not aware of appropriate methods in case of polyploid species. Instead we preferred to be more explicit on the limits of our data set and results. In the introduction we also specified that selfing evolved independently in *C. rubella* and *C. orientalis* and that we don't know if *C. bursa-pastoris* inherited its mating from *C. orientalis* or if selfing evolved a third time.

Another important point is that the correlation between mating systems and competitive ability could be due to confounding factors. We would recommend to i) describe more precisely the differences and common features of the different studied species regarding life-history traits and strategies such as lifespan, dispersal, size of flowers and fruits, size of the vegetative parts, the stability and other characteristics of their natural environment;
ii) discuss possible alternative explanations for their results: for instance, their results could be explained by a trade-off between perenniality and competitive ability. More globally, the authors show an interpretation bias that is widespread in the literature on mating systems evolution, that is that the mating system is the most important driver for life-history traits evolution.

We agree that other life history traits could affect competitive ability. The *Capsella* genus is interesting in that respect because the four species are annual with very similar life form and ecology, except for mating system. We realized that we didn't specify that they were annual species. We now characterize the four species with more details.

We think that the way the authors investigate the effect of the rosette surface on the number of flowers is not entirely satisfactory. In our opinion, a better way would be to incorporate the rosette surface × treatment interaction into the statistical model. We can expect that this interaction will be not significant and in this case the authors would be able to more easily conclude that rosette surface does not explain the response to competition as measured by the number of flowers. As a consequence, Figure 5 would no longer be needed. Moreover, it is unclear why the rosette surface × treatment interaction is not included into models comparing species (Table 3).

In the first version we treated the two variables separately and then we added rosette surface as a covariable because of the apparent contradiction between rosette surface and flower number in Cbp. To simplify and homogenize the analysis we have added the rosette surface as a covariable also for among species comparison and included the interaction as suggested. For completeness we also have added interactions with block effects. As the interaction was not significant we have suppressed Fig 5 as suggested. Note however that the interaction is significant for the among-species dataset.

An alternative explanation about the intermediate results obtained for C. bursa-pastoris could be that this tetraploid species is an hybrid of two species, one outcrosser and one selfer, also tested in this study. Hence, this is not necessarily due to a masking effect of deleterious mutations. Their results do not allow for the disentanglement of the various potential mechanisms underlying the observed pattern. The authors should once again be more balanced in their interpretations and conclusions.

We agree and we have rewritten this part and added the possibility that C. bursa-pastoris has simply inherited characteristics from these two parents.

Minor comments

It is not clear whether the measurements were performed by a single or several experimentators. This should be indicated in order to eliminate the possibility of an experimentator-effect.

All measurements were performed by Xuyue Yang. We now mention it.

We suggest to merge figures 1 & 2, and 3 & 4 to gain space (results are quite clear on those figures, smaller figures would be enough).

Done. We also changed the way we present results as suggested by the other reviewer.

Introduction: "Polyploidy is often [...] formation of the polyploid species.". For readers not used to the literature on polyploidization, it is worth briefly explaining here the underlying mechanisms.

We have added a sentence to explain this claim.

M&M: Define more clearly what are S1 and S2.

Done

M&M: The authors refer to the interaction using both the "∗" and the "×" signs. For clarity, the notations should be unified.

We have checked the whole manuscript and used the sign "x" thoughtout.

Results: Use past or present tense, but not both. Results: "We first compared" instead of "First we compared".
Results, beginning of last paragraph : in the sentence "We then tested whether... among geographic areas", "differences" instead of "difference".
Discussion: "the outcrossing species C. grandiflora" (add "species") Discussion: "row values": What do the authors mean by "row"? or "raw"? Discussion: "without demographic consequences": prefer "with small demographic conequences"

Corrected. Thanks for checking.

Reviewer 2

Review of "Competitive ability of a tetraploid species across its expansion range and comparison withers sister species"
In this paper Yang et al compare fitness proxies of outcropping and diploid and tetraploid selfing capsella species with an without a competitor. The authors find that diploid selfing species have the greatest fitness reduction upon competition with an aster species. The authors find that both diploid selfing species have a proportionately greater reduction in fitness upon competition than the polyploids selfing species and the polyploid seller, which itself has a proportionally greater reduction in fitness upon competition than does the outcrosser. These results are interesting. That said I have numerous comments that would improve the paper

THE TITLE. This paper is written to about much more than the polyploid (C. bp), but this species is the focus of the title. I suggest the authors change this. Perhaps "Mating system, ploidy and range expansion shape the competitive ability of four Capsella species" would be more appropriate.

Thanks for this suggestion. We chose a more general title: "Variation in competitive ability with mating system, ploidy and range expansion in four *Capsella* species"

THE FIGURES.'slope-o-graphs' would be a better way to present all of the data, as opposed to paired box and whisker plots facetted by species (or geography). Additionally the Ic bar plots do not add too much (especially once the data are better presented) and could be removed.
Ic - I realize this has been presented in a separate manuscript, but ratios like this retouch for me to follow / comprehend for numerous reasons. First of all, they can be difficult to interoperate bc its not clear if results are driven by the numerator or denominator (e.g. rosette area in chinese c. bp). Second, such ratios are more naturally interpreted as logs, so i suggest log transformation.

We now present the data as suggested. We have merged Fig. 1 and 2. and Fig. 3 and 4 and we have removed the Ic plots. Instead we present the results as slope graphs. However we have kept the mean value of Ic for each case (just added on the top of each graph) as it is a simple way to have a quantitative view of the effect of competition.

STATS - On the whole the stats are well described and well justified. However, (as above) I am confused by the statistical approach taken to analyze Ic. It seems like this can be interpreted roughly as the interaction species X competition interaction term in the linear model (except as a ratio rather than a linear effect). As such it seems like this should be a post-hoc test rather than an independent one. Also I worry if these ratio data are normal (see above). That said, these are somewhat minor gripes.

We agree that the two analyses are partly redundant. As noticed above we also analyzed Ic as a response variable to be consistent with our first article where it was clearly justified to understand interactions. Here the design is simpler so we agree that the Ic analysis is less justified. We thus removed it and instead did contrast analysis on interactions (using the phia package in R). To use this we also needed to use the glmer.nb function instead of glmmadmb. As mentioned above we still give the point estimate of Ic as a quantitative summary of the effect of competition but tests are directly done on interactions as suggested. The results are qualitatively unchanged.

DIFFERENCES IN SURVIVORSHIP - On page 10 we are told 'The flower number distribution was bimodal with a mode at 0 and another around 1000 (see results). Thus it was analysed in two steps. First, we analysed the proportion of flowering plants with a binomial model and a logit link using the glmer function of the R package lme4 (Bates et al. 2015). Dead plants were included in the non-flowering category. Second, we excluded plants that did not flower and analysed flower number with a negative binomial model and a log link with the glmmadmb function of the glmmADMB package (Fournier et al. 2012; Skaug et al. 2013).' — However, I did not see this bimodal distribution presented, and did not see any results concerning survivorship. Did I somehow miss this?

Indeed the bimodal distribution was not shown. The proportion of dead and non-flowering plants was given in Table 1 but not on Figures because we used a log-scale. We have added the raw distributions in supplementary and we hope it's now clearer in the main text.

DIVERSITY's IMPACT ON THE RESPONSE TO COMPETITION - The authors find a strong positive relationship between sequence diversity and Ic across C. bp. However, as the authors' show this effect can be fully explained by geography, Because these pi values are not independent, I do not think this is a robust result, and I think it should be omitted from the manuscript. In it's place should be a section of the discussion noting that at the population level (n=3) the rank order of population diversity was the same as the rank order of Ic. This would be a more responsible and correct presentation of the results.

We agree. We suppressed Fig 6 and now just comment the ranking.

SCOPE OF EXPLANATION / RELEVANCE The results presented in this manuscript are exciting, and suggest an interesting potential effect of mating system, polyploidy and geographic position on how competition impacts fitness. However this is a very small sample size (4 species), so it might be best to emphasize that this suggests a pattern but many more experiments in other taxa are required.

We agree (see also our comment on phylogenetic control). We now discuss more the limit of our study and suggest more explicitly further directions. We have added a specific part in the discussion to present the limits of the study and the possible directions for improvement: "Limits of the study and further directions"

MECHANISM The authors propose alternative hypotheses to explain these patterns (basically adaptation vs drift). They do a responsible job of not committing to one of these alternatives bc the data do not currently allow it. However, it seems like a set of crossing experiments could untangle these. It would be nice if the authors discussed how future experiments could differentiate these hypotheses.

We have added discussion along this line in the new part we added (see above).

MINOR COMMENT On page 16, the authors state that "selection is expected to be softer when interspecific competition is low." I think the authors are making a mis-statement and that they meant to say "selection is expected to be LESS SEVERE when interspecific competition is low." I suggest staying away from the phrase 'soft selection' because this could be confused for its more technical meaning (which I don't think the authors intend). In fact, according to the technical term, I think that selection is probably best considered 'hard' in the absence of competitors and 'soft' in their absence.

Corrected.