Dear recommender,
We are pleased to submit a revised version of our manuscript uploaded on Bioarxiv under the same DOI: doi: 10.1101/273367. Thanks to the two anonymous reviewers, we clarified and corrected some points listed below, and we hope you will find this version improved. The raw data are now available at DOI 10.17605/OSF.IO/VNWHB.

Elsa Noël (on behalf of all coauthors)

Dear authors

The two reviewers have generally favorable opinions, but each makes substantive suggestions, which in my opinion the authors should address prior to sending a new version of the paper for recommendation.

In addition, the authors need to make the data available in a public format, either as supplemental material in the preprint or in a public data repository (e.g., Dryad).

Charles Baer

Reviews

Reviewed by anonymous reviewer, 2018-03-07 18:38

Both sexual selection and inbreeding can purge genetic load, but the relative impact of these processes has not previously been investigated, to my knowledge. Noel et al. experimentally evolved populations of simultaneous hermaphrodites under conditions that limited selection on male or female function, and the level of inbreeding. By using a hermaphroditic organism they avoided the confounding effect of sexual conflict that have caused problems in related experiments. They find evidence that sexual selection through the male function prevents the accumulation of genetic load, but that regular inbreeding can provide a similar benefit. These results provide a useful confirmation of the hypothesis that sexual selection purges deleterious genetic variation, and have interesting implications for the evolution of mating systems.
The authors report on a well-controlled and labor-intensive experiment, and describe their hypotheses and findings clearly, with appropriate statistical analyses. The Discussion is thoughtful and well-organized. As such, I only have minor comments.

1. The authors note that juvenile survival and inbreeding depression in the control populations did not change between the two experimental time points, suggesting that the initial population was at equilibrium, which is useful in interpreting the changes observed in other groups of lines. It is not clear whether the authors have any data on juvenile survival and inbreeding depression from the start of the experiment (time zero); if so this would be a valuable addition.

Unfortunately, we don't have any measurement of juvenile survival and inbreeding depression in our ancestral population, but Escobar et al (2008) obtained juvenile survival in first-generation laboratory outbred and inbred offspring in seven of the 10 natural populations that were used to create our ancestral population. From Escobar et al's source data, the average juvenile survival at 15 days was 0.544 +/- 0.054 (SE across populations) in outcrossed juveniles and 0.251+/- 0.038 in self-fertilized juveniles ; and the inbreeding depression was on average 0.524+/- 0.077. This is to be compared to the means of the control lines in G20, 0.545+/-0.022, 0.293+/-0.027 and 0.46+/-0.10 respectively (see Figure 1 and Table 3). Our conclusion is that we have no evidence for a significant change in all these traits during experimental evolution

in the control treatment. We have added these data to support our argument in Figure 1 and in lines 253-255 of the Results section.

Escobar, J.S., Nicot, A., and David, P. (2008). The different sources of variation in inbreeding depression, heterosis and outbreeding depression in a metapopulation of *Physa acuta*. Genetics *180*, 1593–1608.

2. To some extent the manuscript presents inbreeding and sexual selection as mutually-exclusive possibilities. The authors discuss the idea that alternating episodes of sexual selection and inbreeding could be highly effective in eliminating genetic load. Another way that these processes might both operate is if there is selection among gametes within selfing individuals. This might occur in plants, where genes are expressed in pollen. There is even reason to believe that a form of sexual selection among cells within yeast tetrads can purge mutations (e.g., Tazzyman et al. 2012, JEB). The authors could consider briefly noting these possibilities in the Discussion.

   Selection in the gametic (or gametophytic) phase is indeed a much understudied phenomenon, and the haploid expression of alleles, combined with the potential strong competition between male gametes (resp. gametophytes), provide opportunities for purging. This is all the more important that this type of purging may eliminate not only mutations on sperm- or pollen-specific traits, but also recessive lethals altering basic metabolic or cellular functions, and therefore could well have positive consequences on juvenile traits expressed in diploid individuals. Male gametes are probably more exposed than female ones to this selection because eggs before fertilization often depend on maternal proteins and RNA produced by the surrounding diploid cells rather than on the expression of their own genes. We added a sentence in the discussion and cited Tazzyman et al.'s model (l. 432-436). We however note that this type of selection may occur in all mating regimes, including not only self-fertilization but also monogamy (therefore it cannot explain the difference between S and F lines, but it may work in both). Thank you for raising this interesting point.

3. Some formatting corrections seem to be necessary in the figures and tables. In Figure 2 there are groups labeled "A1" and "A2", which I assume should be "S1" and "S2". In Tables 1 and 2 the P-values given in scientific notation seem to be missing multiplication symbols, e.g., "3 10-16".

   We corrected the figure with the appropriate labels S1 and S2 and added the multiplication symbols in the tables.

Reviewed by anonymous reviewer, 2018-03-07 18:39

This manuscript reports the temporal change in juvenile viability of several lines of a hermaphroditic snail differing in terms of sexual selection and inbreeding, with the overarching goal of testing the role of sexual selection vs. inbreeding in eliminating deleterious mutations, a question that has rarely been addressed. Overall this is a really nice study, with a careful experimental design and intriguing, original results.

My only relatively major suggestion is to try and provide more explicit theoretical expectations regarding the effects of sexual selection vs. inbreeding, in particular which types of mutations they are likely to purge most effectively. The prediction is clear for inbreeding (recessive mutations), but less so for sexual selection. For example, I think two extreme, caricatural

situations may produce the same observed results: (1) inbreeding and sexual selection purge the same single pool of mutations (~all mutations), such that juvenile survival stays the same in treatments C, M and S or (2) inbreeding and sexual selection purge different, non-overlapping pools of mutations that each contribute equivalently to the genetic load, with the same outcome in terms of relative juvenile survival (=identical for C, M and S, all lines having purged half of the load). These two contrasting situations would however differ in terms of the absolute value of the load, which should be higher in situation (2) where only half of the load is purged in lines C, M or S vs. (1), where pretty much all the load is purged in these lines. A comparison with a fifth treatment, selfing + outcrossing with mass-mating instead of monogamy, would have helped separating the two (note: I am saying this as a criticism of the experimental design, which is impressive enough as it is). I think it can be useful to be able to pinpoint which types of deleterious mutations can be purged by each process, as this would have consequences e.g. to discuss the benefits of mixed mating, but I may be wrong and I would very much appreciate the thoughts of the authors on this point. Obviously the authors are aware of the possibility that the two processes target different types of mutations (e.g. lines 392-418), and address part of this question via the study on recessive mutations/inbreeding depression, but it would be very helpful to introduce this possibility as soon as the introduction.

This lack of theoretical predictions is a real concern, but unfortunately, contrarily to deleterious mutations purged by inbreeding, which have been widely studied, there are no clear predictions on the dominance of deleterious mutations purged by sexual selection (SS).

- *Inbreeding enhances selection on a mutation if its homozygous effect on fitness is much higher than its heterozygous effect. We therefore expect mutations purged by inbreeding to have mild deleterious effects on juvenile survival in the heterozygous state, and much stronger effects in the homozygous state (i.e. they are at least partially recessive). After purging by inbreeding (S lines), the result is an increase in outbred and inbred juvenile survival, but the latter increases more, and inbreeding depression decreases.*
- *Sexual selection purges mutations that have large effects on male competitiveness, and their elimination will affect juvenile survival because of pleiotropy. These mutations are purged under outbreeding, therefore in their heterozygous state. We therefore expect mutations purged by sexual selection to have deleterious effects in heterozygous state on both juvenile survival and male reproduction in competitive conditions. There is no condition on their homozygous effect (and therefore on their recessivity) on any of the two traits. When purged by sexual selection (C and M lines), the effect is to increase outbred juvenile survival, with no particular prediction on inbreeding depression.*
- *Under the hypothesis that the mutations purged by inbreeding and by sexual selection are the same, they would have to combine strong heterozygous effects on male reproduction and partially recessive deleterious effect on juvenile survival. We would then expect C=M=S>F in terms of outbred juvenile survival (which is approximately verified), and C=M=S in terms of inbreeding depression, which is not verified, as we observed lower inbreeding depression in S lines than in C and M. The inbreeding depression in the F lines is hard to predict as the mutations may increase in frequency and eventually approach fixation, so that even if they are very recessive, they will not generate lots of inbreeding depression.*
- *If the two categories of mutations were different (i.e. if alleles that affect male reproduction had pleiotropic, but non recessive effects on juvenile survival), we would expect C=M>S in terms of inbreeding depression (verified), F<C=M and F<S in terms of*

outbred fitness; the outbred fitness of C and M could be higher or lower than that of S depending on how many (and how strong) mutations are in each category.

- *Mutations purged by inbreeding and sexual selection could also partially overlap, but in practice it appears impossible to tell to what extent – except it is not 100%. Patterns of outbred juvenile survival and inbreeding depression suggest that mutations purged in S lines by inbreeding are on average more recessive than those purged in C and M lines by sexual selection. They could be two entirely distinct sets of mutations, or a single common pool, within which a subset is more recessive and sensitive to inbreeding, and another subset is less recessive and more sensitive to sexual selection.*
- *Thus, what we can reasonably infer from the data is that the mutations purged by sexual selection are not exactly the same as those purged by inbreeding. However, the degree of overlap between the mutations purged by sexual selection and inbreeding cannot be precisely determined and depends on the dominance of pleiotropic effects of mutations on different traits (juvenile survival, male reproduction). As noted by the referee, the response to a mixed regime with both selection (on survival) and sexual selection would be worth studying in this respect, as well as the response of male reproduction in the existing lines.*

*We tried to incorporate a short version of these arguments in the Introduction (*lines 85-92*), thus preparing the reader to the discussion on this theme. We also mentioned in the Discussion (*lines 422-436*) the open problem of the diversity of mutation types targeted by different mechanisms of purging and directions for future research.*

On a related note, the authors mostly distinguish two types of deleterious mutations (highly recessive vs. partially recessive, although they hint at other architectures for the load in the discussion); however, a number of sexually-selected traits are likely to be quantitative characters, for which mutations are not unconditionally deleterious. Instead, their effect on fitness depends, among other things, on the genotype at other loci controlling the character. Do the author have any expectations on how polymorphism at QTL may be impacted by sexual selection and inbreeding?

There are no specific models, as far as we know, on the consequences of sexual selection on QTL polymorphism in a sexually selected trait. However, the dominant hypothesis (which is coherent with the purging effect of sexual selection) is that sexually selected traits owe their genetic variance mostly to condition-dependence and the capture of genetic variance that occurs at many loci affecting general vigour, including nonsexual fitness traits such as survival (Rowe and Houle, 1996). Under this hypothesis, the maintenance of variation at sexually selected traits, in spite of very strong directional selection, would be due to the constant renewal of deleterious mutations, rather than to variation at QTLs of the sexual trait itself (i.e. QTLs that would affect how the trait is expressed for a given number of mutations). A clear formulation of this idea can be found in Houle and Kondrashov's model of male sexual ornament evolution (Houle & Kondrashov, 2002). These authors combined a deleterious load model based on mutation-selection at the genome scale (similar to classical previous models by Kondrashov, each individual is characterized by the number of deleterious mutations it carries in his genome), and a quantitative-genetic model for the reaction norm that determines the expression of the ornament as a linear function of the individual load. At a given time, the genetic variance for the ornament within a population emerges from the variation in the load,

rather than from the variation in the reaction norm, because the whole genome is assumed to be a bigger mutational target than the set of genes that code for the reaction norm.

Although this is only a theoretical model without direct application to QTLs, it reflects the general logic of the condition-dependent sexual selection argument and its role in purging. Following this logic, we expect that the evolution under relaxed sexual selection will primarily concern the deleterious load (relative accumulation of mutations) and only secondarily the condition-dependent reaction norm of the sexual traits (and male reproductive success). For example, we would expect:

- *our F lines to accumulate load, and **as a consequence** to exhibit lower male reproductive success under competitive conditions;*
- *the S lines that are purged by inbreeding to avoid mutation accumulation, and **as a consequence** to preserve male reproductive success (when put in competition) although selection on that component was stopped in the S regime.*

If relaxed sexual selection was also affecting the reaction norm itself, we would observe a decreased male reproductive success in the S lines despite their purged load.
Unfortunately, we don't have data on male reproductive success at the 50th generation in S lines, but the results we obtained in the 20th generation (Noël et al 2016) were that, at least in the outcrossed condition, individuals from S lines had a male RS comparable to controls (suggesting that the reaction norm itself had not yet evolved at that time). We are planning more experiments to look at later generations.

We have tried to clarify these aspects in lines 415-420.

Rowe, L. and Houle, D., 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B*, *263*(1375), pp.1415-1421.

Houle, D. and Kondrashov, A.S., 2002. Coevolution of costly mate choice and condition-dependent display of good genes. *Proceedings of the Royal Society of London B: Biological Sciences*, *269*(1486), pp.97-104.

My frustration of not being able to single out easily the expectations may come from the fact that I am not a specialist of sexual selection, but that may be true of many future readers of the manuscript, and I think it would be helpful if the authors can improve this aspect.

We do also feel a form of frustration... There are still many unknowns both in theoretical terms (e.g., QTL distributions) and in how far we can understand the genetic architecture behind the responses we observed. At the same time, we obtained novel findings that bring answers to important questions about genetic purging, sexual selection and inbreeding, and we are impatient to have the theory and data required to explore all the consequences. Frustration and excitement are one and the same, to some extent, the good news is that some of the required information is accessible through feasible experiments in the future. We believe that the corrections we made in response to the points raised above have improved the logical flux of the paper and that these reviews helped us a lot to make the paper more exciting and accessible to the general reader.

A few minor additional comments:

Lines 123-124: this sentence (“occurs both to gain mates and among stored sperm…”) is not clear

Corrected, lines 131-133

Line 229: inbreeding depression is a measure of the effects of inbreeding on fitness. I would therefore replace “sensitivity to inbreeding depression” (which makes no sense) with either “levels of inbreeding depression” or “sensitivity to inbreeding”

Done, line 238

Line 230: I would re-order the sentence to read “was detected between G20 and G50 on average over all lines” (otherwise one may interpret the sentence as no differences among lines)

Done, line 239-240

Figure 2: replace “A1” and “A2” with “S1” and “S2”

Done

Note: I could not find the information that the data are available on an open online repository. This should be added, as I understand this is mandatory for a recommendation in PCI

The raw data have been deposited in the Open Science Framework site. The doi is now given line 491.