

*Dear recommender,*

*Thank you for taking the time to consider our manuscript. We appreciated the comments by both reviewers and did our best to take them into account. In addition, we included new results in the manuscript regarding the effect of inbreeding depression affecting fecundity. We now think that our paper is much improved, and would like you to reconsider it for recommendation in PCI.*

*Please find our detailed answers below.*

*Best regards,*

*Thomas Lesaffre and Sylvain Billiard*

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## **Decision**

by Thomas Bataillon, 2018-10-29 14:35

Manuscript: <https://doi.org/10.1101/420877>

### **Revisions needed for your MS "The joint evolution of lifespan and self-fertilisation"**

I am acting as recommender for PCI Evol Biol for your manuscript "The joint evolution of lifespan and self-fertilisation" Authors: Thomas Lesaffre, Sylvain Billiard <https://doi.org/10.1101/420877> version 1 - <https://www.biorxiv.org/content/early/2018/09/18/420877> for recommendation at PCI Evol Biol.

Your manuscript has now been evaluated by two referees. Both have expertise in theoretical population genetics and an interest for mating system evolution. Both reviewers (see detailed reviews) and myself agree that your manuscript has potential to be an interesting contribution that is suitable for being recommended at PCI Evolutionary Biology. But we all feel that before this can happen, fairly extensive revisions should be made.

Here is a synthetic list of the major points that need imperatively to be addressed:

\* Reviewer 1 has a number of fairly precise requests (labelled 1 and 2 in the review). These have to do with the presentation of the main results as well as request for clarifying the derivation of the theory results.

***We appreciated the comments made by Reviewer #1, we corrected our manuscript accordingly (see details below).***

\* Reviewer 2 has a series of more general comments that are worth considering while revising your manuscript. One important one (labelled comment 2) is how to compare the propensity to evolve selfing while "holding lifetime levels of inbreeding depression constant". I think that is an important point that together with the points raised by reviewer 1 merits to be addressed in quite a bit of depth.

\* Point 4 should be considered at least in the discussion.

\* Point 5 is relatively minor but the rev2 has a point regarding what is empirically known on the genetic basis of inbreeding depression.

***We tried to take into account the comments made by Reviewer #2, especially in the introduction and in discussion. Concerning comment 2, we are aware that assuming fixed inbreeding depression is a strong assumption. However, relaxing this assumption would require a multiloci modifier approach which is far beyond the aim of this manuscript. Furthermore, to our knowledge, multiloci modifier approaches with overlapping generations do not yet exist.***

I very much hope that you can revise your MS to take into account these points.

Best Regards Thomas Bataillon

## Reviews

*Reviewed by anonymous reviewer, 2018-09-19 15:51*

Self-fertilising organisms are generally short-lived in nature, while outcrossers are likelier to be long-lived. Previous theoretical research has investigated why this arises, while assuming a fixed lifespan. Here, selfing is disadvantageous in perennials due to the reduced need for reproductive assurance, and the unmasking of inbreeding depression. In this paper Lesaffre and Billiard use multi-locus models to investigate mating systems evolution if lifespan itself can jointly evolve with lifespan. Under this scenario, selfing causes evolution of shorter lifespans in order to maximise reproduction; yet this phenomenon can exacerbate the impact of inbreeding depression, so selfing is less likely to survive in the long-term.

The novel mathematical arguments are nicely laid out, outlining how the joint evolution of mating systems with other phenomena (in this case, lifespan) can alter expectations of how reproductive strategies can be maintained. The theory seems to be generally correct. That said, I feel a bit more work needs to be done in order to fully clarify and support the main claims in the manuscript.

**(1) Clearer presentation of main results.** The most important finding is that the joint evolution of mating system and lifespan should further select against self-fertilisation compared to a scenario where lifespan is fixed. Yet it's hard to understand this result from Figure 2, which only outlines the steady-state lifespan for different values of inbreeding depression. There is not a figure to show, for example, how the same ID parameters affect the steady-state mating system when lifespan is fixed.

At the very least, this figure needs updating (or a new figure added) to fully demonstrate how the steady-state mating system is affected by evolving lifespan. Such a figure, as well as Figure 2 if it remains, should also include the exact numerical results so the reader is confident that the approximations accurately capture the behaviour of the system.

Figure 2 is also hard to read: the text on each plot is very small and not easy to read. The font sizes should be made bigger. In the legend, it states that "The solid line corresponds to the threshold inbreeding depression condition below which selfing evolves". I presume that the 'threshold inbreeding depression' refers to thresholds for both juvenile and adult inbreeding depression? If so this point should be made clearer.

***In order to better present our results, we completely changed our figures. Figure 2 has been replaced by two new figures. One figure illustrates the three kinds of evolutionary trajectories that may be followed by the coevolution of reproductive effort and the selfing rate. The second figure summarizes the evolutionary outcomes of this coevolution. We modified the main text accordingly. Furthermore, we added new results regarding the effect of inbreeding depression affecting fecundity and added another figure in this section.***

**(2) Further details of the mathematical derivations.** The mathematical arguments seem broadly correct, but I found at least one possible error, and a few cases where more information could be provided to aid mathematical readers in understanding the derivations used.

***We thank Reviewer #1 for pointing out these typos, which are now corrected. We also agree with Reviewer #1 on the fact that more details are needed in the derivations of the results for the evolution of reproductive effort. We made substantial changes to the Appendix II in an attempt to make it clearer.***

• For the equilibrium proportion of selfed individuals  $\Theta^*$  (Equation 1 and A5), I obtain a different solution; I believe the denominator should be  $\alpha(1 - \delta_j)(1 - S_0(e)) + (1 - \alpha)(1 - S_0(e)(1 - \delta_a))$ . I'm unsure if this error is solely typographical, or whether it permeates throughout the rest of their derivations. I suspect the former, since Equation A8 holds for  $S_0(e) = 0$  if the denominator I obtained is used. The authors should carefully check their other equations for errors.

***This error was indeed purely typographical. We corrected it and checked our equations for errors.***

• It would be good to mention somewhere that  $E(\zeta_m) = 0$ .

***Agreed. We added this in Equation A10 (P24).***

• It's unclear how the linkage disequilibrium measurements across life stages (i.e. A17 and A23) are obtained using the multi-locus framework. Does it require defining and working with stage-specific indicator variables (e.g.  $\zeta_{jm} = X_{jm} - p_{jm}$ , and similar for the maternal chromosome)?

***Since we consider single locus models, the only linkage measurement is  $D_{\{m,m\}}$ , which depicts the excess in homozygotes at the modifier locus. To compute its variation across life stages, one does have to use stage-specific indicator variables, since selection affects it differently in juveniles and in adults, and since syngamy only occurs in juveniles. In an attempt to make derivations clearer, we substantially modified Appendix II. We hope it makes this part clearer. We also appended the main text for clarity.***

• How is  $D_{m,m}$  obtained? It seems that one invokes 'Quasi-linkage equilibrium' assumptions; that is the selective forces ( $\epsilon$  and  $p_m$ ) are weak so  $D_{m,m} = D'_{m,m} = D_{m,m}$ . However, I could not recover A25 when using these assumptions. Please provide further information on this derivation.

***We updated Appendix II and the main text to this end. Obtaining  $D_{\{m,m\}}^*$  involves solving  $D_{\{m,m\}}^{\prime} - D_{\{m,m\}} = 0$ , assuming homozygosity at the modifier quickly equilibrates in comparison with allelic frequencies under weak selection.***

• Does the derivation of A26 require  $D^*_{m,m}$  (A25)?

*$D_{\{m,m\}^*}$  is not required since it only appears in the first term in Equation A28, which is always positive. However, one has to calculate it to make sure that it remains positive under weak selection. We updated Appendix II for clarity.*

**(3) Minor issues (P = page number, L = line number)**

- P1, abstract: ‘for instance’ is not needed here and could be deleted.

*Deleted.*

- P4 L73: ‘anterior’ does not make sense in this context. Best to use ‘previous’ instead.

*Corrected P4 L74.*

- P6 L109: ‘overlapping generations’ reads better than ‘generations overlap’.

*Corrected P6 L117.*

- P6 L115: ‘no approximations’ instead of ‘no approximation made’.

*Corrected P6 L123.*

- P7 L126: Would be good to point out that  $\varepsilon \ll 1$ .

*Added P7 L146.*

- P7 Eq 2: It would be nice to briefly explain why this function form of  $S_0(\varepsilon)$  is used (I presume because it’s rather flexible?).

*We added a short sentence to state this. We chose this function because it allows to consider a variety of trade-off shapes, and because it was the one used by Zhang (2000).*

- P8 L158: ‘In addition’ would be a better phrase to use than ‘Besides’.

*Corrected P9 L179.*

- P15 L316: Please delete the ellipsis (...).

*Deleted.*

Reviewed by anonymous reviewer, 2018-09-19 16:17

Review of: The joint evolution of lifespan and self-fertilisation

The authors develop evolutionary models of the evolution of the selfing rate and plant life-history. First looking at the evolution of the lifespan, then the mating system, then both. The results are straightforward - as modeled, the extent of inbreeding depression is (much) higher for perennials than annuals, because the costs of inbreeding depression are approximately multiplicative with lifespan. Because inbreeding depression increases with lifespan, they find that perennials should be outcrossing. Similarly, because selfers can trade their later life inbreeding depression for more success early in life, they selfers should evolve to be annuals. The results are intuitive and well described, and the model predicts a well known observation. This seems like the beginning of a potentially interesting result!! However I do have some concerns. These concerns include (1) Whether then current simple model is useful without the complex realities of (2) The consistency of inbreeding depression across mating system, (3) The lack of senescence. I also wonder (4) if the model is necessary to explain the correlation found in nature.

Before discussing these issues, I note a mysterious omission from the results, that would help readers. Specifically, the major assumption (?or maybe result?) of this manuscript is that the realized inbreeding depression increases with longevity. However, this result was not stated particularly clearly. A simple graph plotting the lifetime inbreeding depression as a function of the survival reproduction trade-off (perhaps clearly delineating the classic  $\delta = 1/2$  threshold) could clarify this result.

***In the “life cycle” section of the methods, we added a paragraph to discuss this issue and make our assumptions more explicit.***

(1) Whether then current simple model is useful. While it is often for theorists to criticize “verbal models” the parameterization of the model (that perennials have a higher level of inbreeding depression, and the results follow simply from this assertion. Similarly selfers can reduce their genetic load by taking their substantial later life inbreeding depression for early life fitness (a classic result in senescence literature... see williams 1957).

While a simple model with easily predictable results can serve some purpose, this seems like more of an idea for a paper than a complete manuscript. I leave it to the authors and editor to decide whether the results here are sufficient for a brief note, or if they would only be useful as a portion of a more extensive manuscript. Perhaps one way to think about this is to ask if there were any surprising results that were not immediately expected by the authors. If the answer is “yes” they may want to share/highlight these.

***We are convinced that a formalized mathematical model, no matter how simple, is always useful to challenge verbal models. One result we did not expect was the existence of two possible ESS for the same inbreeding depression, and extrinsic mortality conditions, depending on the initial conditions. This new result is now highlighted by Figures 2-4.***

Another thing I'm having trouble thinking about is if the authors have shown that perennially prevents the evolution of selfing, or if high inbreeding depression precludes the evolution of selfing (which is well known), and then parameterize a model where lifetime inbreeding depression

increases with expected lifespan. An “apples to apples” comparison would fix the total expected lifetime inbreeding depression to not differ by life-history, and then see if life history & mating system themselves (independent of the increased inbreeding depression experienced in longer lived species that is asserted in this model) itself has any causative effect, or if the result is simply a consequence of higher effective inbreeding depression with lifespan. Of course, it is unclear if an apples to apples comparison is appropriate, or if the authors should just say “we’ve sensibly parameterized a model which shows that lifetime inbreeding depression is elevated in perennials”

***Reviewer #2 is correct when stating that it is not perennality per se that precludes the evolution of selfing, but rather the high lifetime inbreeding depression it generates through inbreeding depression affecting adult survival. We added a sentence at the beginning of the discussion to make this point clearer.***

(2) The consistency of inbreeding depression across mating system The authors note this assumption. It is quite hard to know what to do here. If increases in selfing rate severely impact inbreeding depression (by purging or fixing the load), then the results can be tempered (if inbreeding decreases inbreeding depression), or removed (if inbreeding fully purges the load). Alternatively, if habitual selfing results in outbreeding depression), or enhanced (if fertility/fitness increases with age) the results would be reversed.

However things get more complex - for example, in a community setting there is a major difference between a fixed load (in which this species could be replaced by another) and a purged load, while in population genetics models with stable  $n$ , there is no such difference. Thus while the model as set up is clearly wrong, it’s wrongness may better reflect true biology than a proper model.

Given these challenges, and the concern that without a deeper thought the paper is pretty thin/predictable, thinking hard about allowing the load to change with mating system could make this a more substantial contribution.

***We are aware that assuming fixed inbreeding depression is a strong simplifying assumption. Our goal was to study the coevolution of reproductive effort and selfing using the simplest possible model. In particular, we aimed at highlighting the effects of inbreeding depression affecting various stages of the life cycle. Of course, it would be extremely interesting to model the mutation load explicitly. However, this would require a multiloci modifier theoretical framework with overlapping generations that is not yet developed. In particular, from the theoretical standpoint, very little is known about the behavior of the mutation load in perennial populations, as we pinpoint in discussion.***

(3) The lack of senescence / stand decline.

As the authors introduce at the end of the manuscript (line 325) the models assume no senescence. Clearly including senescence would act to functionally decrease the effective inbreeding depression in maturity and make the proposed effect less severe (although the authors could, for example play with the functional form of senescence and inbreeding depression as well). Again adding some reality tooth’s manuscript would flash it out some (although its not clear that this would give results that aren’t predictable a-priori (as I just predicted them).

***We agree that it would be interesting to incorporate senescence in the current model, but it would deserve a paper on its own and it was not our aim here.***

(4) Is the model necessary to explain the correlation found in nature.

The work is motivated by the association between selfing and lifespan observed in nature. While the results of the model generate this correlation, it is unclear if the model results have anything to do with observations in nature. Above I discussed some of the shortcomings in the modeling. Here I ask if the model is necessary. Selfing plants often live in ephemeral habitats which directly change the probability of surviving to the end of season (“high extrinsic mortality”), and consequently there is no survival across years, even for perennials. This observation could be interpreted as consistent with this model OR it could be argued that these environments are the ones in which selfers thrive and/or can persist in the face of e.g. mate/pollinator limitation. And therefore the “predictions” of the model are generated by ecological factors unrelated to the predictions, rather than the genetic scenario modeled by the authors.

See the introduction of Emms et al 2018 (a paper of tangential relation to this idea), for some key references.

Another reason why it is unclear if this model is necessary to explain the observed association between mating system and an annual life history is noted by the authors - 'long-lived species may endure significantly more mitotic mutations throughout their lives owing to their overall larger stature, which could result in an increase in inbreeding depression as plants do not have a separated germline.' It is important to separate whether a model plausibly predicts some pattern (which this model does, at least as parametrized) and if the processes described in a model play a causative role in generating this pattern (which this may or may not do, even if the model is correctly parameterized).

***We agree with Reviewer #2 that one has to be extremely cautious to not attribute a causative nature to a simple correlation. It is a point we tackle in the discussion, when we discuss the potential confounding effect of perenniality on the observed correlation between mating systems and dispersal strategies. One role of models in evolutionary ecology is to propose potential mechanisms, and to serve a “proofs of concept” for new ideas. One contribution of our model is to provide expectations regarding the ecological and genetic conditions required for a given association between mating system and life expectancy to emerge. In particular, we show in which conditions it is possible to have long-lived selfers. This is new from the theoretical standpoint. We modified the discussion accordingly.***

(5) Additionally there are some deliberate misstatements / incorrect citations.

For example Charlesworth and Willis do not suggest “Inbreeding depression is generally thought to be caused by recessive mildly deleterious mutations segregating at low frequencies in populations”. In fact the point to evidence many rare large effect recessives (although they acknowledge that modest effect partial recessives may also play a role in inbreeding depression).

***Reviewer #2 is right. We corrected our manuscript.***