Dear Mr. Fernández-Pascual:

The above manuscript has now been evaluated by expert reviewers, as well as one of our Associate Editors. Based on their recommendations (included at the end of this e-mail), I regret to inform you that we are unable to publish your paper in Methods in Ecology and Evolution.

However, the Executive Editor thinks your paper is a strong candidate for publication in Ecology and Evolution, a peer-reviewed, open access, interdisciplinary journal providing rapid publication of high-quality research. To learn more about Ecology and Evolution and the article processing charges that apply, please go to https://mc.manuscriptcentral.com/ecologyandevolution. You do not need to revise or reformat your manuscript before transferring it. The Ecology and Evolution Editors may base their publication decision solely on existing reviews and Editor comments from the Methods in Ecology and Evolution, but may require additional peer review.

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Thank you for considering Methods in Ecology and Evolution for the publication of your research. I hope the outcome of this submission will not discourage you from submitting your work to us in the future.

Sincerely,
Dr. Jana Vamosi
Executive Editor, Methods in Ecology and Evolution coordinator@methodsinecologyandevolution.org

Associate Editor Comments to Author:
Associate Editor
Comments to the Author:
The tools presented here may improve the efficiency and repeatability of analyses of seed traits. Given the number of steps that require human examination and input, the description of the tool as "automated" seems unwarranted. **The tool and sample data would be better-implemented as a package to facilitate use.**

More seriously, however, are the statistical and practical concerns raised by both reviewers. The statistical justification for some of the approaches here are not clear. Reviewer 1 is not convinced that this tool is even necessary, although I do think that it could improve reproducibility of analyses. Both reviewers raise statistical concerns, and reviewer 1 in particular has a number of suggestions for more recent model methods.

While the tool described in this manuscript could contribute to scientific reproducibility, it is not now ready for publication. I wish you the best for its further development.

Reviewers Comments to Author:
Reviewer: 1

Comments to the Corresponding Author
Standardised measurement of seed functional traits: automated calculation of germination cardinal temperatures and thermal time using R

Ladouceur et al. Methods in Ecology and Evolution

Monday, March 27, 2017

Title: suggest delete “automated”

abstract, summary and Intro: suggest deleting “urgently”

Abstract: could “the best policy to” be changed to “guidelines for the”

Why limit this analysis to the 50% subpopulation? Subsequent to Garcia-Huidobro, there has been a lot of work done on population germination models that distribute thermal time estimates among different subpopulations, acknowledge variable cardinal temperatures, and even deal with curvilinearity in the optimal temperature range. Most of these models still retain a number of simplified assumptions regarding normality, linearity etc. and allow for greatly expanded inference beyond simple estimation of sub and supra-optimal linear relations of the median subpopulation. Indeed, focusing on a single subpopulation ignores important information about population germination syndromes that relate to germination spread around the mean. I am not sure that much time is saved over traditional methods if it is just a matter of picking sub and supra-optimal points and estimating the parameters of two linear regressions, which can also be done “automatically” in any spreadsheet program.

The printed page numbers at the bottom, and the pdf page numbers at the top are different. I refer to the pdf page numbers in my review.

Page 3, line 2. “underappreciated” and “powerful” both seem to be subject to the term “emerging”. This is kind of awkward. You could almost just change it to “... emerging as a powerful ...” and eliminate “underappreciated”, otherwise, you need to parse the sentence into previously underappreciated, but newly emerging ... .

Page 3, line 8. Standardization refers to the procedures for calculating traits and not the traits themselves, making this sentence a little awkward.

Page 3, line 10. Using R seems to apply to 2 and 3, but it would be the logic of the underlying procedure is what would seem to avoid bias. I would think that as long as the logic was clear, it wouldn’t really matter what tool you used to program the solution.

If you only tested this with two datasets, and one worked and one didn’t, then it would seem that your procedure has a 50/50 chance of working. If you are going to make claims about the utility of the program, shouldn’t you test it on more datasets, and wouldn’t you need to have more on the order of 90-95% success to justify using it?

Is it really your responsibility to introduce new users to R?

Page 4, line 10. “maximized” may be the wrong word to use. You might want to consider changing the wording to “ ... value of these would be optimized if there were a consistent ...”

Page 4 line 15. There are also hydrothermal models out there that are probably more relevant to ecological comparisons. Why not provide an R solution to the more complicated but more ecologically relevant HT models?

Page 5, line 6. There isn’t necessarily any particular evidence that climate change scenarios are going to produce more complex thermal environments. So far, most models show a relatively small shift in mean temperature, and perhaps more extreme (high and low) limits to variability, but day to day variability isn’t necessarily going to change, particularly during the times of year when water is available to the extent that thermal models and not hydrothermal models are expected to be predictive.

Page 5, line 12. I don’t see how R inherently removes bias given that a person had to create the algorithm that was programmed into R. Rather, this procedure just automates what the person programmed and any inherent bias of the programmer is going to still be manifest in the procedure.

In my experience, the main difficulty in simple thermal time analysis (single subpopulation; supra and sub-optimal linear regression) is in having enough thermal data points to have confidence in the regression, particularly at supra-optimal temperature where the drop off in rate can be precipitous and not adequately captured by a typical thermal treatment interval that us usually greater or equal to 5 degrees.

The methodology shouldn’t really read like an instruction manual for parameterizing the R program, but should identify the underlying equations that are subsequently coded in R.

Page 8, line 7. What do you mean by “poor”. I would think that the main issue is that your germination counts are done on a small enough time interval that you can accurately estimate 50% germination. Why not just do what most people do at this stage and interpolate between the germination count below 50% and the

one above 50%? If you have to plot a whole distribution just to estimate days to 50%, then you probably have a measurement interval that is too long.

How is optimization of the population distribution (Weibull, logistic, etc.) any different from optimizing the cumulative curve and estimating 50% (exponential, sigmoid, ...)?

Page 9, line 5. Your description of how the procedure identifies the breaking point is insufficient. This seems to be the key procedure given that once identified, the sub and supra-optimal linear regressions are pretty straight forward.

Page 9, line 14. In the abstract, you only mention the 50% subpopulation, but in the methods note a 10% subpopulation interval. Wouldn't it be a relatively minor step to also parameterize a Probit distribution of thermal times, or explicitly test important assumptions such as constancy of base temperature across subpopulations, or whether or not supra-optimal thermal times are also normally distributed, or are constant?

Page 9, line 19. Isn't post-calculation screening of "fit" going to also introduce bias unless there are some kind of objective criteria for throwing out data?

Page 10 line 13. typo on "degree-days"

Page 10, line 14. This is kind of confusing. If you only have sub or supra-optima data, then of what relevance is identifying the optimal germination temperature. Isn't our whole approach basically sub and supra-optimal linear regression and identification of optimal temperature as the intercept? The only thing that could be different from "traditional" analysis would be how you optimized the two curves in order to identify the break point, although I can think of a number of ways that an automated optimization procedure could bungle that estimate if there were outliers or errors in the identification of germination rate in the treatments just above or below optimal. Those can usually be identified through the process of subsampling within temperature treatments, or actually replicating thermal treatments and looking for the anomalous rate estimates at the individual treatment level. In general, I don't think that there is any real substitute for thoroughly plotting and visualizing the data up front before initiating an "automated" procedure. Often the errors found have nothing to do with "bias" but common problems with data entry errors when transposing information from a data sheet to the computer, recording data in the wrong line on the data sheet, miss-labelling or loading the wrong seed sample in a given petri dish.

Page 11, line 4. I agree that a generalized linear approach is also appropriate for estimating base water potential, but this concept is dropped in this paragraph out of the blue. If you aren't going to provide a more detailed procedure for assessing within-population variability in thermal time, or estimate hydrothermal parameters, you probably don't want to include this paragraph at all.

Page 11, line 17. I don't really see the need to tell people that if they want to characterize both sub and supra-optimal thermal relations that they need to make sure that their lab treatments cover both the sub and supra-optimal thermal range.

Page 12, line 4. I don't understand what you mean by constant temperatures yielding promising results for "testing" cardinal temperatures. There is a general assumption that for the seeds in question, thermal variability, per se, does not affect rate and that constant temperature-derived models can adequately predict variable temperature response, but the only way to test this assumption is to conduct variable-temperature experiments for comparison with model predictions.

I think this is exactly what we say in the next sentence so I have not changed it. Page 12 line 9. I would agree, but that would work only if both the high and low temperature regime were also entirely within either the sub or supra-optimal range.

Figure 3. The previous text implies that "segmentation" is confined to identification of sub and supra-optimal thermal ranges. Your figure shows more than one break point for species A.

I had this same thought after we revised the script for Maria. Figure 4 shows two linear regions and the optimal temperature intercept for specie A, but there was no discussion as to how the procedure partitioned or optimized sub and supra-optimal data points. Also, I don't see why figure 4 wouldn't suffice to also explain the data in Figure 3.

Why are there three segments for t10 in figure 3, species B, but only two in Figure 4?

In general, I think that it would maybe help the thermal-germination community to have a common procedure to identify the treatment threshold for parsing sub and supra-optimal data points, but you don't explain that procedure in your paper.

All of the rest of the paper is just using what are pretty well described procedures for using linear regression to identify cardinal temperature values for a single subpopulation.

Most people who are interested in population variability in thermal time parameters, however, don't calculate them subpopulation by subpopulation, but optimize a probit function to deal with within population variability.

The relative utility of any modelling approach, however, is in how well the models predict variable-temperature response, which is not really addressed here.

Although I think that some people would like to have an R program to facilitate this calculation, the only real utility of the described procedure would be in whatever iterative approximation procedure is used to remove bias from the identification of sub and supra-optimal thermal ranges.

You would also probably need to compare your procedure somehow to that derived from visual inspection of the breakpoint, and simple linear regression for the rest.

The vast majority of people doing thermal time studies aren't really generating so much data that they need an automated procedure, and any recommendation that they not visualize model vs data at every step is a mistake.

Review manuscript MEE-17-03-175
Standardised measurement of seed functional traits:..

The authors discuss a standardised statistical framework to estimate germination cardinal temperatures. The framework relies on a couple of statistical models, including nonlinear and segmented models.

The proposed model avoids subjective assessments to identify sub- or supra-optimal temperature ranges, and thus it could turn out quite useful for practitioners in applied Ecology. Some issues of mine are reported below. The manuscript is, in my opinion, worth publishing, however it could benefit greatly from a statistician that could be involved in the paper/project. Currently there are several mistakes and inaccuracies typical of people 'able' to using sophisticated statistical models but with no sound statistical background.

Major Points

- I apologizes for the probably silly question, but I am not familiar with such application. I am wondering what is the connection between step 2 and step 3a. It seems to me that step 3a (the most important) can be carried out with no information gained in step 2. The authors should discuss this point clearly.
- On page 6 lines 16-17, the authors write: 'The final germination..Figure 1, which needs to be inspected visually for evidence that the temperature treatments used in the experiment do represent the full germination temperature range'. Rather than relying on visual (subjective) inspection (that should be avoided as stressed by the authors) I am wondering if a statistical test could be used. The point is to test if the germination-temperature relationship is linear or piecewise linear (i.e. segmented). The package segmented should include functions to test for the existence of the breakpoint.
- In step 2 the authors use the function `drc` to fit dose response models. In the source file the authors define the function `CGfun` in turn calling, for instance

  ```
  m1 <- drm(G/PG ~ Time, data = x, type = "binomial", fct = LL.2())
  ```

  I see no `weights` argument is specified (which should be 25 in the example in the manuscript). Please make sure if such argument should be specified (I think so.., see `?drm`).
- Figure 3 is not exactly correct (and likewise object `BLfig` in the code file). In fact in some panels (t20, t60,...) the fit is not piecewise linear, and thus 2 breakpoints (erroneously) appear! The issue depends on the function `BLfun()`. In that function I suggest to replace the line

  ```
  data.frame(Treatment=unique(x)$Treatment, Rates=broken.line(seg)$fit)
  ```

  with

```
new.x<- sort(c(unique(x)$Treatment, seg$psi[,2]))
data.frame(Treatment=new.x,
Rates= predict(seg, newdata=data.frame(Treatment=new.x)))
```

- The proposed STEP 3b is to fit separate linear models in the sub- or supra-optimal germination temperature ranges. I am wondering if (and why) it is really necessary. I think it is not.
  For the panels where breakpoint has been estimated, the fits could be very similar to the fits from segmented models. Note *very similar* and not the *same*. In fact fitting two separate regression models for covariate values less or more than the breakpoint does not imply the intersection point *is* the breakpoint. Rather it will be different!
  The linear fits could be added in the appropriate panels of Figure 3. In other words, Figure 4 (and relevant code) could be removed or the authors should motivate it carefully.

Minor Points

- On page 5 line 5: 'keeping the columns, their order, and their column names'. i don't think the order is necessary, provided the names are correct.
- At line 43 in the source code file (i.e. 'supplementary_information_2.R'), name in read.table() is wrong (it misses underscores and a capital letter..)
- At lines 46 and 55 in the code file: there is no reason to print on the console the whole dataframe `dat1` or `dat1[,2]`. the same at line 172 (`GR`). I suggest to use `View` (e.g. `View(dat1)`) to have a look at the dataframes.
- Please explain the column names in the output files ('Table-S2-Germination-rates.txt' and 'Table_S3_Segmented_model.txt', 'Table S4 Linear models.txt')
- It seems the reference of the segmented package is wrong (on page 6, line 9: '(Vito, 2008)'. In fact by typing in R `?segmented`, I get
  Muggeo, V.M.R. (2003) Estimating regression models with unknown breakpoints. Statistics in Medicine 22, 30553071.
  Muggeo, V.M.R. (2008) Segmented: an R package to fit regression models with broken-line relationships. R News 8/1, 2025.
  The authors should include both (right) references in their manuscript.