



# Peer Community In Evolutionary Biology

## Transgenerational plasticity through three generations

**Troy Day** based on peer reviews by **Stewart Plaistow** and 1 anonymous reviewer

Juliette Tariel; Sandrine Plénet; Emilien Luquet (2019) Transgenerational plasticity of inducible defenses: combined effects of grand-parental, parental and current environments. Missing preprint\_server, ver. Missing article\_version, peer-reviewed and recommended by Peer Community in Evolutionary Biology.

<https://doi.org/10.1101/589945>

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Organisms very often display phenotypic plasticity, whereby the expression of trait (or suite of traits) changes in a consistent way as a function of some environmental variable. Sometimes this plastic response remains labile and so the trait continues to respond to the environment throughout an organism's life, but there are also many examples in which environmental conditions during a critical developmental window irreversibly set the stage for how a trait will be expressed later in life. Traditionally, most studies of phenotypic plasticity have considered how an organism's phenotype is altered by the environment that it experiences (called within-generation plasticity) but there is growing interest in how an organism's phenotype is altered by the environment experienced by its ancestors (called transgenerational plasticity) [1]. In the simplest cases an organism's phenotype might be affected by the environmental conditions experienced by its parents. There are several examples of this phenomenon as well, including interesting cases where predator cues experienced by an organism's parents dictate the extent to which it displays a defensive phenotype. Tariel et al. [2] present a study that takes these ideas to the next logical step and examines transgenerational plasticity through three generations. They used a well-studied system of snails (*Physa acuta*) that display inducible defences in response to predator (crayfish) cues. The authors exposed three generations of snails to one of two treatments: the presence or absence of predator cues, and then examined a suite of behavioural and morphological traits associated with predator defence. This allowed them to determine if and how offspring, parental, and grandparental environment influence offspring phenotype. Interestingly, their results do show that transgenerational plasticity can act across multiple generations. The patterns found were complex though and it is difficult at this stage to assess how likely it is that these responses are adaptive. For example, a behavioural trait appears to respond to grandparental but not parental environment, shell thickness responds

to both, and snail weight and a composite index of morphology respond to neither. Exactly what this means in terms of an offspring's fitness, however, is unclear. It is also not immediately clear from the study how predictive a grandparent's environment is of the conditions likely to be faced by an individual. Further work will be needed on these issues to better interpret what this transgenerational plasticity means and to assess if it might be an evolved response to cope with varying predation pressure. It would also be useful to delve more deeply into the developmental mechanisms throughout which this plasticity occurs. Irrespective of these issues, however, the study does reveal that transgenerational plasticity across multiple generations can indeed occur and so cannot be ignored as a source of phenotypic variation.

### **References:**

[1] West-Eberhard, M. J. (2003). Developmental plasticity and evolution. Oxford University Press.

[2] Tariel, J., Plenet, S., and Luquet, E. (2019). Transgenerational plasticity of inducible defenses: combined effects of grand-parental, parental and current environments. bioRxiv, 589945, ver. 3, peer-reviewed and recommended by Peer Community in Evolutionary Biology. doi: [10.1101/589945](<https://dx.doi.org/10.1101/589945>)

## **Reviews**

### **Evaluation round #1**

DOI or URL of the preprint: [10.1101/589945](https://doi.org/10.1101/589945)

Version of the preprint: 1

### **Authors' reply, 18 June 2019**

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### **Decision by Troy Day, posted 06 May 2019**

#### **Revision**

I think this is an interesting paper that demonstrates quite nicely the potential complexity of transgenerational plasticity. The reviewers also felt that the paper makes a useful contribution to this important and growing area of research and they provided a number of very helpful comments. However, they also raised a couple of potentially serious concerns that I think need to be addressed. One has to do with the statistical analysis and false discovery – perhaps taking multivariate approach as suggested would help to alleviate this concern to some extent. The second concern though has to do with pseudo-replication. It is not entirely clear to me how best to deal with this issue but it is clearly something that needs to be addressed (both reviewers mention this point).

I had two other questions:

(1) How repeatable do the authors think their results are? Given the seemingly varied and complicated patterns of response as a function of past environment, one wonders if the patterns would remain consistent across multiple experiments.

(2) Given the results it is difficult to imagine how these responses could be adaptive. One can always make up stories but that isn't very satisfying. It would be nice if the authors could provide their thoughts on the sorts of experiments that might be done to test whether these kinds of patterns are adaptive or simply "noise".

It would be helpful to have legends on Figure 1. Also, it should be stated that the vertical dashed line separates the offspring treatment groups.

**Additional requirements of the managing board:**

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 1, 28 April 2019**

In this study the authors investigate how exposure to predators of grandparents, parents, and self affects several behavioural and shell traits in an aquatic snail. This was determined by running a full factorial design under controlled laboratory conditions. We still know little about transgenerational plasticity, especially for generations earlier than parental ones, so this is an interesting and useful study. The design is adequate, and the sample sizes seem sufficient (although it is not mentioned how many parameters are estimated per trait – see comment below). However, I think the statistical analyses can be improved in several ways and there is especially a risk of overinterpreting the results due to inflated significance under multiple testing. I therefore make several suggestions that I think will be relatively easy to implement and that hopefully will lead to improved and more robust results. This may also change the interpretation and key messages of the study.

L 50: there are a few minor english grammatical mistakes throughout the text, e.g. here faced with or facing

L 60: move "to ...cues" to the end of the sentence

L 85: any idea why this might have happened? How could this have influenced your results?

L 91: might it be easier and shorter to reflect the experimental design, including treatments and sample sizes, in a figure? If possible, also tracing the relatedness of the individuals, i.e. family membership.

L 93: this is a non-native crayfish species – is the response of your snails as large and in the same directions as for native crayfish? Provide some results and references on previous studies showing responses in your snail using this predator treatment.

L 99 and 100: reverse the order of these two sentences

L 103: weighed, and omit weight. Dry or wet weight? Snail + shell? Or just the shell (which makes more sense)?

L 104: photograph

L 106: so is that the thickness of the rim of the aperture, not the shell width?

L 113: it might be a good idea to standardise your variables before modelling, both for estimations purposes (improving collinearity issues) and interpretation (all estimates on the same scale)

L 116: why not just run the full model, and provide all t-, F- or Chi square- and p-values? That provides more information (e.g. for posterior meta analysis), and prevents biased estimates in the remaining variables. See the paper by Forstmeier and Schielzeth cited below.

L 117: how is the relationship between weight and the other variables? I would guess it would be exponential with an exponent close to 3, if so then don't take the ln of weight, but the cube root as a covariate. Check for all your models that the residuals have the desired distributions.

L 118: I lost track of the genetic relatedness between the individuals in your design, but isn't it necessary to model relatedness in each generation (current family, parental family, grandparental family), i.e. as several nested random variables? Even if these higher levels are not significant, I would include them anyway as they take care of pseudo-replication in the design.

Having said that, how many parameters are you estimating? Per parameter you should have at least 5 to 10 data points to obtain reliable estimates.

Another issue is the large number of traits and parameters that are tested in this study (many interactions per trait, and many traits), which could increase the probability of obtaining significant results by coincidence. The authors should study and implement ways to control for this, for example via false discovery rate. There are R packages that implement several options to control for inflation of significance. As an exercise, simply randomise the data of your response variables (but according to the structure of your design, so not across families for example) and re-run the models, and see if you get any significant effects (I predict you would). See e.g. Cryptic multiple hypotheses testing in linear models: overestimated effect sizes and the winner's curse by Wolfgang Forstmeier and Holger Schielzeth.

In view of my suggested improvements in the statistical analyses, and thereby possibly changing the results and interpretation of the study, I have not in detail reviewed the discussion. Nonetheless, the discussion is multifaceted and balanced, and in that sense is acceptable. Figure 1 and table 1 are also good in their design.

**Reviewed by [Stewart Plaistow](#), 01 May 2019**

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