

Evaluation of master theses by Hana Dvořáková: Transgenerational effect in *Taraxacum brevicorniculatum*: test of a novel method of experimental plant DNA demethylation and its practical application in exploring the impact of maternal competition on progeny phenotype

The presented theses are in form of English written manuscript for international ecological journal with expanded literature review. This fact brings about several drawbacks for reviewer as well for student. As a reviewer I am hesitating whether to admire capabilities of the student or whether to be suspicious concerning support provided by supervisor.

So my first question: Who are authors of the manuscript and what their contribution to the study is.

Overall the quality of theses exceed expectations for master student. Message is clear, tested hypotheses interesting, methods appropriate, evaluation and discussion relevant and I expect that it will be interesting paper. Still there are formal and factual things which should be elucidated.

Literature review suffer from terminological inconsistencies. For example adaptation, transgenerational effect, functional traits and epigenetic are sometimes not well used. Moreover I miss mentioning method actually used for assessment of methylation rate.

My questions:

What is "adaptation" and when the term was not properly used in the text.

Why term "functional trait" is used here? Is it necessary or is there another possibility?

Hypotheses and aims of the study are declared as (1) test novel method of demethylation, and, (2) effect of intraspecific and interspecific competition on early growth of progeny. But the method is not as novel as declared. Its usage was published last year by Rendina González et al 2016 in American Journal of Botany. Also second aim is problematic as despite complicated design of the experiment only overall effect of the competition (without distinguishing inter- and intraspecific competition) was evaluated. The leaving part of experiment not analyzed is acknowledged at the end of the discussion.

My question: Can you formulate how novel your experiment is in comparison with work by Rendina González et al 2016 published in American Journal of Botany?

Methods are described in details but reading this section make me very confused. Structure is not wholly logical: in chapter "2.2.2. Plant morphological measurements" experiment 2 is mentioned even when it is described only later on. In chapter "2.2.4. Statistical analyses" 10 days old seedlings and 6 week old plants are mentioned but not plants harvested after 3 weeks. I miss calendar dates when the experiment was performed. Text is full of abbreviations which are not necessary. I totally do not understand why the experiment with 20 plants treated for 6 weeks was established. It is not true that studied species has advantage over native species of dandelions being genetically uniform apomict.

My questions:

What expectations are behind 3 week and 6 week experiments?

Why plants from offspring generation in the Experiment 2 were cultivated only in conditions without competition? Such design prohibited testing one hypothesis, do you know which one?

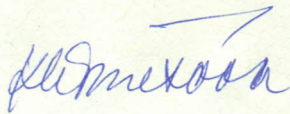
Results are difficult to follow due to including lot of numeral results into text. I would rather prefer to have them in a table. Figures are informative but I would appreciate to have them included in text and not at the end of the theses.

Discussion is not easy to read due to very long paragraphs. It is interesting that student admit that demethylation method was already published for another species and instead of "novel" call her experiment "proper testing the method".

Conclusions are not in accord with aims.

Despite my numerous comments I take a liberty to recommend the thesis for a defense.

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January 11, 2017

Trebon

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Opponent's review of the Master thesis

Title: *Transgenerational effect in Taraxacum brevicorniculatum: test of a novel method of experimental plant DNA demethylation and its practical application in exploring the impact of maternal competition on progeny phenotype*

Author: Bc. Hana Dvořáková

Supervisor: Doc. Francesco de Bello, Ph.D.

The aim of the master thesis is testing the role of parental competition on progeny phenotype and evaluation of the role epigenetic variation in potential transgenerational plasticity in *Taraxacum brevicorniculatum*. Overall, the topic is very timely and the logic of the research is good. I particularly appreciate Hana's ability to connect ecological experiments with molecular analyses. I am sure that the study has very good potential to influence and advance future research on ecological epigenetics because it provides novel method in experimental demethylation of plants (1st study) and confirms that the suggested approach in demethylation can be successfully used in ecological studies (2nd study), although I have some criticism that I have listed below. The thesis is very well written, topic is nicely and thoroughly introduced, objectives are clear and properly tested, results are well presented (I would appreciate figures and tables to be placed into the text not at the end of the thesis) and discussed but some unclarity can be found in methodological description.

My biggest criticisms of the thesis aims at the execution of the first study. Hana provides strong evidence that the new method in plants demethylation is joined with considerably less side

effects than the traditional one but has similar efficiency in DNA demethylation. This is an excellent finding! One of the explanation why the traditional method leads to lower viability of treated plants is that germination of seeds in azacytidine is toxic especially for root tissue of emerging seedlings, which has been demonstrated also by this thesis. Nonetheless, due to the absence of root biomass comparison between germinated and sprayed plants the thesis fails to clearly demonstrate that spraying plants with water solution of azacytidine does not reduce root development of treated plants, which can be one of the strongest benefit of the method. This omission thus reduce explanatory power of possible mechanisms behind the difference among the two methods.

It is also unclear which plants were used for DNA methylation assessment. In the 2.2.3. chapter it is written that DNA from the three-week experiment was used. The doubt I have is about the source material. Was DNA extracted from the harvested and dried plants from the first study? If so, it is unclear why only shoot biomass was measured in the Study 1 whereas root and shoot biomass was used for DNA extraction, i.e. root biomass was available for the biomass measurements too. Also the number of analysed DNA samples is unclear.

I really like the second study because it is cleverly designed and asks several interesting questions. Moreover, biotic interactions are underrepresented in transgenerational studies. In my opinion slightly problematic is resetting of epigenetic memory in the F1 generation. Since azacytidine was applied on already growing F1 plants it could be possible that azacytidine removed also other epigenetic marks that were not involved in transgenerational memory, which could result in evening of plant phenotypes. Therefore, it is not perfectly clear how important the epigenetic memory was in transgenerational effects. There is also wrong denominator in the equation, should be plus instead of minus sign.

Questions:

Is there any better approach how to test the role of epigenetic memory in transgenerational effects in the second study?

Some transgenerational effects are just “passive” consequence of plant physiology, e.g. stressed plant produces low quality seeds, which can result in low performance of progeny. Nonetheless, there is increasing body of evidence that some transgenerational effects are adaptive. Can you predict under which circumstances can be transgenerational effects evolutionary beneficial?

Evaluation:

Despite the criticism, which is duty of every opponent, I am sure that both studies have very good chance to be published in well-established journals and therefore I can only recommend the thesis for the defense.

A handwritten signature in black ink, consisting of several loops and a long tail, positioned on the left side of the page.

Průhonice, 5. 1. 2017