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OPPNONENT REVIEW

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Master's thesis of **Jeni Sidwell**

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Overview

The Master's thesis "*Trade-offs in parasitoid resistance and competitive ability in* Drosophila" provides insights into the responses of fruit flies populations to stressors coming from the environment (limited food resources) and biotic interactions (parasitoid resistance). As such, the research work describes an important pattern in evolutionary ecology, which is how phenotypes change under new conditions and whether trade-offs can be identified in varying phenotypes.

The dissertation's Introduction to trade-offs, experimental evolution, and the *Drosophila* system gives a comprehensive account of current knowledge. The dissertation, I believe, is of very good quality and the results merit scientific publication in an international journal.

Dissertation goal and methods

The thesis has two specific goals: 1) investigate whether any trade-off exist after phenotypic responses to environmental stresses, specifically, parasitoid resistance vs competitive ability; 2) estimate which phenotype ("evolved" or control) performs better under multiple environmental conditions encompassing parasitoids, limited food resources, a combination of both, and a control environment.

Overall, the dissertation tackles the specified goals using a series of state-of-the-art methods in experimental evolution, taking advantage of a massive experimental protocol to compare two *Drosophila* species, two parasitoid species, and a factorial combination of treatments for 12 – 16 fly generations. Indeed, the experiments were carefully planned, standardized, and well executed.

The data analyses are technically sound, and the conclusions are solid. It is understandable that in some cases, significance in the form of p-values < 0.05 has not been reached, but this is more related to sampling size and peculiarities of each experiment rather than a bad experimental design.

Dissertation results and merits of the student

The master student did a great job describing and discussing the main results of the thesis. The student correctly interprets the methods strengths and the caveats associated with experimental evolution. A very

few passages and terms would have benefited from further clarification (e.g., competitive ability: it could have been further dissected as competitive larvae feeding faster, fighting for resources, developing faster or requiring less resources for development; clarify whether competition for resources might really be an adaptive response in nature, or whether a higher pay off would be to invest in dispersal ability; timescale: is 12 – 16 fly generations enough to disentangle adaptive evolution?). Nonetheless, these are just simple clarifications and matter of further discussion which do not detract the significance of the thesis.

The student has significantly contributed to the thesis by revising the literature, generating and analyzing the data, and interpreting the results. All of them done in a high scientific standard.

Significance of the thesis

The thesis topic is original, and the results point towards a promising research avenue using a system that has rarely been studied in experimental evolution (2 *Drosophila* species other than *D. melanogaster*). Although most of the research questions are necessarily exploratory because of the limited knowledge of the system (food resources, parasitoids, fly species), the student did an excellent job by framing it into a research endeavor including expectations backed up by the recent literature and proposing interpretations based on a solid experimental setup.

In the conclusions, the student suggested that evolutionary adaptations drove phenotypic responses in their fly populations. However, this might need re-phrasing, as the student is aware throughout the text that the results might not necessarily be explained by adaptations but plasticity or trade-offs. During the thesis defense, I also would like to hear more about the future directions, in which the student proposed further measures of phenotypes and genotypes.

Overall evaluation of the thesis

The student presents a very good thesis backed with carefully thought experiments, using relatively rare study systems (2 *Drosophila* species from Australia). The research topic is timely as we need to know more on how species and communities will respond to increased environmental changes.

The dissertation is also built up on a very exhaustive laboratory work. The analyses presented in the study are sufficient to tackle the goals of the thesis and the interpretation of results have been done following an up-to-date literature reference. Therefore, I think that the dissertation meets the conditions for the defense.

Questions for the student

1. Is there any relation between your definition of trade-off and the concept of maladaptation?
2. What specific behavior (e.g., feeding fast, aggressiveness) or physiology (e.g., faster development) do you think drives the “competitive ability” in your study system?
3. Why do you think that competition for resources in *Drosophila* larvae is an adaptive response? Would not a better adaptive explanation be investing in dispersal ability when resources are limited?

4. Do you think the timescale and space used in your experiments are enough to provide insights into adaptive mechanisms (generation of new mutations, drift, allele fixation driven by selection)? Or are you only able to measure species responses to the environment given phenotypic plasticity and trade-offs, without invoking adaptations?

5. One of the expectations of the thesis is: *does increased performance in one condition cause decreased performance in the other condition?* But based on previous evidence stated in the Introduction, is it not expected that increased larval competitive ability somehow increases performance in resistance (more wounds in crowded environment, better parasitoid resistance)?

6. It is not clear when were the flies collected in the field. Such information is given for the *Drosophila* parasitoids (2017 – 2018). In addition, do you think the flies after several generations in the lab before your experiments have lost genomic variation important for the expression of phenotypes and traits under study?

7. In the Discussion, it is stated that: *The general pattern reveal that the control populations were better competitors than the evolved populations when exposed to the reduced food conditions*. But would not it depend on the species identity? For *D. birchii*, it seems that there is no evidence of control being better competitors than "evolved" in neither experiment 1 nor 2.

8. In the Discussion, it is stated that: *Competitive ability was more imperative to survival than parasitoid resistance in these species when faced with this combination of conditions*. However, it seems the opposite for *D. sulfurigaster*; more "evolved" flies emerged in the combination of conditions in both experiments 1 and 2.

9. In the results from *D. birchii*, it is mentioned that *some illness in the flies or infertile wasps were to blame for the unexpected outcomes*. Were not the experiments run in parallel with *D. sulfurigaster*? If so, any issue with infertile wasps might be ruled out, as it seems that wasps were viable in those other experiments.

10. In the Future directions section, it is mentioned that genotyping would be an invaluable method in follow up experiments. Do you think genotyping to understand adaptation and evolutionary change would be better measured under artificial selection and backcrossing of contrasting phenotypes? Or would you still do genotyping in experimental evolution setups?

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