Review

Thank you for your patience and for your interesting work and manuscript who piqued my interest…

General comments

In this paper the authors present two experiments that have been done on bulb mite populations. In this species, well fed juvenile males are more likely to become "fighter" adults and nutritionally deprived juvenile males usually become smaller "scrambler" adults. In a nutshell, there is a threshold of "juvenile condition" that determine the type of adult morphs the juvenile males will become.

We also learn that before becoming adults, some mites (males and females) can transform themselves into a transient specific morph called "deutonymph". Based on previous observations that male deutonymphs always become fighters, the authors explain that deutonymph probably come from individuals of good condition despite being placed in poor environmental conditions.

The authors explain that the developmental bifurcation between fighters and scrambler which corresponds to a form of developmental plasticity can either be considered as a form of **mitigating** plasticity or of **anticipating** plasticity, both of which are supposed to be adaptive plasticity; also they never clearly mention that. No other alternative interpretations are considered.

In the case of mitigating plasticity, the low condition juvenile males will become scramblers to avoid paying a dangerous physiological cost supposedly associated with the development of a costly fighter morphology.

In the case of anticipating plasticity, as far as I understood, the juvenile males will "decide" to become a fighter or a scrambler depending on the potential future advantage in terms of reproductive success associated to each of these forms which I guess should depend on 1) their size, 2) the predicted number, type and size of potential male competitors.

The authors claim that they want to study how these two types of plasticity can "fuel the eco-evolutionary population change in response to perturbation", although it is not always what they mean in practice by that.

The authors then explain that the body condition threshold that determines the development into a scrambler or a fighter will change differently depending on the type of plasticity as a response to a demographic perturbation which is here some harvesting of juveniles.

They have done two experiments. One in which they applied different treatment of juvenile harvesting and followed the population dynamics and several phenotypic traits. And a common garden experiment to study whether some traits have evolved after the first experiment.

The first experiment consists in harvesting different types of juveniles in some populations to generate these "perturbations"

The harvesting treatments were (1) harvesting of 100% of the deutonymph each week, (2) harvesting of 50% of the deutonymph each week, harvesting of juveniles other than deutonymph “at the same percentage” which reads (3) 100% of juveniles or (4) 50% of juveniles, and (5) no harvesting.

The authors have then assessed the potential effects of the different harvesting treatments on 1) the body length of deutonymphs, fighter males, scrambler males and adult females, 2) the number of individuals of each life stage, 3) the total population size,

The authors then interpret the observed responses in terms of change in the proportion of fighter males and male body size as a consequence of the above-mentioned two alternative types of developmental plasticity, which is either mitigating when the metamorphosis into fighter or scrambler males depends only on the condition (size?) of the larvae, or anticipatory if the larvae can modify their developmental pathway (toward fighter or scrambler adults) according to the perceived change in their demographic environment.

The second experiment was a common garden experiment to assess if some life history traits had evolved after the first experiment.

The authors have individually isolated 420 offspring produced by 3 females sampled from each population and followed them until maturity to record the number of individuals that became deutonymphs, tritonymphs (a new form that has not been mentioned before??) and the sex and morph of adults. These morphs have been sized but the individual growth trajectories have not been measured, nor have the age and size at maturity although the authors have predicted that these traits could have evolved depending on the harvesting treatments.

Before presenting these experiments, the authors make some predictions but unfortunately I found their explanation and the links with the experimental design pretty hard to follow. Here is what I manage to understand:

In the case of mitigating plasticity, I understood that if the idea is to avoid paying unaffordable physiological costs, I would predict the threshold to remain constant despite some changes in the juvenile density, given that the cost paid should depend on the juvenile condition at metamorphosis. But the authors predict that the threshold will increase, using a complex reasoning involving a series of more or less speculative causalities mixing plastic, demographic and evolutionary responses and mixing different time scales not clearly identifiable. For instance, they argue that harvesting juveniles will lead to an early maturation which I interpret as a long-term evolutionary response. Although it seems pretty ambitious to expect to observe such evolution of the maturation reaction norms on such a short time scale. But beyond that, one could well imagine that the harvesting treatments will change the juvenile density, competition, probably also growth trajectory and condition and maybe also their maturation strategy, but I do not understand why this is supposed to modify the condition threshold if it is determined by physiological cost associated with the production of the fighter phenotype.

In the case of anticipating plasticity, although it is not explained in these words, I understood that harvesting the deutonymphs which are supposed to become fighters will give a signal to the other nymphs that it may be more advantageous to become a fighter event if you have relatively low condition given that the competition between fighters will be less harsh. Thus the condition threshold could plastically vary and become smaller, which is not expected to be the case when harvesting concerns the other types of juveniles (also one could also say that the proportion of fighter males will increase if only the deutonymphs survive and thus it could be more advantageous to become a scrambler even if you are in relatively good conditions).

> The study these two alternative hypotheses relies on the identification of the "condition threshold". But how "juvenile condition" is measured is not clearly defined: is it body size, growth rate, corpulence? Condition seems to be equivalent to body size given that the author measure the size of the different morphs but then how can one tell if two juveniles have different conditions given that their size is susceptible to change as they grow.
>
> Moreover, the authors seem to quietly abandon the idea of studying the sliding of this "body condition threshold" and seem to replace this idea by the measurement of "fighter expression" expressed sometime as "proportion of fighters". But this is very different from the above-mentioned condition threshold, given that for instance higher growth rate could well enable more juvenile to cross the threshold and metamorphose into a fighter without any modification of the threshold itself.
>
> Rather than displaying the mean scrambler and fighter size on different and differently formatted graphics which prevent any comparison (4B, 4C; 5A with a missing figure showing the fighter size in the second experiment), the author could rather show the distribution of the fighter and scrambler size and study how their joint distribution, which should reveal in its centre the famous threshold, is modified by their treatments.
>
> It is also difficult to understand the rationale under the different harvesting treatments. More information is required on the population size and structure of the control populations and how it is affected by the harvesting treatments. How many juveniles, how many deutonymphs, how many male and female adults? How does it vary with time, etc.
>
> Why did the authors harvested the same % of deutonymphs and juveniles rather than the same number? I guess that the deutonymphs represent only a small part of the juveniles and thus the demographic effect of harvesting 100% deutonymphs versus 100% juveniles are not at all comparable. Thus, how to rule out the idea that the effect of the treatment is simply a demographic effect rather than a selection of individuals according to their condition or their potential future?

I found the paper quite dense, and at some moments pretty difficult to understand especially for someone not familiar with the very interesting but somehow quite complex biological system.

I think that an effort should be made to try to better explain what has been done.

Understanding the links between the general framework, the theoretical predictions, the biological system and the experiment design is not easy.

I thought that it would help to have detailed presentation of the biological system before presenting the general framework, or at least to better explain the links between the two. For instance, there is a cognitive leap from lines 67 to 68.

Comments on the figures

Figure 1

Please explain how fitness curves have been measured. It could also help to provide the empirical data on which this figure is based on. The “fitness” of each type of male depends on its body condition (body size?) prior to metamorphosis, but shouldn’t it also depend on the adult density and especially on the relative proportion of the different type of males which is not directly linked to the “condition” axis. Thus, it is a bit difficult to understand what the fitness lines really represent. Or it may be useful to explain that these fitness crossing lines represent the fitness of each type of morph measured in a similar specific fixed environmental condition.

> How it is possible to know the fitness of a fighter of weak condition given that they do not exist. -> you could distinguish in the graph the fitness functions that have been measured from those that are extrapolations (dotted lines).
>
> Condition is not clearly defined. Condition of what? If it is body size, then this should be explained. It is not clear what the distribution of condition is in the population. If the condition=size, then is this distribution equivalent to the population size structure that includes all the different stages/ages.

515 -> “deutonymph harvesting will reduce the size of the condition distribution” I am not sure to understand this sentence. Do you mean reduce the population size?

I found it difficult to understand what the blue and red lines represent. Is it the size distribution in the populations, including all the different individuals? If this is the case, I do not understand how the population size (grey area), can increase after a harvesting event (D, F), especially after juvenile harvesting given that deutonymphs seem to be quite rare among the other juveniles. Could you explain what you mean by “after” compared to “before”. As it is, ”after” can be understood as after harvesting but this is probably not the case. But it is probably a “long-term after” especially if you expect some demographic and evolutionary response. It may help to clearly distinguish the two time-scales: how the population will look like just after harvesting and what could be the long-term demographic responses. Then it could be nice to compare these predicted changes in the size distribution to the observed size distributions in your population at different time scales.

I must admit that it is difficult to follow the reasoning given that it is quite complex and relies on mulpliple causalities. Juvenile harvesting -> males will mature earlier (through a plastic or evolutionary response?) -> more scramblers (in proportion or total number?) -> “this will increase scrambler fitness” (I do not understand why, and fitness compared to what?) -> evolution of a higher threshold (not clear why).

Then it is written that the scramblers mature earlier, live longer and produce more offspring than fighters (in which conditions?), and thus the population size will increase. But if scrambler are so performant, why do fighters exist? And what about the density dependence? Is the link between these performant scramblers and a change in population size empirically verified?

In a nutshell I found the reasoning difficult to follow, probably because this reasoning is based on many causal relationships, different time scales (just after harvesting, after demographic response, after possible genetic evolution) and responses of different natures (evolution and plasticity) in a system where there is necessarily some complex demographic feedback, and it is difficult to understand how they can act (a figure could help present how the different possible causalities intertwine).

I think that it would help to represent the different times scales, and to represent similar data derived from the experiments (for instance the size distribution in the populations and their change after harvesting and during the experiment).

Given that this figure is supposed to help make clear predictions, it would be useful to add some predictions that are directly linked to the traits that will be analysed (body size rather than the threshold).

- ➔ In case of mitigating plasticity, we expect to observe an increase in total population size after harvesting compared to controls, a smaller number but bigger fighters after both deutonymph and juvenile harvesting.
- ➔ In case of anticipatory plasticity, we predict to observe a smaller population size in harvested populations compared to controls, the same proportion of fighters compared to controls but smaller fighters only in the deutonymph harvesting treatments.

The figures of the results should be shaped to present the data in a way to facilitate the comparison with the above-mentioned predictions which is not the case right now.

Figure 2.

Given that the number of populations is rather limited, it would be nice to show the population dynamic of each population and to add some information on the size distribution or of the cumulative number of the different groups of individuals.

Cumulative bar plots could be used for instance to show the temporal changes of the population size and structure

You could also add an arrow each time harvesting takes place. And it would also be nice to show the different periods on the graphs.

As it is now, the figure 2 does not show the between-population variability of total population size or of the population structure, both demographic traits being important to grasp to understand what is happening.

Please also explain why the first part of the population dynamics are missing in for the J-D100 and J-D50 treatments.

It is a good idea to use different colors for the different treatments but it would be nice to use the same colors in all the figures.

Figures 3 and 4.

I found it difficult to follow what is going on because the way the results are presented changes from one panel to another. If I do understand the rationale behind the idea of grouping together some treatments, depending on the result of the statistical contrasts, I do not think that it is a good idea to present the data as it is done on the figure because 1) it requires too much brain effort to understand what is going on each time one switches from one panel to another and 2) it is not possible anymore to directly compare the results to your predictions. For instance, we need to be able to compare the different treatments to the control treatment which is now grouped together with other harvesting treatments.

Thus I suggest representing the result of the models with the interaction and thus to have an independent estimate for each of the 5 treatments and to use the same colors as the one used in figure 2. Figure 3 A and B could be fused together.

Moreover, to better represent the real amplitude of the between period and between treatment's variations, I think that it would be more relevant to scale the y axis of the graphs from zero. Plotting the 95%Ci rather than se could also help to easily see which are the treatments that significantly differ and this could help draw lines between the graphs and the results of the statistical analysis.

Other comments

I allow myself to write down some other comments that came to me while reading your manuscript in case it could be useful to you.

Title

I am not sure that the "leave a male polyphenic signature" in the title is clear enough.

I would rather recommend a title like "The short- and long-term effects of selective juvenile harvesting on the demography and developmental trajectory in the male polyphenic bulb mite"..

Abstract & Introduction

I think that the abstract and introduction could be improved to clarify things. Use shorter sentences when possible. It would help to use more empirical examples to illustrate certain theoretical concepts and ideas that are not always easy to understand.

Although some efforts have been done to explain the hypothesis and prediction, notably using the figure 1, I had difficulties understanding the presented predictions (see comments on figure 1). It is difficult to understand what represents and on what are based the fitness curves. The condition distribution is also not clear. Is it equivalent to size distribution within a population?

L2-3 First sentence long and complex. -> Shorten / split?

L6 Do you consider that there are only these two types of plasticity?

What do you mean by "underlie different ecoevolutionary population dynamics"?

L7-10 the transition to your system, to the selective harvesting and male polyphenism without explanation is a bit abrupt. -> give more information on the system and the question that arise before presenting the experiment.

30 "large major *individuals"*

33 if the difference are typical, tell us what the difference are.

34 "Difference in individual development…"-> what do you mean by "difference"? Do you already refer to plastic of genetic modification of the condition threshold? Please clarify what will affect the population structure size and growth.

35-38 Could you illustrate with some empirical examples?

39 It could help to present what you mean by "the perturbation of developmental trajectories" upstream.

41 “perturbation *of* individual development”?

42 could you illustrate this idea with an example?

46 It could help to present the two types of developmental plasticity using some examples before remobilizing these concepts here. Or maybe first explain that you consider that DP can be either anticipatory of mitigating and then present the two alternatives.

47 Mechanism *is* the evolutionary result -> *can be interpreted as?*

54-56 I do not understand the difference with anticipating plasticity, which also constructs phenotypes that are successful at reproducing. Please clarify the difference between the two types of plasticity. You could illustrate each type with an example.

63 Please clarify. I do not understand what you mean by “a plasticity that is regulated by the dynamic of population density…” If the plasticity is the existence of a condition threshold, you mean that this threshold is itself plastic and will vary depending on the population dynamic and level food competition? I would have rather considered that these demographic factors will affect the conditions of the individuals which will determine their position relative to the condition threshold which itself remains constant. As it is written right now, it is not clear what you mean to explain.

64 Why do you write that the condition distribution informs on population size? I do not understand the link between the two.

65-67 This is not clear for me. What do you mean by “fuel the ecoevolutionary population change”. Can you be more specific. I am afraid that this formulation can have several interpretations.

67-68Llarge cognitive leap. It may help to present the biological system in more details earlier.

69 “impact eco-evolutionary population responses” -> as above, please explain what you mean by providing for example what are the precise demographic traits that you ambition to measure and on which timescale.

73 “good condition juveniles” -> given that this is central to your experiment and reasoning it could be useful to better explain what you mean by that. How do you know that a juvenile in your population has a large resource budget? Body size or corpulence could be a proxy but I guess that body size increase with juvenile age, thus how can you tell if a small juvenile is a nutrition-deprived individual or a well fed but just young individual?
-> it may help to have a figure showing the lifecycle of your mite and the different stages, etc.

74 The growth rate could indeed be a proxy of the juvenile condition given its age, but can you measure that in your populations/experiments? But the link between growth rate and condition at metamorphosis is not straightforward given that a condition deprived individual could reach metamorphosis with the same condition as a well-fed individual. It could just take longer to reach this stage.

76 Could you cite between brackets what could be these unfavourable environmental conditions (higher density?).?

81 “carries development costs…” -> it is maybe more cautious to write something like “linked to/associated with a reduced size…”, given that formally demonstrating that it is a “development cost” may be a bit challenging.

83-87. May be first explain that Deutonymphs always mature as fighters and then give your interpretation.

91 remove “thus”?

94 what do you mean by “juvenile performance”?

94-100 difficult to follow. You could clarify when you consider that the threshold will “evolutionary change” or when it may be plastically modified depending on the environmental conditions.

105 Could you try to rephrase this more simply?

107 You refer here to the genetic evolution of the maturation threshold?
minor -> scambler?

108 “the *evolution* of scrambler expression” -> could you consider using another word to avoid confusion with genetic evolution?

108 “Mean fighter expression”: please explain what you mean. How do you measure it? Is it the proportion of juveniles that become fighter, the total number?

109 reasoning difficult to follow given that you could also imagine that harvesting-> less juveniles -> less competition -> more resources-> higher growth rates and increased juvenile conditions -> more fighters.

113 Do the scrambler mature earlier or at the smaller size?

113-114 It is written as if it was true in all environmental conditions. But I imagine that the scrambler’s performance is regulated by the density, the proportion of fighters, etc. Otherwise, why do fighters exist if the scramblers mature earlier, live longer and produce more offspring.

116 ((-> (

In a nutshell I found your reasoning difficult to follow given the intertwining of time scales and potential demographic feedback loops.

Methods.

More details are required to better understand the experimental design (a figure could help).

It is not clear why the authors choose to analyse time as discrete three periods rather than as a continuous covariable (using gam for instance).

Why did you choose to harvest the same percentage rather than the same number of juveniles versus deutonymph to keep the demographic impact constant?

124 please explain what the tritonymph are and give some information on the duration of these stages (and the other stages also… through a figure for instance) and the mean proportion in your populations.

124 an ->and

126 unfavourable conditions -> also high density?

131 -> do you expect to have a lot a genetic variability in your populations?

136 Please indicate here that harvesting has been done on a weekly basis (the information is otherwise provided on line 167).

150 What do you mean by “from the source population with founder mites”?

156. explain what the time periods are before mentioning them. Why did you choose to split the experiment into three time periods? Why not just show the time trajectories of the measured traits to show how they continuously vary with time?

157 “up to five” -> how many on average?

161-163 could you illustrate these measurements with some pictures?

165 “dynamics to stabilize” -> What do you mean? Dynamics of what? Do you mean population size and age/size/morph structure?

172-173 Isn't it the condition threshold that is supposed to remain stable rather than “fighter expression” which I read as the number of fighters produced, which itself could increase if there is a higher juvenile growth rate and juvenile condition due to a lower number of juveniles due to the harvesting?

175-177 Please clarify why you expect such evolution given that the two types of plasticity are supposed to be adaptative. Plasticity may be sufficient?

188-189 The effect on which traits?

200 Please justify the three periods. Why three, why the limits, why not consider time as a continuous variable?

205 Did you use Poisson models? Did you include the number of females as a covariable? Why not analyse the fighter expression as a proportion using a binomial model (number of fighter males versus scramblers)?

207 You analyse the size of the different types of individuals and the number of deutonymphs. What are your predictions on these traits as a function of the different treatments and different types of plasticity?

211 In the analysis of number of deutonymphs, did you add the total density as a covariable, given that in the introduction it is explained that more deutonymphs are expected when the conditions are harsh (higher density, higher competition?).

215 In the life history assay why didn’t you measure the age at maturity rather than mean adult size given that one of your predictions was that this trait would evolve?

Results.

The models and their simplifications should be grouped together into tables to clearly present what has been done.

235 Given that the interaction is marginally significant, it would be nice to have an unconstrained view (in the figure) of what is going on in your data, showing the prediction of

the models with the interaction. The interaction may become significant if you pool together some treatments as you have done later.

235 When you present some results could you also give the magnitude of the effects: “Population size was *on average 3%* highest when…”

139)) ->)

240 need a test for the difference between periods. Given that you choose to split the time period into three periods rather than analyzing time as a continuous covariable, you should maybe say “population size different significantly among periods” rather than “increased over time”.

242 verify the chisq symbols.

246 What is this test? Given that there is a significant interaction between periods and harvesting treatments, shouldn’t you have to do this test independently for each period?

To better understand the statistical analysis you could also refer to a table where you report all the different statistical models that you have done.

246 You say that “the proportion of fighters was always low when 100% of deutonymphs were harvested” but this is not the case because it is always higher than 80% which is not low... You could maybe rephrase with something like “<u>on average</u>, the proportion for fighters varied between ~85% and 93%, and we found that during the first two periods, it was slightly but significantly lower (-5%) in the D100 treatment (~85%) compared to the other treatments (~90%).”

249 The mean proportion was lower during the third period does not mean as it is stated that the “proportion significantly decreased toward the end of the experiment”. It could well increase during the third period. Formulations that suggest that time has been measured continuously should be avoided. (cf. 251, 259, 262, 268, 279 etc.).

262 Isn't a statistical test missing to support this sentence?

273 Explain what is this test. Shouldn’t you also show that there is no significant interaction between D50-JD100 and period?

I do not understand how it is possible to have the higher number of deutonymph in the treatment were 100% of the deutonymphs are removed each week.

You provide many measurements on the size of the morphs but there isn’t a clear hypothesis or predictions regarding these traits.

286 You provide here the mean size. It would be nice to provide the mean values of each of the trait that you study.

289 Refer here to figure 5B.

292-298 You present non-significant results. This approach can be understood but is in contradiction with the previous approach which consisted of simplifying the models and grouping treatments together even when the effects were marginally significant…

Discussion

Discussion may have to be updated to take into account the potential above-mentioned suggestions of modifications.

Nothing really is mentioned about the amplitude of the highlighted effects. I think that this should be mentioned and discussed.

More could be said on the potential mechanism on which anticipatory plasticity may rely on? How does it work on other systems?

You could also discuss the limits of your experimental setup, the traits that you have missed that could be interesting to track down, the type of experiments that remains to be done to clarify things.

? 302 male alternative male…

324 Why didn't you measure age/size at maturity?