Project 14314 Privman and Purcell

I enjoyed reading this proposal. It is interesting and well-organized overall. As a friendly reviewer, the major issue I notice is a lack of details in the Experimental Design sections. I have noted specific examples in the margins. This seems like a potentially significant point for reviewer criticism. Another area is to explain explicitly why this study benefits from two sources of ant populations with similar gradients but on two continents. This is a critical point for a collaborative grant and should be clear. Another potential issue is whether you argue that the proposal is distinct or an extension of the previous BSF. These are detailed in the comments below and in the margins. I hope the comments are helpful!

Summary Comments

1. Section numbering. For easier reference to individual sections of the proposal, I strongly recommend numbering all sections. While most sections could be characterized as major sections, there are also subsections. For example, under Experimental Design, there are multiple subheadings that subheadings should be numbered. The numbering scheme for major sections could be 1., 2., 3., etc.. Subsections could be numbered as 1.1, 1.2, 1.3, 2.1, 2.2, etc. Logical numbering will assist you in communicating specific sections to reviewers.

2. Compactness. This is well-written overall. I tried to compact the writing for clarity. This includes the use of alternative words and terms for length and clarity. I also split complex sentences into simpler ones. As scientists, we tend to think in complex ways. yet we want to be precise. Because we write how we think, this can translate into wordy writing in the interest of precision. This pattern can result in long, complex sentences that take time even for expert reviewers to comprehend, which can interrupt the reading flow or even be an annoyance. In general, I suggest limiting each sentence to no more than two concepts. Alternatively, if a sentence requires more than three full lines of 12-pt text, it is probably too long to be easily understood. I hope this tip helps in the future!

3. What is the advantage of studying ants in two locations? As a friendly reviewer, it is not stated why the use of two species having similar environmental gradients is desirable. What can you learn that is unique by studying in Israel and UCR? For example, does the approach allow you to determine general adaptive mechanisms versus those specific to local populations or environments? This could be addressed at line 49 in the Background section or at line 81. Since the dual study is a crux of the collaboration, I suggest stating it at both locations without being too redundant to emphasize the point. This is one of your main justifications.

4. Hypotheses. At the beginning of the Objectives, I suggest stating the hypotheses you are addressing with your three objectives. The proposal should be hypothesis-driven. For example, the second objective could easily be stated as a hypothesis to be addressed. You might consider stating each hypothesis followed by the objective that addresses that hypothesis. This will result in three hypotheses and three associated objectives.

5. Image quality. I suggest replacing Figure 1 with a higher-quality image. Low-quality images are harder to read because they are blurry when enlarged. They also communicate that the figure is an afterthought plucked from a publication PDF or PP slide. I am also guilty!

6. Related to Comment 3 (line 103). This is a good explanation of what you can do with the collaboration. I understand that you are planning to sequence the genomes across the two species across similar gradients to determine supergene traits that are genetic and less variable vs those that vary with environmental conditions. However, the underlying concept is not clearly stated. As noted in Comment 3, I suggest being more specific about what you will learn directly from this comparison. What are you comparing? How will this parallel investigation allow you to disentangle extrinsic vs intrinsic conceptually? The proposal will provide novel and general insights into genetics and evolution, as stated in the next sentence. But how will it provide this? For example, the next sentence (line 105) could address this by stating, “We will identify supergene sequences with low variability between the similar gradients to those displaying variability within each of the two gradients. This comparison will permit us to identify and quantify supergenes that vary due to extrinsic environmentally based selection pressures versus supergenes with less variation due to intrinsic genetic mechanisms”. This is just an example to demonstrate the concept. This content is for the PIs to determine.

7. Significance. As a friendly reviewer, perhaps the Significance could be improved.

The significance, as stated, appears to be:

1. We know little about supergenes (reworked sentence from line 108).

2. You have a unique approach that will tell you about supergenes.

3. You will gain insights into supergene dynamics.

4. The proposal is timely because of climate change (last sentence).

1 - 3 are intrinsic to the proposal. That is, they relate to direct knowledge you will gain about supergenes in your system. I suggest thinking more about broader implications. Is there significance to the field or biology in general? Does the research have broader implications for genetics and biology? Can you extrapolate your results to other species beyond ants, such as plants or animals, including humans? Can your system serve as a model for other species, giving it wider appeal? While climate change is cited, it is used somewhat generically. How will the results address climate change more specifically? For example, could insect behavior or supergene evolution due to climate change affect humans?

8. Study systems (line 113). As organized, this section seems out of place as a separate major section. I suggest this be a subsection (4.1) under Experimental Design (4). This seems logical. OK?

9. Sampling section (line 150). I suggest explaining why 200 colonies with five ants per colony is the appropriate sampling strategy. What do these numbers mean statistically? What are the limits of these statistics? As a friendly reviewer, I would ask for details about why these numbers were chosen and what statistical power they bring. For example, can you provide a sense of resolution regarding the supergenes you are tracking? Are there examples in the literature where similar sample sizes achieved desirable results?

10. Sampling Legend Figure 3. There are four transects in the US and three in Israel, with 10 sampling sites for each country. How do these transects relate to the 200 colonies and five individuals sampled from each colony? I suggest describing or clarifying this for reviewers. Also, how are you planning to sample the colonies? How will ants be sampled and preserved for DNA? Will you preserve ants in liquid nitrogen or alcohol? How will they be stored before processing? How soon will they be processed? How will DNA be extracted? Can you reference your methods? As a friendly reviewer, I suggest the experimental plan should provide detailed explanations of how each project step will be done. There is sufficient text space within the 15-page limit for such details.

11. Sampling (line 150). I suggest explaining why 200 colonies with five ants per colony is the appropriate sampling strategy. What do these numbers mean statistically? What are the limits of these statistics? As a friendly reviewer, I would ask for details about why these numbers were chosen and what statistical power they bring. If I understand correctly, you propose to measure allele frequencies at collection sites across your gradient. Can you provide a sense of accuracy at the sites based on sample size?

12. Genomic sequencing details (4.3). I suggest adding more detail in this section with specifics about read length, pooling, and ddRADseq, for instance. My sense is that it lacks details for non-expert reviewers and experts who want to assess the skill set of the labs involved. You are likelier to be faulted for lack of detail than too much.

13. Hypotheses. Proposals, in general, should be hypothesis-driven. I suggest you provide a specific set of hypotheses you are testing via the Aims. For example, you might incorporate hypotheses to be tested into each of the three Aims starting at line 62. Alternatively, you can place hypotheses numerically just prior to the Aims. I do not see any specific mention of hypotheses in the submission guidelines, but I suggest you confirm that. But as a suggestion, I suspect it can only help with the clarity of the proposal.

14. Software tool details (lines 176, 216, 220). I suggest providing a brief overview of the samba tool for non-experts. What does it do? How does it work? At line 181, you describe the advantage of the tool. You may wish to consider providing a brief example of this correction. Similarly, at line 216, what does the SLiM tool do, and how does it work? At line 220, briefly, what do the fastimcoal2 and DIY-ABC tools do, and how do they work?

15. Supergene function (line 121). As a friendly reviewer, supergenes are being examined as variable genomic regions. In this sense, the identity of genes within the clusters is not essential knowledge. However, for geneticists and molecular geneticists, it seems important to know something about the genes found within the supergene regions. This is not discussed, making the descriptions of supergenes seem abstract because they are not rooted in function. Did you identify any specific genes in the regions? Were they linked in any way functionally? Are the functions consistent with expectations for environmental adaptability, including the queen number? Does this knowledge enhance your analyses and conclusions?

16. Population density details (line 232). I suggest providing more detail here. What is the specific range of population densities are proposed? Why are those densities chosen? Are the different social forms referring just to the queen number?

17. Final statement (line 235). I suggest an ending with a paragraph that explains the expected outcome of the experimental plan. What are valuable results that will be achieved? I would also suggest restating the significance of those results for scientific knowledge and specific practical outcomes. This will leave the reviewers with your summary of the outputs and their benefits as the finale.

18. Resource details (line (245). I suggest more detail. While it can be inferred what the labs will contribute, I suggest stating explicitly what tasks will be performed in the different labs. Also, I suggest stating explicitly what skills students will learn from each laboratory. This will give reviewers a better idea of the split in research effort and student benefits for the US and Israel.

19. Resources. Also, does BSF favor new proposals or extensions of previous work? If they favor new proposals, I suggest emphasizing strongly that this proposal presents work distinct from the previous grant with a novel set of objectives and outcomes.

20. Vegetation gradients (line 265). To this point, only precipitation gradients were discussed. If you are proposing to study precipitation and vegetation, I suggest that these two gradients be introduced earlier in the proposal. As is, this may cause reviewer confusion. If you are planning to study more than two gradients, perhaps a Table would assist in explaining to reviewers exactly what gradients are planned. This could include information on sampling site locations, perhaps, if this is relevant.

21. Appendix A (line 283). Why is this an appendix? It seems better placed as part of the preliminary results. Also, the Preliminary Results section seems better placed before the resources section (line 236). As written, the only results shown in Preliminary Results are for Figure 2, which is not a primary data result.