Reviewer: 1

Synopsis: The authors compare Jackson's chameleons from an invasive population on Oahu with a native population from Kenya. The invasive population is the consequence of an accidental release in 1972. The Hawaiian lizards have attributes that suggest that they have evolved in response to an environment that is free of snake and avian predators, both of which are common in Kenya. The males in the Hawaiian population attain brighter coloration than those from Kenya when confronted by another chameleon. The magnitude of the difference between their bright and cryptic color patterns is enlarged, and they are less cryptic in response to the threat of avian or snake predators.

Details: They quantify luminance and chromatic contrast of individuals from each population against the background of their respective environments then model how conspicuous they would be to either a snake or avian predator. This modelling takes into account the spectral sensitivity of the predator – birds have better color vision than snakes. The qualitative trends all show higher contrast in lizards from Hawaii than in those from Africa. The differences are not always significant, but the trends are consistent. The magnitude of the difference in chromatic contrast between when lizards are presented with another chameleon (and hence are brightly colored) versus when they are presented with the image of a predator (and hence are cryptically colored) is greater in the Hawaiian than African lizards. All of these trends are consistent with the Hawaiian lizards having evolved in response to a release from the threat of predation by predators with good color vision. An implicit assumption is that more brightly colored males have a selective advantage in this setting, or that the coloration seen in Africa represents a compromise between the advantage of bright coloration in behavioral interactions and the disadvantage associated with becoming more conspicuous to predators.

The paper is quite clearly and well written and well-illustrated. There are three issues that concern me. One is that the differences in coloration reported in the paper represent a confound of the differences in color attained by the lizards and the background against which the color is perceived. This is the way they should be evaluated because it is the way they are perceived by conspecifics and potential predators, but If they want to claim that lizard coloration has evolved, then they need to go a step further and separate out the contributions of background versus lizard to the results reported in the body of the paper. At present, the only thing we know for sure after reading what would be the published portion of the paper is that the differences in chromatic contrast between the cryptic vs. display color patterns is greater in the Hawaiian than African lizards (Fig. 3D). I note from their description of data collection and modeling of color in the natural environment in the supplement that they collected data on the color of lizards and the background separately, then combined these data to generate a "virtual" measure of how the animals would be perceived in their natural environment. This means that it is possible to present results on the lizard coloration along plus make inferences about the relative importance of lizard color versus background by modeling African and Hawaiian lizards on both African and Hawaiian backgrounds.

Now that I have read through the supplement I see that they describe doing this on the top of p. 24. The results appear in Table S5. They only looked at Hawaiian lizards on both backgrounds and it seems that there is not a significant background effect, but this does not answer my question. I am envisioning a model that includes both lizard populations on both backgrounds because I want to know if the perceived differences between the

two populations is independent of the background against which the lizards are seen. The effect of population and the population by background interaction are the tests that would interest me since those are the ones that effectively de-confound the effects of population and background. Why wasn't this done? I also think it is important to describe these extra tests in the main body of the paper since many readers are likely to raise the same question.

My second concern is that Africa is represented by one population. Granted this was a huge amount of work but what they have done carries the implicit assumption that the difference between the two populations in this study is an ancestral-descendant comparison, meaning that there is no variation in coloration among African populations that could have resulted in those that were introduced into Hawaii were different from the Kenyan population in this study from the start. Is it possible to address the extent to which there is variation among populations in Africa?

My third concern is that the differences reported here are based on measurements made on wild lizards that developed in different environments. This means that the results represent a confound of genetic differences between the two and whatever environmental differences were encountered in Hawaii versus Africa. Is it possible that some environmental effects could contribute to coloration? There is good precedent for the effects of environment, particularly diet, on the coloration of birds and fish so this is at least a possibility. Common approaches to de-confounding genes and environment are reciprocal transplants (certainly not feasible here) or common garden rearing (which is feasible). There is precedent for Science publishing such results without the benefit of any such effort to address possible confounding of genotype and environment. I am thinking of papers on Anolis lizards, but they date to the late 1990's and early 2000's. I appreciate the novelty of this work and would like to see it published here, but I also think it would benefit from whatever can be added to address, and possibly discount, the potential role of the environment in contributing to these differences among populations in coloration. I also wonder if, with this passage of time, the bar should be set higher than it was 20 years ago.

Most of the remainder of the supplement does an excellent job of showing that the color differences extend over the full body of the lizard. The color of each portion of the body is not the same so they repeated all of their analyses, but with separate color measurements on four different regions of the body, which were then included in the models as repeated measurements on the same individual. They do a very good job of showing that the chromatic differences are pretty uniform across the body. The differences in luminance are more complex but not in a way that changes the story since the main body of the paper reports that the differences between populations in the change in luminance if less dramatic than the chromatic change.

Did the authors ever quantify the repeatability of color measurements? If so, that is worth adding to the supplement.

Reviewer: 2

Hello Dr. Whiting and colleagues,

This well written manuscript represents some exciting global fieldwork, with completely robust up to date visual modelling methods, and a unique, interesting, and appropriate study system for the hypothesis being tested (introduced chameleons in a nearly predator free environment).

My main concern with the paper is that I am not completely convinced that the observed differences in coloration are the result of rapid evolution/selection, as opposed to either developmental plasticity, or the founder effect. With regards to plasticity it reminds me a bit of the series of Losos papers on limb length in Anolis sagrei. The initial thinking was that there was evidence of rapid evolution of limb length after island introductions, however later work found that limb length showed developmental plasticity depending on habitat/ branch thickness (Losos et al. 2007).

There are a number of factors that could potentially influence possible developmental components of the coloration (considering their maximum display color). Differences between the Kenya and Hawaii sites such as humidity/rainfall (which seem to be very different), temperature, seasonality, and prey types/abundance could have a possible influence on development of coloration. Additionally, I could also imagine that population density/frequency of interactions might play a role in development of coloration as influenced by hormones such as cort and T.

Unfortunately the best way to really clarify this and isolate evolution vs developmental plasticity would be captive rearing/common garden experiments, which are beyond the scope of revisions and would likely represent an entire new project (which I would still love to see!). Within the scope of the current manuscript, I think it is highly important to address the question of developmental plasticity, especially within the context of rapid evolution, citing papers such as those in the Losos series, as well as some which might give specific insight into chameleon development. Is there evidence for or against any of the variables considered above affecting the development of coloration in chameleons, or other lizards? Overall I would use much more limited/hesitant phrasing throughout the paper, that this may represent an example of rapid evolution in the wake of character release, but more work is still needed to confirm (common garden).

Another concern I have is about the timing of fieldwork, where the Kenyan individuals were studied in April while Hawaiian ones were measured in Jan/Feb. There is one mention in the text of a “breeding season,” and certainly some lizards develop new or brighter coloration during the breeding season, so it leads me to question how the sampling seasons relate to possible breeding seasons in this species (and in each locality). To address this I would need to see a bit more info about breeding seasons in this species as well as if there is evidence of seasonal color change (representing their peak display color, not rapid color change) in chameleons more generally. Also, is there data on temperature during trials (to show that they were generally similar)?

Regarding the founder effect, I do wonder if the introduced chameleons represented a more attractive/brighter selection given that they were destined for the pet trade, although the fact that they were directly imported from Kenya and not from captive stock is a good sign. I would expand the sentences on the founder effect just a bit for thoroughness, but I doubt there is more info available about the initial chameleons which is worth including.

I hope that my review is helpful to you and provides you with interesting perspectives to consider and ways to improve your manuscript. The visual modelling work presented here is top notch and I would be excited to see more work on this system in the future.