DEPARTMENT OF
BIOLOGICAL SCIENCES
Faculty of Science
and Engineering

MACQUARIE University
SYDNEY·AUSTRALIA

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Dear Dr. Vignieri,

We are contacting you in reference to our manuscript "Invasive chameleons released from predation display more conspicuous colors" (abm0862). We realise a month has passed since our paper was rejected. However, we have now had the opportunity to properly evaluate the reviewers' comments. It is notable that the reviewers found our results significant and exciting, as indicated by their positive comments:

Reviewer 1: "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."

Reviewer 2: "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)."

We believe that some of their comments reflect a lack of understanding of chameleon biology (entirely understandable) and dynamic color change—likely exacerbated by the short format of Science Reports. Our goal here is to address the major concerns highlighted by the reviewers with the hope that you will consider a resubmission.

Reviewer 1 & 2's comments can all be addressed through additional analyses and simple clarification, which we provide to each of their major points below:

1. The first point raised was that the conspicuousness of a lizard's display is confounded by background. Reviewer 1 suggested a model that included both lizard populations on both backgrounds. We have now re-analysed the data according to this suggestion. As predicted, the differences in conspicuousness of dynamic signals between Hawaii and Kenya does not depend on background environment in any context (population by background interaction effect: **Luminance**: male-male display– $F_{1, 48.7} = 0.005$, $p = 0.944$; courtship – $F_{1, 40} = 1.09$, $p = 0.303$). We have also re-parameterised an interaction model to compare how conspicuous a Hawaiian chameleon is against a Kenyan (population origin) background showing in detail how these differences do not depend on the background. These additional analyses are easily incorporated into the manuscript and effectively remove the effect of background on signal conspicuousness.

2. The second concern was the number of populations we sampled. We are unsure why this would be an issue because we sampled from the source population. Furthermore, this is a generalist species that is very unlikely to consist of multiple small populations in the area of the source population. (You can find them in suburbia, for example.) The reviewers also raise some minor points such as whether the animals were sampled during the breeding season and why the differences in the timing of field work. We can easily offer more details but in brief, we did this work during the reproductive season, which is different in Kenya and Hawaii. Importantly, the chameleons all exhibited very strong behavioral responses during courtship and male-male competition, consistent with reproductive behavior.

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3. Both reviewers raised the potential for developmental plasticity or environmental effects to explain the difference between Kenyan and Hawaiian populations. For example, both reviewers mention diet as a potential factor, and Reviewer 2 lists climatic variables (temperature, rainfall/humidity). It is important to highlight that the measured colors and color change represent a dynamic response to specific stimuli (conspecific, model predator) in contrast to the 'fixed' color signals of many birds, fishes and lizards that have been shown to be affected by diet and other environmental factors. Additionally, the mechanisms underlying the rapid color response of chameleons don't involve dietary pigments, and luminance change is partially structural (see Teyssier et al. 2015, Photonic crystals cause active colour change in chameleons. *Nat. Commun.* 6, 6368).

Both reviewers refer to the anole lizard system (papers led by Jonathan Losos) when mentioning the possible role of plasticity and environmental effects. Losos' work dealt with limb morphology which, unsurprisingly, was affected by the developmental environment and the diameter of perches available to young anoles during development. Our study deals with dynamic color change, which is a form of phenotypic plasticity, and unlikely to be similarly constrained.

For these reasons, we maintain that environmental effects (other than predation) are unlikely to account for *all* of the differences in the color responses we observed between populations. Nevertheless, we acknowledge that we cannot definitively exclude this possibility. Moreover, plasticity (including color plasticity) can evolve, and can itself be an adaptive response. Indeed, we explicitly state in the manuscript that the observed results are consistent with local adaptation, in which phenotypic plasticity, which can also evolve rapidly, may also play a role. We suggest that the question of whether the population differences are genetic or environmental (most likely both) is somewhat a moot point. What is significant about our work is the demonstrated pattern of character release in a dynamic signal. We can do a better job of making this clear in a revision.

4. The last point raised was the possibility that the observed patterns of character release could be due to a founder effect, especially given that the chameleons were collected for the pet trade. While we acknowledge this possibility in the manuscript, it is exceedingly unlikely. Chameleons are almost always encountered in a camouflaged color state because they are solitary and only use display colours during relatively brief social encounters. Therefore, this is not a species in which hunters could select the most brightly coloured individuals. Nevertheless, even if pet traders were selecting for larger animals, our analyses control for any relationship body size might have on colour. Finally, the fact that all the changes we document are in an adaptive direction make it very unlikely that they can all be explained by a founder effect.

In light of our response to the main concerns raised by the reviewers, we hope you will consider a resubmission and look forward to hearing back from you.

Kind regards,

Martin Whiting, on behalf of all authors
Professor of Animal Behaviour