Peer Review File

Observing character displacement from process to pattern in a novel vertebrate community

Corresponding Author: Dr James Stroud

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Character displacement has a substantial contribution to shape ecological communities, but usually we can only investigate it from its results. Here, a lizard system composed of two introduced species provides a unique opportunity for dissecting the initial steps of a supposedly long process. Authors integrate results from morphometrics, habitat use, behaviour and population genetics quite nicely to demonstrate fast phenotypic shifts in response to another species close in the niche space. My main criticism is that shift are solely attributed to natural selection with, in my opinion, no sufficient evidence. If these aspects are clarified and criticisms adequately the manuscript may attain the standards of the journal.

Detailed comments:

Title
Considering that natural selection is not unambiguously demonstrated (see below), it should be replaced by a more neutral term (e.g. “Rapid adaptive shifts…”). In any case “drives” should be replaced by “drive”.

Introduction
Line 50
Authors should be careful suing terms. Invasiveness mean negative repercussions on native biota. If such evidence cannot be provided, the term introduced would be preferred. Indeed, the distinction is not much relevant for the analyses.

Line 54
Please, describe the distribution of both species in Florida. Do they differ? Do they follow any environmental pattern?

Line 72
The distances between these locations are relevant to determine how independent are these sites.

Line 73
Please, indicate a reference for the number of generations of add the info in Table 1.

Line 90
Apparently, other Anolis sp. are present. This should be clarified in the experimental design, because character displacement could be limited/enhanced by the presence of third parts.

Results
Lines 146-147
You certainly detected shifts in limb morphology, but you cannot test if this is due to selection to phenotypic plasticity or both with your data. In principle, considering the fast rate observed, and the experimental evidence already available for Anolis (even in A. sagrei) one would favour phenotypic plasticity. Maybe you could use the shape of the character distribution and the variance as proxies, but no information is provided.

Kolbe, J. J. and Losos, J. B. 2005. Hind-Limb Length Plasticity in Anolis carolinenesis. - J. Herpetol. 39: 674-678.

Langford, G. J., Macedonia, J. M., Bessette, C. W., Matey, J. L., Raboin, B. A., Schiffmacher, A. E. and Reynolds, B. J. 2014. Phenotypic Plasticity in the Relative Hind-Limb Growth of Lab-Reared Anolis sagrei: Replication of Experimental Results and a Test of Perch Diameter Preference. - J. Herpetol. 48: 228-232.
Losos, J. B., Creer, D. A., Glossip, D., Goellner, R., Hampton, A., Roberts, G., Haskell, N., Taylor, P. and Ettling, J. 2013. Evolutionary Implications of Phenotypic Plasticity in the Hindlimb of the Lizard Anolis sagrei. - Evolution 54: 301-305.

Of course, fast responses to selection have been reported in Anolis (even in A. cristatellus), but genomic evidence was provided, which is not the case here.

Winchell, K. M., Campbell-Staton, S. C., Losos, J. B., Revell, L. J., Verrelli, B. C. and Geneva, A. J. 2023. Genome-wide parallelism underlies contemporary adaptation in urban lizards. - PNAS 120: e2216789120.

Lines 156-157
You correlated morphological changes between forelimb and hindlimb.

Lines 167-168 and 173-174
I admit this may suggest selection, but evidence for habitat-mediated phenotypic plasticity is also strong. If interspecific interactions promoted habitat shift as you demonstrated, then plastic changes in limb morphology will arise even earlier than selection removes maladapted morphologies. Of course, there is evidence that limb length is heritable, but this is not enough because there is also evidence the limb development may be affected by habitat structure. You need additional evidence (genomics, common garden) for disentangling both forces.

Line 184
Replace “Natural selection“ by “Adaptive responses” or something similar.

Line 190
Same.

Discussion
Lines 212-218
Authors should tone down the claim for natural selection while no stronger evidence (including genomics and or common garden experiments) is available.

Line 224
Consider that in introduced, species populations, and particularly those of A. cristatelus, could be not attaining the carrying capacity, hence, effecting to the (exploitative) competition.

Line 230
Developmental plasticity is here invoked as a delaying force, but why not as accelerating one? Discuss it with literature date.

Methods
Line 262
Please, indicate if other Anolis sp. are present in the study sites and which potential predators exist namely compared to those present in Cuba and Puerto Rico.

Line 271
Justify why this method of analysis of trophic ecology was preferred compared to stable isotopes or metabarcoding. I understand you were interested in prey size and mass, and not only in OTUs.

Line 285
Indicate the precision of the calliper.

Line 297
Did such transformation ensure Normality? Did you test for homoscedasticity? This is not trivial because character displacement could induce variation in variances besides means.

Line 346
Were limb-SVL residuals calculated separately for each species? Would you expect and sexual shape dimorphism is this trait? If not the case, justify with references.

Reviewer #2

(Remarks to the Author)
In "Rapid shifts in natural selection drives character displacement in the wild", Stroud et al. study character displacement between sympatric populations of Anolis sagrei and A. cristatellus. They have both a "pattern" side to their study, wherein they compare sympatric and allopatric populations in their limb morphology and "habitat" use (time spent locomoting on different strata/substrates), and a "process" side to their study, wherein they compare the same set of morphological variables before and after an invasion of A. cristatellus into existing A. sagrei populations they had been studying. The

authors did not observe phenotypic shifts in the latter part of the study, but did observe strong shifts in selection strength over the period of their study; Breeder's equation plus additional sources of information suggest such a short period of study would be insufficient to observe shifts in phenotype.

The paper is excellent and I have mostly only minor point. One point approaching "major" is the lack of connection between the interspecific interactions/network graph portion of the study, and the rest of the study. My comments below make it clear how this aspect of the study could be improved.

Line 23: is lacking -> are rare

Line 36: "to faciliate coexistence" - I know the authors don't mean this exactly, but this phrasing makes it sound like there is a purpose behind divergent evolution like this. Probably wouldn't notice if it was buried in the manuscript, but starting off the first sentence like this caught my attention.

Lines 70-71: Missing a short phrase here, e.g. "To expand on this preliminary finding,"

Lines 110-111: What are the nature of these interspecific interactions? This is a substantial oversight in the manuscript as is. Nowhere is the question of behavioral dominance addressed. What are we to infer from Fig 1E? This figure and portion of the study strikes me as a missed opportunity. In general, the purpose/story of this figure is not explained, and I suspect there are better ways to arrange nodes to tell the untold story. Furthermore, what is happening during these interactions? Is one species consistently displacing the other from a perch? Do they just give each other the stink eye? Dewlaps? Tell us more. Based on selection differences described later (and shifts in community level abundance), I would naively posit that one species is behaviorally dominant to the other, but nowhere is this mentioned, which leads me to believe this is not the case. State this somewhere!

Line 118: Do you mean Fig. 2?

Line 173: I would not call this incidental.

Lines 177-181: I would not really consider this a result. Maybe something to integrate into the discussion? Strikes me as something that was inserted to preempt reviewer criticism, not an actual finding.

Line 239: the Martin and Martin warbler papers from Arizona are worth referencing here.

Line 280: Could the word individual here be replaced with something more appropriate such as specific or particular?

Lines 313-325: I'm confused by this. An interspecific interaction is--? Being within 3m of each other, and nothing else? This is not clearly stated, but if that is what is meant, is this a useful definition? Please tell us more here.

Lines 329-337: Tell us more about interactions, or lack thereof, between these species and the study species.

Reviewer #3

(Remarks to the Author)
In this study, Stroud and colleagues report on a pattern of character displacement between two anolis species introduced to Florida at different times in the last century, and taking advantage of a recent sympatric site, they document changes in selection in one species in the direction of the presumably evolved changes they observed in longer established sympatric sites.

Character displacement is almost always studied from a historical perspective, by comparing traits of the competing species in areas of allopatry and sympatry. While experiments can be used to further investigate the process of character displacement, it is very rare to catch this process ongoing in nature as the authors have done here. I found no issues with the studies methods or analysis, and the paper is interesting, engaging, and makes an important contribution towards our understanding of character displacement.

I do have a few topics for the authors to consider, and that I hope will be helpful. First, the authors organize the manuscript by distinguishing results focused on the pattern vs the process of ecological character displacement. I found this to be useful, but it did make clear to me that there are remaining gaps in our understanding of the process of character displacement in this system. While it appears clear from the results that A. cristatellus is the cause of the altered selection regime for A. sagrei, what ecological interactions are responsible are not as clear.

The authors attribute natural selection in sagrei (and the changes in morphology and habitat use in older sympatric populations) to resource competition with cristatellus. However I think the data supporting this are lacking. For instance, there is no shift in diet between allopatric and sympatric populations. The authors seem to use the shifts in perch height to imply resource competition but I would argue a more likely explanation would point to antagonistic interactions between the two anolis species (antagonistic character displacement). While there has long been debate about the definitions and terminology associated with character displacement, it is generally agreed that the pattern of character displacement can be the outcome of different, distinct ecological interactions (Grether et al. 2009; Pfennig and Pfennig 2012; Stuart et al 2017). In this view, I would argue that it is not clear that the authors are documenting “ecological” character displacement, since it appears that resource use has not diverged. And so, I suggest following the suggestion of Stuart et al 2017, and solely referring to these results as character displacement.

A distinct but related point: there is very little discussion in the paper of the ecological interactions causing character displacement here, beyond the suggestion that it is due to competition for resources. I think that this topic deserves to be included in the Discussion and perhaps in the Introduction as well - what could be the underlying ecological interactions - e.g., resource competition, interspecific aggression - and what is the supporting evidence among the alternative hypotheses?

Other comments:

1. Line 227. Is there any other evidence for evolution as the underlying cause of character displacement in the older sympatric populations? I agree that the absence of divergence in the new sympatric site compared to the older sympatric sites does suggest that phenotypic plasticity is not responsible for the older divergence but this is not the strongest evidence that character displacement has indeed evolved - alternatively, the recently sympatric sagrei population could simply have low plasticity compared to other populations (not saying this is true but it is possible). I suggest the authors more clearly state the evidence for the evolution of character displacement in this system vs. other possibilities.

2. Are the sympatric sites independent? That is, has character displacement (the older sympatric sites) occurred independently more than once here or did it perhaps evolve once in sagrei and spread? The molecular data does not show that allopatric and sympatric populations cluster into two groups, supporting independent origins, but this could be made more clear in the text.

3. A map of the study sites would be very helpful I think.

Sincerely,
Ryan Martin

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
I reviewed an earlier version on this work. The second version has included most of my suggestions and clarified some of unexplained aspects. In particular, I am glad to see that the claimings for natural selection, across the text and in the title, are now more proportional to the evidence and that alternative scenarios for short evolutionary timeframes (phenotypic plasticity) as discussed. Authors are also to be congratulated for providing clear information on the location and composition of the communities, including the non-target anole species. Overall, I consider the manuscript is now suitable for the journal.

Detailed comments:
Line 189
Replace “Table 1. Natural selection during…” by “Morphological shift attributable to natural selection during…”.

Line 195
Replace “Table 1. Natural selection on Anolis sagrei…” by “Morphological shift attributable to natural selection on Anolis sagrei…”.

Line 272
A. sagrei and A. cristatellus should be in italics.

Reviewer #2

(Remarks to the Author)
The revised article is great and addresses my previous minor concerns. I look forward to seeing it in print. Also:

Line 105: despite sounding correct due to the last letter of the specific epithet, I believe perch/perches should both be plural, or not, for consistency.

Line 268: missing a word

Reviewer #3

(Remarks to the Author)
The authors have carefully addressed my comments on the first submission, and I have no further questions or concerns. This is an interesting and exciting study, and while there are still pieces to be explored (the ecological cause(s) of character

displacement here, and direct evidence of evolutionary divergence), this study makes a strong and important contribution to the field.

Below we respond to each of the reviewer's comments point-by-point. We have also attached a 'tracked changes' copy of our manuscript so that all edits are obvious and identifiable.

Start summary: Reviewer #1
Character displacement has a substantial contribution to shape ecological communities, but usually we can only investigate it from its results. Here, a lizard system composed of two introduced species provides a unique opportunity for dissecting the initial steps of a supposedly long process. Authors integrate results from morphometrics, habitat use, behaviour and population genetics quite nicely to demonstrate fast phenotypic shifts in response to another species close in the niche space. My main criticism is that shift are solely attributed to natural selection with, in my opinion, no sufficient evidence. If these aspects are clarified and criticisms adequately the manuscript may attain the standards of the journal.
End summary: Reviewer #1

Thank you for your thorough review and positive feedback on our manuscript. Regarding your main concern about attributing shifts solely to natural selection without sufficient evidence, please see below for our extensive revisions on this topic. Through the revised manuscript, we have toned down our claims where appropriate and acknowledge alternative explanations. Thank you for your detailed review. Our revisions in response to your comments and feedback have improved our manuscript.

Start Comment 1: Reviewer #1
Title: Considering that natural selection is not unambiguously demonstrated (see below), it should be replaced by a more neutral term (e.g. "Rapid adaptive shifts…"). In any case "drives" should be replaced by "drive".
End Comment 1: Reviewer #1

Thank you for your suggestion regarding the title. We have made the following title edit which removes the explicit assumption that our observation of rapid shifts in selection pressures in a novel contact zone is responsible for driving the established pattern of trait divergence in other communities (i.e., character displacement):

"***Connecting process to pattern: Divergent natural selection and character displacement in a novel vertebrate community***"

We chose this title because our data do show that changes in natural selection in a novel contact zone (from no selection on hind limb length to strong positive directional selection for longer hind limbs; Fig 3) coincides with the arrival of A. cristatellus. The direction of change in selection aligns with the broader landscape patterns of character displacement (longer hind limbs in sympatric communities; Fig 2) – we include "Connecting process to pattern:" to align the reader with the focus of the study.

Start Comment 2: Reviewer #1
Introduction (Line 50): Authors should be careful suing terms. Invasiveness mean negative repercussions on native biota. If such evidence cannot be provided, the term introduced would be preferred. Indeed, the distinction is not much relevant for the analyses.
End Comment 2: Reviewer #1

We appreciate your point about using the appropriate terminology. You are right that the term 'invasive' can be loaded, and so we have edited all reference to 'invasive species' in our manuscript to 'non-native species'.

Start Comment 3: Reviewer #1
Line 54: Please, describe the distribution of both species in Florida. Do they differ? Do they follow any environmental pattern?
End Comment 3: Reviewer #1

In our Methods section 'Study system' we have provided information on the distributions of both species in Florida. While these species differ in the extent of their distributions in Florida, possibly due to their differences in arrival date, the environmental correlates of their distributions are very similar. We have supported this using information from both field guides (e.g., Krysko et al. 2019), as well as a suite of empirical studies that range from those collecting detailed microhabitat data (e.g., Kolbe et al. 2016, Battles et al. 2018, 2019, Stroud et al. 2019) and landscape scale assessments (e.g., Clements et al. 2019) and potential habitat suitability (Mothes et al. 2018). We made edits as follows, with pertinent sentences highlighted in bold and underlined:

Methods

Character displacement: the pattern

Study system

Anolis sagrei and A. cristatellus are small (40-70 mm snout-to-vent length; Fig 1A), diurnal lizards that diverged ca. 50-60Ma and do not hybridize. These species are highly convergent in ecology, morphology, and behavior and classified in the same 'trunk-ground' ecomorph group19. Anolis sagrei was first recorded in Miami, south Florida USA, in the mid-1940s (45,46); A. cristatellus in the early 1970s (31,32,46). **<u>Anolis sagrei is widespread in Florida,</u>**

while A. cristatellus is limited to only Miami-Dade County (21,46). In their native ranges, both A. sagrei and A. cristatellus are habitat generalists and occupy similarly structured environments (47,48). In south Florida, both species occupy a gradient of habitat types from closed canopy forest to urban environments (21,46,49–52) and are estimated to have very similar statewide predicted habitat suitability (53). We studied ecological resource use (habitat use and diet), movement behavior, and morphology in nine sites across Miami (three allopatric sites per species and three sympatric sites that include both species; Extended Data Table 1).

Refs

Battles, A. C., Moniz, M. & Kolbe, J. J. Living in the big city: preference for broad substrates results in niche expansion for urban Anolis lizards. Urban Ecosystems 21, 1087–1095 (2018).

Battles, A. C. & Kolbe, J. J. Miami heat: urban heat islands influence the thermal suitability of habitats for ectotherms. Global change biology 25, 562–576 (2019).

Clements, S. L., Catania, S. V. & Searcy, C. A. Non-native species dominate herpetofaunal community patterns in both native and non-native habitat patches in urban Miami-Dade County. Biological invasions 21, 1775–1788 (2019).

Kolbe, J. J. et al. Determinants of spread in an urban landscape by an introduced lizard. Landscape Ecology 31, 1795–1813 (2016).

Krysko, K. L., Enge, K. M. & Moler, P. E. Amphibians and Reptiles of Florida. (University of Florida Press, 2019).

Mothes, C. C., Stroud, J. T., Clements, S. L. & Searcy, C. A. Evaluating ecological niche model accuracy in predicting biotic invasions using South Florida's exotic lizard community. Journal of biogeography 46, 432–441 (2019).

Rand, A. S. Ecological distribution in anoline lizards of Puerto Rico. Ecology 45, 745–752 (1964).

Scherrer, R., Donihue, C. M., Reynolds, R. G., Losos, J. B. & Geneva, A. J. Dewlap colour variation in Anolis sagrei is maintained among habitats within islands of the West Indies. J of Evolutionary Biology 35, 680–692 (2022).

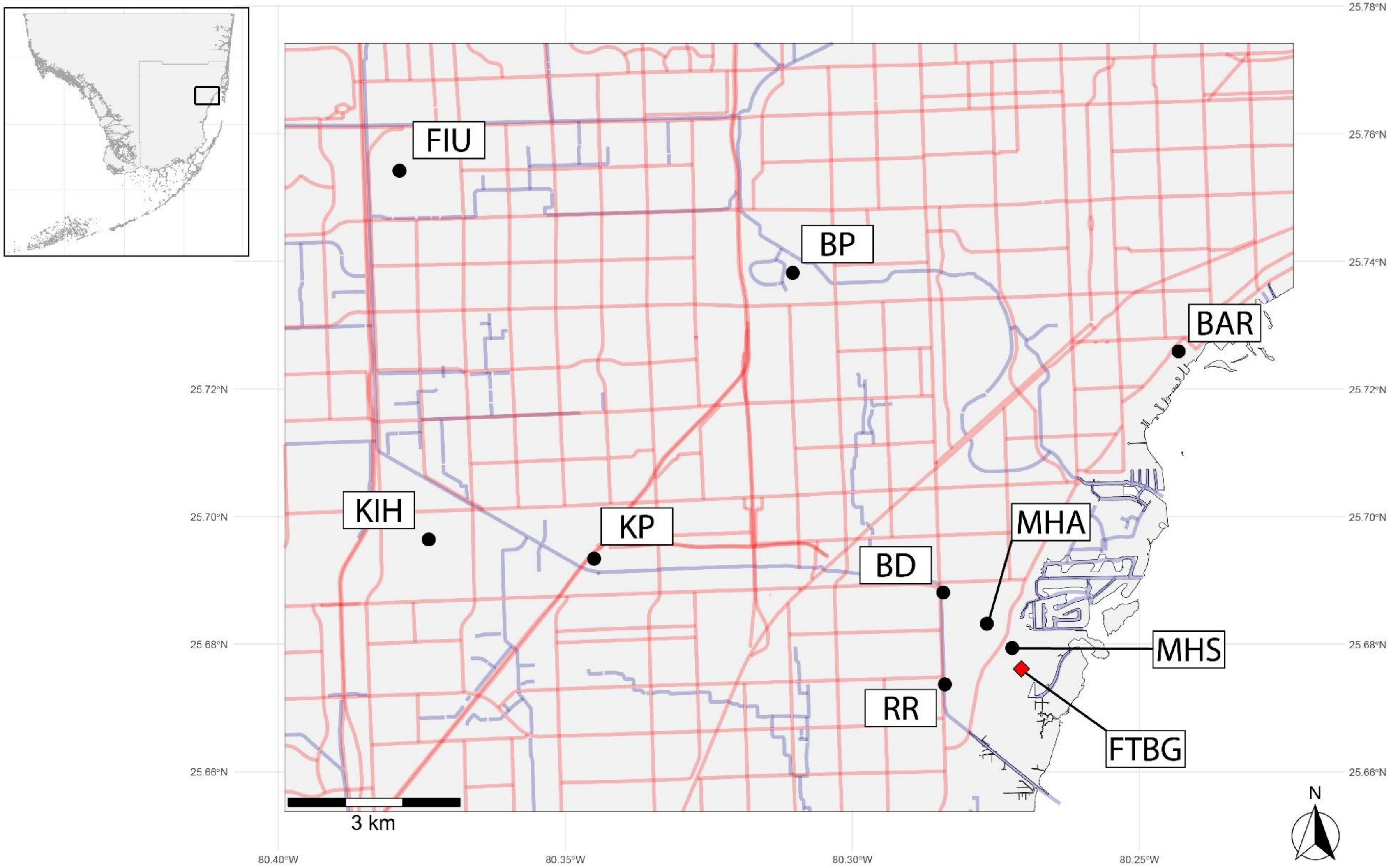
Stroud, J. T. et al. Behavioral shifts with urbanization may facilitate biological invasion of a widespread lizard. Urban Ecosystems 22, 425–434 (2019).

Start Comment 4: Reviewer #1
Line 72: The distances between these locations are relevant to determine how independent are these sites.
End Comment 4: Reviewer #1

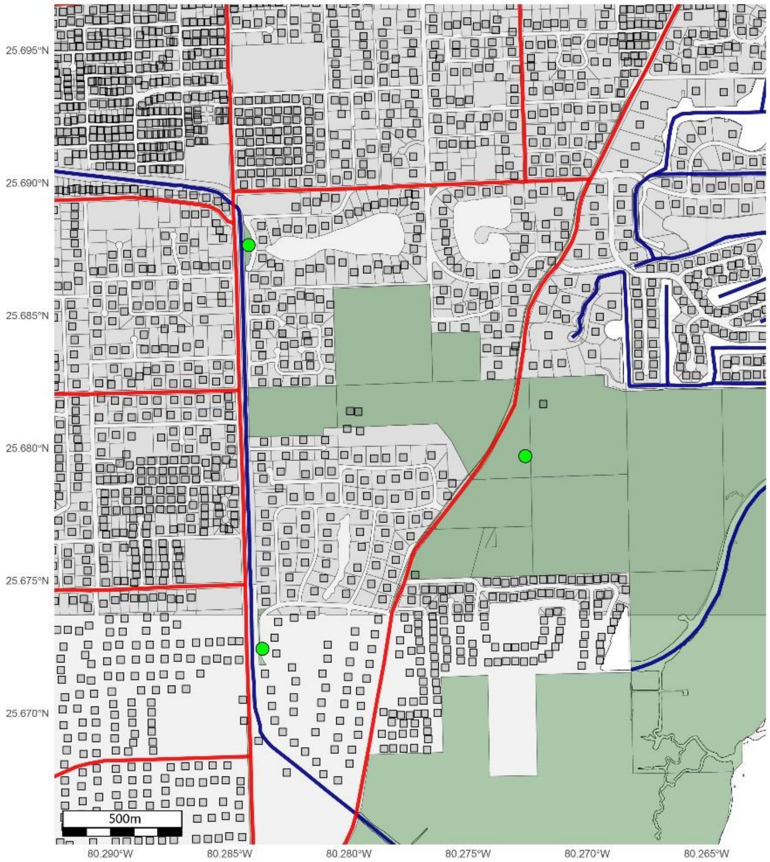
We have created a map of these sites as also recommended by Reviewer 3 (**Extended Data Figure 1**; below). The selection of sympatric sites is limited by the distribution of *A. cristatellus* and the availability of long-term (>30 year) sympatric communities. Nevertheless, we selected 3 that maximize distances between locations, and which also have major barriers to dispersal between them (e.g., roads, canals; see additional map below). We have also calculated the geographic straight-line distances between sites and included that in the Supplementary Material as a table (**Extended Data Table 2**). The sympatric sites are located on average 1.47km from each other (straight-line distances Red Road Canal (RR)-Banyan Drive park (BD) = 1.60km; Red Road Canal (RR)-Matheson Hammock park (MHS) = 1.32km; Matheson Hammock park (MHS)-Banyan Drive park (BD) = 1.50km). These locations are also intersected by a complex matrix of human development (see additional map below).

Refs

Salzburg, M. A. Anolis sagrei and Anolis cristatellus in southern Florida: a case study in interspecific competition. Ecology 65, 14–19 (1984).



Extended Data Fig. 1. Location of study sites: see Extended Data Table 1 for information on connecting site code to site name. Red lines are major roads; blue lines are canals. Inset map of south Florida; black box highlights the study region.



Zoomed map of sympatric sites (green points; Banyan Drive park [BD], Matheson Hammock Park [MHS], Red Road canal [RR]) showing their distribution within a complex matrix of roads, streets, and properties. Our admixture analysis (main manuscript Results and Extended Data Fig 2) identified no genetic structure separating sympatric or allopatric sites, suggesting gene flow between these sympatric locations is low or at least comparable to gene flow with allopatric sites which are located much further away (Extended Data Table 2).

Start Comment 5: Reviewer #1
Line 73: Please, indicate a reference for the number of generations of add the info in Table 1.
End Comment 5: Reviewer #1

We have added the following citations to support this statement, which provide details on the history of species at these locations in south Florida:

Bell, L.N., 1953. Notes on three subspecies of the lizard Anolis sagrei in southern Florida. Copeia, 1953(1), pp.63-63.

Kolbe, J. J. et al. Determinants of spread in an urban landscape by an introduced lizard. Landscape Ecology 31, 1795–1813 (2016).

Salzburg, M. A. Anolis sagrei and Anolis cristatellus in southern Florida: a case study in interspecific competition. Ecology 65, 14–19 (1984).

Start Comment 6: Reviewer #1
Line 90: Apparently, other Anolis sp. are present. This should be clarified in the experimental design, because character displacement could be limited/enhanced by the presence of third parts.
End Comment 6: Reviewer #1

We explicitly mention this in the "*Study site*" description in the section "Character displacement: the process" section (pertinent sentence bold and underlined):

Character displacement: the process

Study site

We measured survival of A. sagrei from 2016-2019 on a 6,000m2 island located within the Fairchild Tropical Botanical Gardens (FTBG) in Miami, Florida USA (Extended Data Table 1), that contains three other Anolis species (73), all of which are substantially more arboreal than both A. sagrei and A. cristatellus; the trunk anole (A. distichus), the arboreal green anole (A. carolinensis), and the giant arboreal specialist A. equestris. **All species are present at all locations studied in our comparative analysis** (21). No changes in structural habitat or other environmental factors (e.g., other lizard species) occurred during this study, with the exception of the arrival of A. cristatellus.

However, we now realize that information would be better presented earlier in the manuscript. Thank you for pointing this out. We now explicitly state this in "Character displacement: the pattern" section in the "*Study system*" subsection, with associated edits to the "Character displacement: the process" section. We also provided additional references to which provide ecological information to support the ecological distinctiveness of these other Anolis species to the two focal species of this study (A. sagrei and A. cristatellus). We have made edits as follows, with the pertinent sentences highlighted in bold and underlined:

Methods

Character displacement: the pattern

Study system

Anolis sagrei and A. cristatellus are small (40-70 mm snout-to-vent length; Fig 1A), diurnal lizards that diverged ca. 50-60Ma and do not hybridize. These species are highly convergent in ecology, morphology, and behavior and classified in the same ‘trunk-ground’ ecomorph group19. Anolis sagrei was first recorded in Miami, south Florida USA, in the mid-1940s (45,46); A. cristatellus in the early 1970s (31,32,46). Anolis sagrei is widespread in Florida, while A. cristatellus is limited to only Miami-Dade County (21,46). In their native ranges, both A. sagrei and A. cristatellus are habitat generalists and occupy similarly structured environments (47,48). In south Florida, both species occupy a gradient of habitat types from closed canopy forest to urban environments (21,46,49–52) and are estimated to have very similar statewide predicted habitat suitability (53). We studied ecological resource use (habitat use and diet), movement behavior, and morphology in nine sites across Miami (three allopatric sites per species and three sympatric sites that include both species; Extended Data Table 1). **All sites that contain three other Anolis species, all of which are substantially more arboreal than both A. sagrei and A. cristatellus; the trunk anole (A. distichus), the arboreal green anole (A. carolinensis), and the giant arboreal specialist A. equestris (21,46,54-57).**

Character displacement: the process

Study site

We measured survival of A. sagrei from 2016-2019 on a 6,000m2 island located within the Fairchild Tropical Botanical Gardens (FTBG) in Miami, Florida USA (Extended Data Table 1). **As with the locations in our comparative analysis, three other Anolis species are also present in this community (A. carolinensis, A. distichus, and A. equestris; see ref[26])**. ~~, that contains three other Anolis species73, all of which are substantially more arboreal than both A. sagrei and A. cristatellus; the trunk anole (A. distichus), the arboreal green anole (A. carolinensis), and the giant arboreal specialist A. equestris. All species are present at all locations studied in our comparative analysis21.~~ No changes in structural habitat or other environmental factors (e.g., other lizard species) occurred during this study, with the exception of the arrival of A. cristatellus.

Additional refs

Giery, S.T., Lemoine, N.P., Hammerschlag-Peyer, C.M., Abbey-Lee, R.N. and Layman, C.A., 2013. Bidirectional trophic linkages couple canopy and understorey food webs. Functional ecology, 27(6), pp.1436-1441.

Giery, S.T., Vezzani, E., Zona, S. and Stroud, J.T., 2017. Frugivory and seed dispersal by the invasive knight anole (Anolis equestris) in Florida, USA. Food Webs, 11, pp.13-16.

Ljustina, O. and Stroud, J.T., 2019. Little evidence for size-structured habitat use in a diverse Anolis community. Anolis Newsletter VII, pp.136-143.

Start Comment 7: Reviewer #1
Results (Lines 146-147): You certainly detected shifts in limb morphology, but you cannot test if this is due to selection to phenotypic plasticity or both with your data. In principle, considering the fast rate observed, and the experimental evidence already available for Anolis (even in A. sagrei) one would favour phenotypic plasticity. Maybe you could use the shape of the character distribution and the variance as proxies, but no information is provided.

Kolbe, J. J. and Losos, J. B. 2005. Hind-Limb Length Plasticity in Anolis carolinenesis. - J. Herpetol. 39: 674-678.
Langford, G. J., Macedonia, J. M., Bessette, C. W., Matey, J. L., Raboin, B. A., Schiffmacher, A. E. and Reynolds, B. J. 2014. Phenotypic Plasticity in the Relative Hind-Limb Growth of Lab-Reared Anolis sagrei: Replication of Experimental Results and a Test of Perch Diameter Preference. - J. Herpetol. 48: 228-232.
Losos, J. B., Creer, D. A., Glossip, D., Goellner, R., Hampton, A., Roberts, G., Haskell, N., Taylor, P. and Ettling, J. 2013. Evolutionary Implications of Phenotypic Plasticity in the Hindlimb of the Lizard Anolis sagrei. - Evolution 54: 301-305.

Of course, fast responses to selection have been reported in Anolis (even in A. cristatellus), but genomic evidence was provided, which is not the case here.

Winchell, K. M., Campbell-Staton, S. C., Losos, J. B., Revell, L. J., Verrelli, B. C. and Geneva, A. J. 2023. Genome-wide parallelism underlies contemporary adaptation in urban lizards. - PNAS 120: e2216789120.
End Comment 7: Reviewer #1

Thank you for raising the important point about the potential role of phenotypic plasticity in the observed shifts in limb morphology. We agree that our data do not allow us to distinguish between adaptive evolution and phenotypic plasticity. Our data do show evidence that natural selection for longer hind limbs occurs in the novel community, although we are obviously unable to test if selection is also responsible for the differences in limb morphology in the established communities that we sampled (as those incipient stages of novel contact occurred decades ago). A clear way to test if our observed limb differences of A. sagrei in established sympatric versus allopatric populations would be to conduct a common garden breeding experiment to investigate limb length heritability. While this is beyond the scope of the current manuscript (as collecting wild lizards and generating F2 lineages in laboratory common garden conditions would require two-three years), we have included the following new paragraph in our study that discusses these alternative explanations. We also directly reference the studies that you mention:

Our observed differences in limb length between established sympatric and allopatric communities (Fig 2) could also have been the result of processes other than adaptive evolution by natural selection. For example, previous studies of laboratory populations of Anolis lizards have observed phenotypic plasticity in hind limb length (34,38,39). As all prior evidence for limb length plasticity of Anolis lizards is derived from captive populations with access to only a limited range of structure habitat types (e.g., treatments with either only ‘narrow’ or ‘wide’ perches; refs[38,39]), it is unclear if wild populations also demonstrate such plasticity. Future tests of the plasticity versus adaptive evolution hypothesis of limb length in Anolis lizards would benefit from common garden experiments that explicitly measure trait heritability under controlled conditions.

Start Comment 8: Reviewer #1
Lines 156-157: You correlated morphological changes between forelimb and hindlimb.
End Comment 8: Reviewer #1

For this test (lines 156-157) we were not investigating correlated morphological changes, but correlational selection on forelimb and hind limb lengths (i.e., Fig 3). This is a standard approach to investigate correlational selection on related traits using the statistical framework developed by Lande & Arnold (1983). This is sometimes referred to as covariance or selection on trait combinations.

Ref

Lande, R. and Arnold, S.J., 1983. The measurement of selection on correlated characters. Evolution, pp.1210-1226.

Start Comment 9: Reviewer #1
Lines 167-168 and 173-174: I admit this may suggest selection, but evidence for habitat-mediated phenotypic plasticity is also strong. If interspecific interactions promoted habitat shift as you demonstrated, then plastic changes in limb morphology will arise even earlier than selection removes maladapted morphologies. Of course, there is evidence that limb length is heritable, but this is not enough because there is also evidence the limb development may be affected by habitat structure. You need additional evidence (genomics, common garden) for disentangling both forces.
End Comment 9: Reviewer #1

We agree that additional genomic evidence, and especially common garden experiments, will be the best way to disentangle these processes and find out which is the predominant driver of our observed trait differences. Our previous new paragraph (see Comment 7 above) also provides these suggestions for future studies. However, we do not believe our study provides evidence for habitat-mediated phenotypic plasticity, although other laboratory studies have found evidence for this.

Start Comment 10: Reviewer #1
Line 184: Replace “Natural selection“ by “Adaptive responses” or something similar.
End Comment 10: Reviewer #1

We respectfully disagree with this suggestion. This table is showing the results of direct tests of natural selection on a population (and not adaptive responses or change), so the current title is the most appropriate.

Start Comment 11: Reviewer #1
Line 190: Same as Comment 10.
End Comment 11: Reviewer #1

As in Comment 10, the results presented in this table (Table 2) are referring to estimates of natural selection directly measured in the wild, so the current table title is the most appropriate.

Start Comment 12: Reviewer #1
Discussion (Lines 212-218): Authors should tone down the claim for natural selection while

no stronger evidence (including genomics and or common garden experiments) is available.
End Comment 12: Reviewer #1

We believe the current text here is appropriate as our data do show significant shifts in biased survival related to variation in limb length in our focal study population (Table 1, 2; Fig 3), *sensu* a standard working definition of natural selection (as described statistically in Lande & Arnold 1983, Schluter 1988, Brodie et al. 1995; and compiled and analyzed meta-analytically in Siepielski et al. 2009, 2013, 2017). Natural selection was estimated using standard protocols for evolutionary field studies, as described in our Methods section '*Measuring natural selection*', and used in other evolutionary field studies (e.g., Darwin's finches, Grant & Grant 2014, Beausoleil et al. 2023; guppies, Reznick & Travis 2019; Caribbean pupfish, Martin & Wainwright 2013, Martin 2016), as well as those from Anolis field studies from our group (e.g., Stroud et al. 2023, Lapiedra et al. 2018, Losos et al. 2004).

As in previous comments, we agree that common garden experiments or genomic data would be excellent evidence for testing if the shifts in natural selection that we observed have led to evolutionary change.

Beausoleil, M.O., Carrión, P.L., Podos, J., Camacho, C., Rabadán-González, J., Richard, R., Lalla, K., Raeymaekers, J.A., Knutie, S.A., De León, L.F. and Chaves, J.A., 2023. The fitness landscape of a community of Darwin's finches. Evolution, 77(12), pp.2533-2546.

Brodie, E.D., Moore, A.J. and Janzen, F.J., 1995. Visualizing and quantifying natural selection. Trends in Ecology & Evolution, 10(8), pp.313-318.

Grant, P.R. and Grant, B.R., 2014. 40 years of evolution: Darwin's finches on Daphne Major Island. Princeton University Press.

Lande, R. and Arnold, S.J., 1983. The measurement of selection on correlated characters. Evolution, pp.1210-1226.

Lapiedra, O., Schoener, T.W., Leal, M., Losos, J.B. and Kolbe, J.J., 2018. Predator-driven natural selection on risk-taking behavior in anole lizards. Science, 360(6392), pp.1017-1020.

Losos, J.B., Schoener, T.W. and Spiller, D.A., 2004. Predator-induced behaviour shifts and natural selection in field-experimental lizard populations. Nature, 432(7016), pp.505-508.

Martin, C.H. and Wainwright, P.C., 2013. Multiple fitness peaks on the adaptive landscape drive adaptive radiation in the wild. Science, 339(6116), pp.208-211.

Martin, C.H., 2016. Context dependence in complex adaptive landscapes: frequency and trait-dependent selection surfaces within an adaptive radiation of Caribbean pupfishes. Evolution, 70(6), pp.1265-1282.

Reznick, D.N. and Travis, J., 2019. Experimental studies of evolution and eco-evo dynamics in guppies (Poecilia reticulata). Annual Review of Ecology, Evolution, and Systematics, 50, pp.335-354.

Schluter, D., 1988. Estimating the form of natural selection on a quantitative trait. Evolution, 42(5), pp.849-861.

Siepielski, A.M., DiBattista, J.D. and Carlson, S.M., 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. Ecology letters, 12(11), pp.1261-1276.

Siepielski, A.M., Gotanda, K.M., Morrissey, M.B., Diamond, S.E., DiBattista, J.D. and Carlson, S.M., 2013. The spatial patterns of directional phenotypic selection. Ecology letters, 16(11), pp.1382-1392.

Siepielski, A.M., Morrissey, M.B., Buoro, M., Carlson, S.M., Caruso, C.M., Clegg, S.M., Coulson, T., DiBattista, J., Gotanda, K.M., Francis, C.D. and Hereford, J., 2017. Precipitation drives global variation in natural selection. Science, 355(6328), pp.959-962.

Stroud, J.T., Moore, M.P., Langerhans, R.B. and Losos, J.B., 2023. Fluctuating selection maintains distinct species phenotypes in an ecological community in the wild. Proceedings of the National Academy of Sciences, 120(42), p.e2222071120.

Start Comment 13: Reviewer #1
Line 224: Consider that in introduced, species populations, and particularly those of A. cristatelus, could be not attaining the carrying capacity, hence, effecting to the (exploitative) competition.
End Comment 13: Reviewer #1

Yes, we agree that there could be a lag time associated with population growth, as observed in many species' introductions. We would therefore expect exploitative interspecific competition coefficients between A. cristatellus and A. sagrei to strengthen through time as the A. cristatellus population grows.

Start Comment 14: Reviewer #1
Line 230: Developmental plasticity is here invoked as a delaying force, but why not as

accelerating one? Discuss it with literature date.
End Comment 14: Reviewer #1

We have added the following edits to this section, including two new references that discuss the relationship between developmental plasticity and adaptive evolution:

Given the detection of such strong directional selection on hind limb length, it was surprising to not observe subsequent evolution of longer hind limbs in this A. sagrei population when re-measured after approximately four to five generations (June 2022). We suspect that too few generations have passed to observe the magnitude of hind limb divergence in this focal population as we recorded in our comparative populations (i.e., Fig 2). Given the observed strength of directional selection on hind limb length (Table 1), limb length differences similar to those recorded between our sympatric and allopatric populations would be expected to occur after 14.4-18.6 generations. The sympatric populations in our comparative analysis have likely been established for 35-50 generations[17,21,31], suggesting our observed differences in hind limb lengths could have resulted from evolution by natural selection. Such evolutionary lag times can occur in natural populations, for example due to weak trait heritability33, developmental plasticity[34,35] **(which could also, conversely, accelerate adaptive evolutionary responses[36,37])**, or if the strength of selection varies through time, even if the direction remains constant[38]. For example, elsewhere in Florida, morphological evolution of the native green anole (A. carolinensis) in response to increased arboreality in the presence of invading A. sagrei were apparent after approximately 20 generations[39], providing comparative temporal support for the evolutionary dynamics that we predict are occurring in our study.

[36] Beldade, P., Mateus, A.R.A. and Keller, R.A., 2011. Evolution and molecular mechanisms of adaptive developmental plasticity. Molecular ecology, 20(7), pp.1347-1363.

[37] Paaby, A.B. and Testa, N.D., 2018. Developmental plasticity and evolution. Nuno de la Rosa L, Müller G, editors. Evolutionary Developmental Biology: A Reference Guide. Cham: Springer.

Start Comment 15: Reviewer #1
Methods (Line 262) Please, indicate if other Anolis sp. are present in the study sites and which potential predators exist namely compared to those present in Cuba and Puerto Rico.
End Comment 15: Reviewer #1

We have added the following information to the methods section in which we describe our study sites (see bold and underlined lines below). It is challenging to estimate if predator communities are similar or different in Miami compared to the native ranges of A. sagrei and A. cristatellus (although, functionally, it is probably very similar). However, more pertinent to our study, we can more confidently discuss the consistency and similarity of predator species present in our study sites.

Methods

Character displacement: the pattern

Study system

Anolis sagrei and A. cristatellus are small (40-70 mm snout-to-vent length; Fig 1A), diurnal lizards that diverged ca. 50-60Ma and do not hybridize. These species are highly convergent in ecology, morphology, and behavior and classified in the same ‘trunk-ground’ ecomorph group19. Anolis sagrei was first recorded in Miami, south Florida USA, in the mid-1940s (45,46); A. cristatellus in the early 1970s (31,32,46). Anolis sagrei is widespread in Florida, while A. cristatellus is limited to only Miami-Dade County (21,46). In their native ranges, both A. sagrei and A. cristatellus are habitat generalists and occupy similarly structured environments (47,48). In south Florida, both species occupy a gradient of habitat types from closed canopy forest to urban environments (21,46,49–52) and are estimated to have very similar statewide predicted habitat suitability (53). We studied ecological resource use (habitat use and diet), movement behavior, and morphology in nine sites across Miami (three allopatric sites per species and three sympatric sites that include both species; Extended Data Table 1). **All sites that contain three other Anolis species, all of which are substantially more arboreal than both A. sagrei and A. cristatellus; the trunk anole (A. distichus), the arboreal green anole (A. carolinensis), and the giant arboreal specialist A. equestris (21,46,54-57). Although direct predation of Anolis lizards was rarely observed during this study, potential predator species were consistent across study communities (JStroud pers. obs.). Likely common predators include common snakes (e.g., Coluber constrictor) and birds (e.g., Buteo lineatus, Cyanocitta cristata, Melanerpes carolinus, Mimus polyglottos, Quiscalus sp.), all of which were present in each study site (JStroud pers. obs.).**

Start Comment 16: Reviewer #1
Line 271: Justify why this method of analysis of trophic ecology was preferred compared to stable isotopes or metabarcoding. I understand you were interested in prey size and mass,

and not only in OTUs.
End Comment 16: Reviewer #1

Our data collection consisted of stomach contents directly dissected from Anolis lizards that we collected as specimens. We were interested in the fine details of interspecific differences in dietary ecology. While stable isotopes are excellent for disentangling broad patterns like interspecific differences at the community level (e.g., see Giery et al. 2013), the approach would likely not provide the resolution that we needed given the similarity of environments among all of our study sites. Similarly, DNA metabarcoding of fecal samples, as used in other community ecology studies of Anolis lizards (e.g., Kartzinel and Pringle 2015, Pringle et al. 2019), was not necessary for our study because it would be unlikely to provide us with substantially different information to direct analysis of stomach contents (as we did). If the Editor would like us to explicitly justify in the manuscript text why we used this approach instead of either stable isotopes or DNA metabarcoding of fecal samples then we would be happy to do so.

Ref

Giery, S.T., Lemoine, N.P., Hammerschlag-Peyer, C.M., Abbey-Lee, R.N. and Layman, C.A., 2013. Bidirectional trophic linkages couple canopy and understorey food webs. Functional ecology, 27(6), pp.1436-1441.

Kartzinel, T.R. and Pringle, R.M., 2015. Molecular detection of invertebrate prey in vertebrate diets: Trophic ecology of C aribbean island lizards. Molecular Ecology Resources, 15(4), pp.903-914.

Pringle, R.M., Kartzinel, T.R., Palmer, T.M., Thurman, T.J., Fox-Dobbs, K., Xu, C.C., Hutchinson, M.C., Coverdale, T.C., Daskin, J.H., Evangelista, D.A. and Gotanda, K.M., 2019. Predator-induced collapse of niche structure and species coexistence. Nature, 570(7759), pp.58-64.

Start Comment 17: Reviewer #1
Line 285: Indicate the precision of the calliper.
End Comment 17: Reviewer #1

We have added the following edit as recommend (pertinent information in bold and underline)

Morphology

We measured limb length (forelimb and hind limbs) and body size (snout-vent length [SVL]) using digital calipers (Neiko 01470A, **accurate to 0.01mm**; all measurements by JTS) of both A. sagrei (n = 127 allopatry, n = 115 sympatry; n = 23-53 individuals per site; Extended Data Table 2) and A. cristatellus (n = 104 allopatry, n = 107 sympatry; n = 24-46 individuals per site).

Start Comment 18: Reviewer #1
Line 297: Did such transformation ensure Normality? Did you test for homoscedasticity? This is not trivial because character displacement could induce variation in variances besides means.
End Comment 18: Reviewer #1

Yes, all model assumptions (including normality of model residuals and tests for homoscedasticity) using the *check_model* function in the R package *performance*. We included this information in the Methods section under *Statistical analysis* (pertinent information highlighted in bold and underlined)

Statistical analysis

All statistical analyses were conducted in R v4.1.262 using RStudio v2021.09.163. We used linear mixed effects models (lmer in R package lme464) to test for differences in ecology (perch height and prey size), morphology, and movement behaviors between allopatric and sympatric populations of each species. All models included treatment (i.e., allopatric vs. sympatric) as a fixed effect and site as a random effect to incorporate between-site variation37,65. All morphological trait analyses included body size as a covariate. All continuous variables were log-transformed, except perch height and movement behaviors which were square-root transformed (see ref. 37). **All model assumptions were checked using the check_model function in R package performance66**. We measured niche overlap of prey items (at the family level) using Schoener's proportional similarity index (D), using the niche.overlap function in the R package spaa67. Schoener's D values > 0.6 infer significant overlap in diet68.

Start Comment 19: Reviewer #1
Line 346: Were limb-SVL residuals calculated separately for each species? Would you expect and sexual shape dimorphism is this trait? If not the case, justify with references.
End Comment 18: Reviewer #1

All data analysis in this project was only conducted with data from male lizards (both the comparative analysis of morphological differences between established populations and our direct measurements of natural selection in the novel community). The only exception is the inclusion of females in Fig 1E (visualization of spatial proximity of males and females of both species in a focal sympatric community). Therefore, we did not need to account for sexual size dimorphism in any of our morphological analyses. All analyses in our study relating to limb length considered only A. sagrei, therefore when limb-SVL residuals were calculated it was only for a single species (A. sagrei).

Start summary: Reviewer #2
In "Rapid shifts in natural selection drives character displacement in the wild", Stroud et al. study character displacement between sympatric populations of Anolis sagrei and A. cristatellus. They have both a "pattern" side to their study, wherein they compare sympatric and allopatric populations in their limb morphology and "habitat" use (time spent locomoting on different strata/substrates), and a "process" side to their study, wherein they compare the same set of morphological variables before and after an invasion of A. cristatellus into existing A. sagrei populations they had been studying. The authors did not observe phenotypic shifts in the latter part of the study, but did observe strong shifts in selection strength over the period of their study; Breeder's equation plus additional sources of information suggest such a short period of study would be insufficient to observe shifts in phenotype.

The paper is excellent and I have mostly only minor point. One point approaching "major" is the lack of connection between the interspecific interactions/network graph portion of the study, and the rest of the study. My comments below make it clear how this aspect of the study could be improved.
End summary: Reviewer #2

Thank you for your thorough and supportive comments! We are happy to respond to all of your specific comments below.

Start Comment 1 Reviewer #2
Line 23: is lacking -> are rare
End Comment 1: Reviewer #2

Thank you for catching this grammatical error. We changed "is lacking" to "are rare" in the revised manuscript to ensure clarity and correctness (pertinent section in bold and underline):

Abstract

Ecological character displacement, whereby shifts in resource use between competing species leads to adaptive evolutionary divergence, is widely considered an important process in community assembly and adaptive radiation. However, most evidence for character displacement has been inferred from macro-scale geographic or phylogenetic patterns; direct tests of the underlying hypothesis of divergent natural selection driving character displacement in the wild ~~is lacking~~ **are rare**. Here, we document character displacement between two ecologically-similar lizards (Anolis sagrei and A. cristatellus) experiencing novel contact. We identify directional selection during the incipient stages of sympatry in a new community that corresponds to repeated trait divergence across multiple established sympatric sites. By identifying the role of natural selection as character displacement unfolds, we connect how natural selection operating at short timescales may drive broader patterns of trait distributions at larger spatial and temporal scales.

Start Comment 2: Reviewer #2
Line 36: "to faciliate coexistence" - I know the authors don't mean this exactly, but this phrasing makes it sound like there is a purpose behind divergent evolution like this. Probably wouldn't notice if it was buried in the manuscript, but starting off the first sentence like this caught my attention.
End Comment 2: Reviewer #2

We have removed these words from the Introduction, which now reads as follows:

Introduction

~~To facilitate coexistence, e~~ Ecologically similar species typically differ in resource use and morphology when co-occurring. Character displacement, the process by which adaptive divergence by natural selection drives such ecomorphological patterns, has long been assumed responsible for many such scenarios1–5. Evidence for character displacement has classically been drawn from broadscale patterns of geographic or phylogenetic trait distributions, whereby closely-related species or those that co-occur in space differ in resource-acquiring traits1,6–8. However, although robust statistical patterns are often detected that fit with the expectations of character displacement9–13—and mechanisms proposed to explain them14, the nature of these macro-scale comparative data typically preclude direct examination of underlying processes4,6,8,15. As such, there remains confusion about the underlying processes that drive character displacement in the wild16.

Start Comment 3: Reviewer #2
Lines 70-71: Missing a short phrase here, e.g. "To expand on this preliminary finding,"
End Comment 3: Reviewer #2

We have added the suggested edit to this sentence as follows (pertinent information in bold and underline):

Ecological character displacement: The pattern

Previous research had suggested character displacement might be occurring between A. sagrei and A. cristatellus in Miami, south Florida, via divergence in perch height17. However, this research was limited to a single location where sympatry had only been established for approximately five years17, preventing the investigation of longer-term evolutionary responses. **To expand on this preliminary finding, we** ~~We~~ selected nine sites across Miami (three allopatric sites per species and three sympatric sites that include both species; Extended Data Table 1), where species had been established for over 30 generations17,20,21, to explore the geographic consistency of character displacement. We predicted that divergence in habitat use would occur in all sympatric sites as previously recorded17; A. sagrei perches lower and exhibits increased terrestriality in sympatry, whereas A. cristatellus perches higher.

Start Comment 4: Reviewer #2
Lines 110-111: What are the nature of these interspecific interactions? This is a substantial oversight in the manuscript as is. Nowhere is the question of behavioral dominance addressed. What are we to infer from Fig 1E? This figure and portion of the study strikes me as a missed opportunity. In general, the purpose/story of this figure is not explained, and I suspect there are better ways to arrange nodes to tell the untold story. Furthermore, what is happening during these interactions? Is one species consistently displacing the other from a perch? Do they just give each other the stink eye? Dewlaps? Tell us more. Based on selection differences described later (and shifts in community level abundance), I would naively posit that one species is behaviorally dominant to the other, but nowhere is this mentioned, which leads me to believe this is not the case. State this somewhere!
Start Comment 4: Reviewer #2

Start Comment 10: Reviewer #2
Lines 313-325: I'm confused by this. An interspecific interaction is--? Being within 3m of each other, and nothing else? This is not clearly stated, but if that is what is meant, is this a useful definition? Please tell us more here.
Start Comment 10: Reviewer #2

These are all excellent questions! As these two comments are similar, we have combined our answers to them here (Comments 4 and 10).

The aim of data collected and presented in Fig 1E was to demonstrate that, despite perch height divergence in sympatry, these species—A. sagrei and A. cristatellus—are still overlapping in space, providing the potential for direct behavioral interactions. While it is possible that one species is behaviorally dominate over the other, we do not have the data to determine whether that is the case. It would be valuable for future studies to attempt to quantify this, however it is beyond the scope of this study.

In response to your request for information on the ‘interactions’ that underlie the construction of our two-species social network figure, we have now clarified the language we use to describe these on lines 394-402. These networks are based on proximity, by far the most commonly used and validated proxy for a social connection between individuals across multiple species (see Croft et al (2008), Davis et al (2018), Farine & Whitehead (2015), Krause et al (2015). The critical component to constructing proximity networks is determining the inter-individual distance threshold, and its nature, that constitutes a biologically meaningful association (Davis et al, 2018). Here, our criteria for assigning an ‘edge’/connection between two individuals was that they were within 3m, and direct line of sight of one another. The rationale for this is based on two main lines of evidence. Firstly, *Anolis* lizards primarily communicate via visually conspicuous displays, which range from subtle, such as lateral undulation of the head (‘headbobbing’), to intermediate, e.g. movement up and down of the entire body (‘pushups’), to the most visually elaborate; the extension of a colorful throat fan called a dewlap (‘dewlapping’). Anoles conduct these communication behaviors frequently in both the presence and absence of detected receivers (i.e., conspecifics, congeners, or potential predators). For example, in south Florida, in the absence of any experimental stimuli, *Anolis sagrei* will naturally conduct ~3 pushups/minute, ~3-5 dewlap extensions/minute, and 3-4 head bobs/minute (when individuals are observed in the wild for 30 minutes; Simon 2007). Secondly, previous work modelling *Anolis* visual acuity and detection distances of conspecifics demonstrate that even the most common and least conspicuous visual displays of *A. sagrei* (headbobs) can be detected between 3.7m to 5.8m away, depending on attenuation of display salience due to local predation risk (Steinberg et al, 2014). Given the frequency by which *Anolis* lizards display via dewlap extensions and headbobs, coupled with being relatively sedentary, we made the (conservative) assumption that at least one individual within a pair situated within 3m and direct line of sight of one another would have detected and identified the other, and so were highly likely to be communicating (we used the term ‘interacting’) to some extent. Whilst we believe our criteria for assigning edges is thorough compared to the standard in network studies, of course it does not allow us to determine the specific nature of these associations (aggressive, sexual, etc), as is a standard limitation of using undirected association networks such as these. Nevertheless, the utility of our network approach in demonstrating that lizards are engaging in some form of interspecific interaction within our sympatric sites shows that interference competition is a plausible mechanism contributing to the selection patterns and divergence we demonstrate.

Steinberg, D.S., Losos, J.B., Schoener, T.W., Spiller, D.A., Kolbe, J.J. and Leal, M., 2014. Predation-associated modulation of movement-based signals by a Bahamian lizard. Proceedings of the National Academy of Sciences, 111(25), pp.9187-9192.

Croft, D. P., James, R., & Krause, J. (2008). *Exploring animal social networks*. Princeton University Press.

Davis, G. H., Crofoot, M. C., & Farine, D. R. (2018). Estimating the robustness and uncertainty of animal social networks using different observational methods. *Animal Behaviour*, *141*, 29-44.

Farine, D. R., & Whitehead, H. (2015). Constructing, conducting and interpreting animal social network analysis. *Journal of animal ecology*, *84*(5), 1144-1163.

Krause, J., James, R., Franks, D. W., & Croft, D. P. (Eds.). (2015). *Animal social networks*. Oxford University Press, USA

Simon, V. B. (2007). Not all signals are equal: male brown anole lizards (Anolis sagrei) selectively decrease pushup frequency following a simulated predatory attack. *Ethology*, *113*(8), 793-801.

Start Comment 5: Reviewer #2
Line 118: Do you mean Fig. 2?
Start Comment 5: Reviewer #2

Yes! Thank you for catching this. We have made the edited from Fig 1E to **Fig 2**.

Start Comment 6: Reviewer #2
Line 173: I would not call this incidental.
Start Comment 6: Reviewer #2

We have removed '**Incidentally**' from the start of this sentence.

Start Comment 7: Reviewer #2
Lines 177-181: I would not really consider this a result. Maybe something to integrate into the discussion? Strikes me as something that was inserted to preempt reviewer criticism, not an actual finding.
Start Comment 7: Reviewer #2

Thanks for the comment. I can understand why this text could be interpreted as a reviewer preemption, but the truth is actually the opposite: we wanted to be as transparent as possible in regard to what data we had available for this portion of the study. Ideally, we would have the data to show changes in habitat use of A. sagrei in real time in response to the invasion of A. cristatellus (i.e., if the invasion of A. cristatellus in this A. sagrei population was planned or pre-empted). Unfortunately, we do not have that, and so we wanted to be up front about it. Nevertheless, we appreciate your feedback and have removed these sentences from the results section.

Start Comment 8: Reviewer #2
Line 239: the Martin and Martin warbler papers from Arizona are worth referencing here.
Start Comment 8: Reviewer #2

Thank you for this suggestion, we have added reference to the following two studies to this sentence in the manuscript:

Martin, P.R. and Martin, T.E., 2001. Ecological and fitness consequences of species coexistence: a removal experiment with wood warblers. Ecology, 82(1), pp.189-206.

Martin, P.R. and Martin, T.E., 2001. Behavioral interactions between coexisting species: song playback experiments with wood warblers. Ecology, 82(1), pp.207-218.

Start Comment 9: Reviewer #2
Line 280: Could the word individual here be replaced with something more appropriate such as specific or particular?
Start Comment 9: Reviewer #2

We have changed ‘individual’ to ‘single’ (see below) and hope that this edit improves clarity:

Movement behavior

As perch height observations only represent habitat use for a single time point, we conducted behavioral observations on natural movement and habitat use of 178 A. sagrei across all sites (n = 28-31 unique individuals per site; 8-20mins per lizard, median = 15 mins; total observation time = 2,609 minutes; Extended Data Table 3). As we were most interested in understanding the relationship between movement behavior and habitat use, we recorded the frequency of movements between separate trees (i) across the ground and (ii) above the ground, as well as the frequency of (iii) changes in perching location within a **single** ~~n individual~~ perch and (iv) movements between perches on the same tree (e.g., from tree trunk to branch, or branch to branch).

Start Comment 11: Reviewer #2
Lines 329-337: Tell us more about interactions, or lack thereof, between these species and the study species.
Start Comment 11: Reviewer #2

We have edited this section to include more information on the non-study Anolis species also present in this location (pertinent revisions to this comment in bold and underlined; this section also contains revisions in response to comments from Reviewer 1 [see above] which are just in bold).

Study site

We measured survival of A. sagrei from 2016-2019 on a 6,000m2 island located within the Fairchild Tropical Botanical Gardens (FTBG) in Miami, Florida USA (Extended Data Table 1). **As with the locations in our comparative analysis, three other Anolis species are also present in this community (A. carolinensis, A. distichus, and A. equestris; see ref[26]). These species are all substantially more arboreal than either A. sagrei or A. cristatellus, are classified as members of different ecomorph groups (A. carolinensis, ‘trunk-crown’; A. distichus, ‘trunk’; A. equestris, ‘crown-giant’)[19], and occupy different morphological space[26] and trophic niches in south Florida[59].** No changes in structural habitat or other environmental factors (e.g., other lizard species) occurred during this study, with the exception of the arrival of A. cristatellus.

Start summary: Reviewer #3

In this study, Stroud and colleagues report on a pattern of character displacement between two anolis species introduced to Florida at different times in the last century, and taking advantage of a recent sympatric site, they document changes in selection in one species in the direction of the presumably evolved changes they observed in longer established sympatric sites.

Character displacement is almost always studied from a historical perspective, by comparing traits of the competing species in areas of allopatry and sympatry. While experiments can be used to further investigate the process of character displacement, it is very rare to catch this process ongoing in nature as the authors have done here. I found no issues with the studies methods or analysis, and the paper is interesting, engaging, and makes an important contribution towards our understanding of character displacement.

I do have a few topics for the authors to consider, and that I hope will be helpful. First, the authors organize the manuscript by distinguishing results focused on the pattern vs the process of ecological character displacement. I found this to be useful, but it did make clear to me that there are remaining gaps in our understanding of the process of character displacement in this system. While it appears clear from the results that A. cristatellus is the cause of the altered selection regime for A. sagrei, what ecological interactions are responsible are not as clear.

The authors attribute natural selection in sagrei (and the changes in morphology and habitat use in older sympatric populations) to resource competition with cristatellus. However I think the data supporting this are lacking. For instance, there is no shift in diet between allopatric and sympatric populations. The authors seem to use the shifts in perch height to imply resource competition but I would argue a more likely explanation would point to antagonistic interactions between the two anolis species (antagonistic character displacement). While there has long been debate about the definitions and terminology associated with character displacement, it is generally agreed that the pattern of character displacement can be the outcome of different, distinct ecological interactions (Grether et al. 2009; Pfennig and Pfennig 2012; Stuart et al 2017). In this view, I would argue that it is not clear that the authors are documenting “ecological” character displacement, since it appears that resource use has not diverged. And so, I suggest following the suggestion of Stuart et al 2017, and solely referring to these results as character displacement.

We appreciate your positive feedback on our study and its contribution to our understanding of character displacement and are pleased to hear that you found the manuscript interesting and engaging. Thank you for your constructive feedback and helpful recommendations, which we address in detail below. We look forward to incorporating your suggestions in the revised manuscript.

Specifically, we agree that the data needed to support ecological character displacement (i.e., exploitative resource competition of a known resource type) are unavailable to us in this study. We connect shifts in behavior and resource use evidence that competition is occurring, but, as you say, this could take many forms: exploitative (e.g., space, food) or interference (e.g., agonistic interactions). Because of this, we have followed your advice and removed our direct references to our study being a case study of ecological character displacement and instead refer to it as just ‘character displacement’ (e.g., in the manuscripts main subheadings and all figure legends).

A distinct but related point: there is very little discussion in the paper of the ecological interactions causing character displacement here, beyond the suggestion that it is due to competition for resources. I think that this topic deserves to be included in the Discussion and perhaps in the Introduction as well - what could be the underlying ecological interactions - e.g., resource competition, interspecific aggression - and what is the supporting evidence among the alternative hypotheses?

This is an excellent point. With the data that we have available, it is difficult to distinguish the relative roles of resource competition versus agonistic interactions in driving perch height divergence and changes in morphology. Ultimately, it seems like that it could be a combination of both. We included the diet data to be completely transparent about what is happening in this system: despite divergence in perch height, foraging patterns (i.e., food resource dynamics) do not appear to change. If exploitative competition for food was driving perch height divergence, then we might have also expected to see shifts in these diet data. It is possible that competition for other resources are driving this pattern (e.g., perch type or availability, or thermal microsites) but we do not have data to explore this. Similarly, we included the network data—constructed using direct line-of-sight distances—to show that individual of these species are overlapping in close proximity, which presents the opportunity for interspecific aggression to occur. However, we also do not have the data on the frequency, intensity, or response to interspecific aggression which would allow us to determine its role in driving species-level patterns of habitat use. We have included the following paragraph in our discussion in response to this comment that provides an important discussion of ecological vs. agonistic character displacement, what we can and cannot say with our data, and how the relative roles of each (i.e., exploitative versus interference competition) could be better investigated in future studies:

Our study provides a clear insight into the effect of A. cristatellus on the behavior of habitat use and movement behaviors of A. sagrei (Fig 1). However, what interspecific interactions are responsible for driving this pattern of ecological divergence? Classically, interspecific exploitative competition for limited resources has been considered the primary mechanism responsible for instances of character displacement (i.e., ecological character displacement)[1,2,5]. In other case studies of ecological character displacement, exploitative competition for limiting food resources is shown to drive changes in resource consumption, leading to character displacement[42,43]. However, our analysis of diet data did not provide evidence for this happening in our study. It would be valuable for future studies to investigate if competition for other resources that could be limiting, for example the availability of perch types or thermal microsites, is occurring between A. sagrei and A. cristatellus. Alternatively, agonistic species interactions could be responsible for our observed character displacement via interference competition[44–46]. Our social network data show that individuals of these species occur in close proximity when in sympatry (Fig 1E), presenting the opportunity for aggressive interspecific interactions. However, the extent to which these species are aggressively interacting, either in frequency or intensity, remains unclear. It would be valuable for future studies of character displacement more generally[16], but also our study system of A. sagrei and A. cristatellus specifically, to explicitly test the role of these different mechanisms to better understand the underlying mechanisms responsible for driving character displacement in the wild.

1. Brown, W. L. & Wilson, E. O. Character displacement. Systematic zoology 5, 49–64 (1956).

2. Grant, P. R. Convergent and divergent character displacement. Biological journal of the Linnean Society 4, 39–68 (1972).

5. Stuart, Y. E. & Losos, J. B. Ecological character displacement: glass half full or half empty? Trends in ecology & evolution 28, 402–408 (2013).

42. Grant, P. R. & Grant, B. R. Evolution of character displacement in Darwin's finches. science 313, 224–226 (2006).

43. Grant, P. R. & Grant, B. R. 40 Years of Evolution: Darwin's Finches on Daphne Major Island. (Princeton University Press, 2014).

44. Grether, G. F., Losin, N., Anderson, C. N. & Okamoto, K. The role of interspecific interference competition in character displacement and the evolution of competitor recognition. Biological Reviews 84, 617–635 (2009).

45. Grether, G. F., Peiman, K. S., Tobias, J. A. & Robinson, B. W. Causes and consequences of behavioral interference between species. Trends in Ecology & Evolution 32, 760–772 (2017).

46. Grether, G. F. et al. The evolutionary consequences of interspecific aggression. Annals of the New York Academy of Sciences 1289, 48–68 (2013).

16.Stuart, Y. E., Inkpen, S. A., Hopkins, R. & Bolnick, D. I. Character displacement is a pattern: so, what causes it? Biological Journal of the Linnean Society 121, 711–715 (2017).

End summary: Reviewer #3

Start Comment 1: Reviewer #3
Line 227. Is there any other evidence for evolution as the underlying cause of character displacement in the older sympatric populations? I agree that the absence of divergence in the new sympatric site compared to the older sympatric sites does suggest that phenotypic plasticity is not responsible for the older divergence but this is not the strongest evidence that character displacement has indeed evolved - alternatively, the recently sympatric sagrei population could simply have low plasticity compared to other populations (not saying this is true but it is possible). I suggest the authors more clearly state the evidence for the evolution of character displacement in this system vs. other possibilities.
End Comment 1: Reviewer #3

This was also identified by Reviewer 1 and we added a new paragraph discussing alternative processes that could have also led to our observed patterns (e.g., plasticity). Thank you for raising this important point about the evidence for the evolution of character displacement in the older sympatric populations. We agree that the absence of divergence in the recently sympatric site compared to the older sympatric sites is not the strongest evidence for evolved character displacement, and we acknowledge the possibility that the recently sympatric A. sagrei population could have low plasticity.

Start Comment 2: Reviewer #3
Are the sympatric sites independent? That is, has character displacement (the older sympatric sites) occurred independently more than once here or did it perhaps evolve once in sagrei and spread? The molecular data does not show that allopatric and sympatric populations cluster into two groups, supporting independent origins, but this could be

made more clear in the text.
End Comment 3: Reviewer #3

Thank you for this comment, we have made the following edits on your recommendation:

Our comparative analyses showing divergence in habitat use and associated morphological traits in established sympatric A. sagrei and A. cristatellus populations suggest that character displacement has occurred (Figs 1,2). **Our molecular data show that allopatric and sympatric A. sagrei populations do not cluster into two groups, which suggests that the ecomorphological changes we observed in our sympatric communities occurred independently (Extended Data Figure 2).** However, these data preclude investigation of the underlying processes that led to this pattern. The formation of a new sympatric community via the invasion of A. cristatellus into an established A. sagrei allopatric population allowed us to study the incipient stages of character displacement in real-time.

Start Comment 3: Reviewer #3
A map of the study sites would be very helpful I think.
End Comment 1: Reviewer #3

We have now added a map of all study sites to our supplementary materials (Extended Data Figure 1).

Our responses are in blue; reviewers' comments or other Editorial requirements are in black.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

I reviewed an earlier version on this work. The second version has included most of my suggestions and clarified some of unexplained aspects. In particular, I am glad to see that the claimings for natural selection, across the text and in the title, are now more proportional to the evidence and that alternative scenarios for short evolutionary timeframes (phenotypic plasticity) as discussed. Authors are also to be congratulated for providing clear information on the location and composition of the communities, including the non-target anole species. Overall, I consider the manuscript is now suitable for the journal.

Detailed comments:
Line 189
Replace "Table 1. Natural selection during..." by "Morphological shift attributable to natural selection during...".

Line 195
Replace "Table 1. Natural selection on Anolis sagrei..." by "Morphological shift attributable to natural selection on Anolis sagrei...".

We cannot make these revisions as they would be incorrect. Neither of these tables demonstrate morphological shifts. Table 1 is a table of (natural) selection coefficients and associated p-values on various morphological traits (following Lande & Arnold 1983); Table 2 is a table of (natural) selection differentials and trait values with optimal fitness (i.e., highest survival probability). It would be incorrect to complete the minor revisions as requested by Reviewer 1; the data presented are literal estimates of natural selection on the traits of interest. The current legends are the clearest way of describing the data in the table.

Line 272
A. sagrei and A. cristatellus should be in italics.

Thank you for catching this – we made the changes as pointed out.

Reviewer #2 (Remarks to the Author):

The revised article is great and addresses my previous minor concerns. I look forward to seeing it in print. Also:

Line 105: despite sounding correct due to the last letter of the specific epithet, I believe perch/perches should both be plural, or not, for consistency.

Thank you for catching this, we have edited it as instructed.

Line 268: missing a word

Thank you also for catching this, you are correct we had missed a word! Here is the edited sentence, with the addition in **bold**:

As opportunities to study novel contact between similar species in the wild are rare, few convincing **case studies** exist that have observed character displacement unfolding in the wild (but see refs. [42,47,48]).

Reviewer #3 (Remarks to the Author):

The authors have carefully addressed my comments on the first submission, and I have no further questions or concerns. This is an interesting and exciting study, and while there are still pieces to be explored (the ecological cause(s) of character displacement here, and direct evidence of evolutionary divergence), this study makes a strong and important contribution to the field.