Character displacement and community assembly in Anolis lizards

Citation

Stuart, Yoel Eli. 2013. Character displacement and community assembly in Anolis lizards. Doctoral dissertation, Harvard University.

Permanent link

http://nrs.harvard.edu/urn-3:HUL.InstRepos:11156787

Terms of Use

Share Your Story

Accessibility

Character Displacement and Community Assembly in *Anolis* Lizards

A dissertation presented

by

Yoel Stuart

to

The Department of Organismic and Evolutionary Biology

in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy
in the subject of
Biology

Harvard University
Cambridge, Massachusetts
March 2013

Professor Jonathan B. Losos   Yoel Eli Stuart

Character Displacement and Community Assembly in *Anolis* Lizards

ABSTRACT

At broad scales, community ecologists study how biogeographic factors like
environmental dissimilarity and geographic distance influence community assembly and
composition. At small scales, community ecologists study how one or several species interact to
determine habitat partitioning and coexistence. In this dissertation, I present studies at both
scales. Chapter One investigates community assembly across the Caribbean, Central, and South
American radiations of *Anolis* lizards and Eleutherodactylid frogs to test whether oceanic islands
are unique in their assembly processes. Such uniqueness is suggested by high levels of endemism
on islands; however, comparable levels of endemism can be found in mainland communities. I
modeled the rate of species turnover between mainland communities, with respect to geographic
distance and environmental dissimilarity, and then used the mainland model to predict turnover
among islands. Turnover among island communities was significantly higher than predicted from
the mainland model, confirming the long-held but untested assumption that island assemblages
accumulate biodiversity differently than their mainland counterparts. Chapter Two reviews the
evidence for ecological character displacement (ECD), an evolutionary process whereby two
resource competitors diverge from one another in phenotype and resource use, facilitating
coexistence in a community. I find that, despite current scientific opinion, the evidence for ECD
is equivocal; most cases of ECD pattern fail to rule out processes alternative to resource
competition that could create the same pattern. I conclude that better evidence may come from
real time tests of ECD. Chapters Three and Four describe just such a test in small island
populations of *Anolis carolinensis*. In Chapter Three, I find that small island populations of *A.*

carolinensis that have come into sympatry with a novel competitor, the invasive *A. sagrei*, shift their habitat use to become more arboreal, compared to allopatric populations. Consistent with prediction, individuals from sympatric populations have larger toepads with additional adhesive scales – a common adaptation to arboreality in *Anolis*. In Chapter Four, I describe a common garden experiment that finds that the observed toepad divergence is an evolved response, suggesting rates of divergence for toepad area and scale number on par with well known examples of contemporary evolution.

TABLE OF CONTENTS

I dedicate this dissertation to my sister, brother, and parents for their love and support.

And to my wife, because she is the best, plus one.

ACKNOWLEDGEMENTS

I am ever grateful to my earliest scientific mentors: R. Grosberg, M. Dawson, C. Hom, B. Cameron, V. Chow, and E. Prieto. I am fortunate to have made so many intelligent, earnest, and kind friends at Harvard and elsewhere: L. Mahler, M. Muñoz, E. Kay, A. Kamath, A. Masterson, M. Pickering, S. Campbell-Staton, A. Young, J. Weber, E. Kingsley, E. Jacobs-Palmer, N. Dappen, N. Losin, B. Berger, E. Reid, J. Stephens, M. Baldwin, A. Harrison, L. Revell, and B. Langerhans, T. Ord, T. Sanger, A. Algar, A. Freedman, D. Collar, A. Herrel, R. Barrett, B. Peterson, R. Pringle, E. Becker, M. Aja, and many others. Special thanks to N. Delaney.

Thank you to those who made field work for this dissertation possible: J. Brocca, M. Landestoy, M. Legare, J. Lyon, J. Stiner, C. Carter, J. Rosado, J. Woodward, J. Martinez, and T. Takahashi, and especially J. McCrae whose generous lending of his considerably bigger, faster, and safer boat allowed me to complete Chapters 3 and 4, especially after my own boat broke down. (Thank you to F. Gregg who rescued us when McCrae's boat, of course, broke down.)

I thank my dissertation committee, Scott Edwards, Hopi Hoekstra, and Marcus Kronforst, whose sage and patient advice over the years has been invaluable; my outside committee member, Todd Campbell, who pioneered the spoil islands described in Chapter 3 and graciously welcomed me to them, whose field time, guidance, support, and enthusiasm were priceless.

My advisor, Jonathan Losos, deserves the most special of thanks. I have learned so much about science from him. He is among the smartest, most knowledgeable, and clearest thinkers I know, and he is a patient, savvy, discerning, and ever-helpful advisor. Which is fortunate for me because he used all of those attributes to get me to this point.

Introduction

Yoel E. Stuart

Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology

26 Oxford Street, Cambridge, MA 02138

Community ecology is the study of the ecological, evolutionary, and biogeographical factors that govern how groups of species assemble and coexist in communities [1]. At the continental scale, community ecologists study the biotic and abiotic factors that determine general patterns of species diversity. The best known metric of species diversity is species richness, or alpha diversity, which describes the number of species that live in a community. There are many factors that influence species richness [2]. For example, island biogeography theory suggests that, all else equal, communities that encompass a large area are also more likely to receive immigrants that introduce new species restore species lost to stochastic extinction; this is especially true if the focal community is not too far from other communities with similar environments [3,4]. The species richness of a community is also expected to increase with increasing area because larger areas will harbor a greater diversity of biotic and abiotic habitats and such ecological opportunity may even promote *in situ* speciation within the community [3,4].

Another metric of species diversity is species turnover, or beta diversity. Species turnover measures how communities change across space. Generally, species turnover is expected to increase with geographical distance because species that have limited dispersal abilities will not be able to move between distant communities [5-7]. Moreover, greater distance between communities increases the likelihood of there being an impassable barrier to dispersal. Environmental dissimilarity between communities also influences species turnover. Turnover is expected to increase with greater environmental dissimilarity because fewer species will have broad enough niche requirements or enough adaptive phenotypic plasticity to survive in two very different environments [5-7]. By studying the processes, like those mentioned above, that govern alpha and beta diversity across space and time, community ecologists are developing a better

understanding of the causes of diversity. In Chapter One, I study the evolution of diversity in two Neotropical vertebrate radiations by testing how geographic distance, environmental dissimilarity, and a water barrier influence species turnover between island and mainland communities.

I start with the observation that oceanic islands are remarkable for their high endemism, suggesting that islands promote unique assembly processes; however, comparable levels of endemism can be found in mainland communities, suggesting that islands may not be as unique as usually assumed. To test this assumption, I study two well-documented, species-rich vertebrate lineages both distributed across the Caribbean and mainland Central and South America: *Anolis* lizards and Terrarana frogs. With similar evolutionary histories [8,9], these lineages serve approximately as biological replicates of one another, allowing us to compare and contrast how patterns of species turnover relate to biological characteristics of the two lineages. For each clade, I model how mainland assemblies turn over their species composition with geographic distance and environmental dissimilarity. I then use these models to predict turnover between mainland and island communities and among island communities, given their geographic distance and environmental dissimilarity, and ask how well predictions from the mainland model match observed turnover involving islands. If the mainland model accurately predicts island turnover, then islands may not be as unique in their assembly processes as is often assumed. If islands are not well predicted, then we have provided additional evidence that island assemblages accumulate biodiversity differently than their mainland counterparts.

Community ecologists also work at small scales, studying the interactions of two or a few organisms to understand how each influences the other. A major goal at this scale is to understand the niche: the set of ecological and environmental conditions required by a species

[10,11], or alternatively, the ecological role that a species plays in a community [12,13]. How niches evolve in response to predation, mutualism, and competition from other species in a community is directly related to processes of extinction, coexistence, succession, and the accumulation of biological diversity [14].

In Chapters 2-4, I investigate an evolutionary mechanism thought to be an important facilitator of species coexistence: character displacement. Character displacement is the process of divergence in resource use and morphology between two interacting species [15,16]. Traditionally, the driving interaction is taken to be resource competition, but other species interactions like intraguild predation, agonistic encounters, apparent competition, or reinforcement may also drive character displacement [17]. In Chapter Two, I review the evidence for prevalence and importance of character displacement driven by resource competition, or Ecological Character Displacement (ECD). The current scientific opinion for ECD is very positive and resource competition is commonly invoked to explain differences among species without rigorously testing the claim. To examine whether positive opinion for ECD has surged ahead of the available data, I compiled case studies from two influential reviews of ECD as well as my own review of the literature, and measured these case studies against six criteria meant to ferret out strong cases of ECD [18].

In Chapter Three, I describe a test for the evolution of character displacement in real time that addresses each character displacement criterion discussed in Chapter Two (while remaining agnostic to the exact type of species interaction that may drive character displacement). I take advantage of a natural experiment created by a species invasion to ask whether small-island populations of *Anolis carolinensis* have adapted to the recent invasion of *A. sagrei*, an ecologically similar species with which *A. carolinensis* likely interacts strongly. Based on the

wealth of knowledge about *Anolis* habitat use and ecomorphology*,* I predicted *a priori* that island populations of *A. carolinensis* sympatric with the invader would respond by shifting their habitat use to become more arboreal, a common habitat partitioning strategy in anoles. I also predicted that those higher-perching populations should show larger toepads with additional adhesive scales, a common adaptation to arboreality in anoles. In Chapter Four, we tested the genetic basis of an observed morphological response in toepad area and scale number by conducting a common garden experiment with the offspring of gravid, wild-caught females from the islands. Persistence of field differences under common garden conditions would be indicative of an evolved response by *A. carolinensis* to novel interactions with *A. sagrei*, as predicted by character displacement theory.

REFERENCES

1. Morin, P.J. (1999) *Community ecology*. Blackwell Science

2. Whittaker, R.J. *et al.* (2005) Scale and species richness: towards a general, hierarchical theory of species diversity. *J. Biogeog*. 28, 453-470.

3. MacArthur, R.H. and Wilson, E.O. (1967) *The theory of island biogeography*. Princeton University Press

4. Losos, J.B. and Ricklefs, R.E. eds. (2009) *The theory of island biogeography revisited*. Princeton University Press

5. Buckley, L. B. and Jetz, W. (2008) Linking global turnover of species and environments. *Proc. Nat. Acad. Sci. USA* 105: 17836-17841

6. Fattorini, S. (2010) The influence of geographical and ecological factors on island beta diversity patterns. *J. Biogeog*. 37, 1061-1070

7. Qian, H. and Ricklefs, R. E. (2012) Disentangling the effects of geographic distance and environmental dissimilarity on global patterns of species turnover. *Global Ecol. Biogeogr.* 21, 341-351

8. Nicholson, K.E., *et al.* (2005) Mainland colonization by island lizards. *J. Biogeog.* 32, 929-938

9. Heinicke, M. P. *et al.* (2007) Major Caribbean and Central American frog faunas originated by oceanic dispersal. *Proc. Nat. Acad. Sci. USA* 104: 10092-10097

10. Grinnel, J. (1917) The niche-relationships of the California thrasher. *Auk* 34, 427-433

11. Hutchinson, G.E. (1957) Concluding remarks. *Cold Springs Harbor Symp. Quant Biol.* 22, 415-427

12. Elton, C. (1927) *Animal ecology*. Sidgwick and Jackson

13. MacArthur, R.H. and Levins, R. (1967) The limiting similarity, convergence, and divergence of coexisting species. *Am. Nat.* 101, 377-385

14. Chase, J.M. and Leibold, M.A. (2003) *Ecological niches: linking classical and contemporary approaches.* University of Chicago Press.

15. Brown, W.L., and Wilson, E.O. (1956) Character displacement. *Syst. Zool.* 5, 49-64

16. Grant, P.R. (1972) Convergent and divergent character displacement. *Biol. J. Linn. Soc.* 4, 39-68

17. Schluter, D. (2000) *The ecology of adaptive radiation.* Oxford University Press

18. Schluter, D. and McPhail, J.D. (1992) Ecological character displacement and speciation in sticklebacks. *Am. Nat.* 140, 85-108

Chapter 1:

The island-mainland species turnover relationship

Yoel E. Stuart[1], Jonathan B. Losos[1], and Adam C. Algar[2]

[1] Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology,

Harvard University, 26 Oxford Street, Cambridge, MA 02138

[2] School of Geography, University of Nottingham, Nottingham NG7 2RD, UK

ABSTRACT

Many oceanic islands are notable for their high endemism, suggesting that islands may promote unique assembly processes. However, mainland assemblages sometimes harbor comparable levels of endemism, suggesting that island biotas may not be as unique as often assumed. Here, we test the uniqueness of island biotic assembly by comparing the rate of species turnover among islands and the mainland, after accounting for distance decay and environmental gradients. We modeled species turnover as a function of geographic and environmental distance for mainland (M-M) communities of *Anolis* lizards and Terrarana frogs, two clades that have diversified extensively on Caribbean islands and the mainland Neotropics. We compared mainland-island (M–I) and island-island (I–I) species turnover to predictions of the M–M model. If island assembly is not unique, then the M–M model should successfully predict M–I and I–I turnover, given geographic and environmental distance. We found that M–I turnover and, to a lesser extent, I–I turnover were significantly higher than predicted for both clades. Thus, in the first quantitative comparison of mainland–island species turnover, we confirm the long-held but untested assumption that island assemblages accumulate biodiversity differently than their mainland counterparts.

INTRODUCTION

Oceanic islands and archipelagos are often characterized by high rates of endemism [1] that likely result from rapid speciation (anagenetic and cladogenetic) on islands of sufficient area and isolation [2-7]. For example, the Hawaiian Archipelago hosts several classic adaptive radiations including silverswords and honeycreepers. The oft-cited pattern of high island endemism suggests that a unique combination of processes may govern assembly on islands. However, many mainland areas also house endemic biotas with endemism levels that can rival the classic adaptive radiations of oceanic islands [8], especially in mountainous habitat islands characterized by dispersal barriers and steep environmental gradients [9]. Even in less mountainous regions, differences in habitat type, climatic gradients, or spatial separation can lead to substantial turnover across space [10,11]. The existence of mainland communities that harbor similar levels of endemism to islands suggests that island and mainland assembly may be more similar than currently recognized and that islands may not be the unique generators of diversity they have long been assumed to be.

Here, we focus on species turnover along geographic and environmental gradients to test for an island effect on biotic assembly. Mainland–island species diversity relationships have been, and remain, a stimulus of evolutionary and biogeographical theory [7,12-14]. However, the predominant focus has been on the species richness of individual islands relative to the mainland. In contrast, the mainland–island relationship for species turnover remains undescribed and unexplored. To remedy this, we compare rates of species turnover within the mainland (M–M) to rates of mainland–island (M–I) and island–island (I–I) turnover for two species-rich herpetofaunal radiations in the Caribbean and Neotropics: *Anolis* lizards and Terrarana frogs.

The rate of species turnover has generally been modeled as a function of geographic distance and environmental dissimilarity [10,11,15]. Large geographic distances may generate high species turnover by lowering the probability of species exchange through dispersal and increasing the probability of anagenetic speciation through reduced gene flow, while environmental dissimilarity can lead to high turnover as a result of environmental filtering or ecological speciation during local adaptation to different environments. Thus, high species turnover between islands or islands and the mainland is not necessarily indicative of unique island assembly; M–I and I–I species turnover may be high but still consistent with M–M turnover for a given geographic isolation and environmental dissimilarity.

Alternatively, there may be an added 'island effect' on species turnover beyond that expected from geographic distance and environmental dissimilarity that stems from the inhospitable overwater dispersal barrier surrounding islands; comparatively reduced dispersal, limited gene flow, and increased ecological opportunity on islands may drive unique assembly of island floras and faunas. In this case, the M–M model would poorly predict M–I and I–I species turnover. To our knowledge, this island effect has never been quantitatively tested.

METHODS

(a) *Study organisms*

Anolis lizards (Iguanidae) and Terrarana frogs (*sensu* [16]; Leptodactylidae) have radiated extensively in the Caribbean and New World tropics, with approximately 400 and 850 species, respectively. Both clades are insectivorous, include species that are arboreal, terrestrial, or partially aquatic [16,17], and lay direct-developing eggs [17,18]. Phylogenetic and biogeographic reconstructions suggest that both clades originated in the mainland Neotropics,

colonized the Caribbean islands, and back-colonized the mainland from the Caribbean once, where they radiated again [19,20].

(b) *Mainland and Caribbean faunas*

(i) *Species lists*

We defined terraranans according to two recent studies [16,20] and anoles following [21]. We built Caribbean species lists for both clades in November 2011 by cross-referencing published lists [16,17,20,22] against the online databases CaribHerp (www.caribherp.org), HerpNet (www.herpnet.org), and Amphibian Species of the World 5.5 (http://research.amnh.org/vz/herpetology/amphibia). For mainland terraranans, we considered the 865 terraranan species with IUCN range maps (www.iucnredlist.org/technical-documents/spatial-data), 714 of which were mainland species. For mainland anoles, we used the 203 species range maps built by [21].

(ii) *Island Species Composition*

Using the resources mentioned above, we determined the anole and terraranan species composition of Caribbean islands for which we could obtain environmental data, resulting in 65 islands for anoles and 46 islands for terraranans (19 islands that contained anoles did not contain terraranans; Table S1.1). For comparison we also extracted terraranan island species composition from the IUCN range maps (there are no island IUCN range maps for anoles). Species presences that likely resulted from human introductions were excluded.

(iii) *Mainland Species Composition*

We used an island-shaped cookie-cutter approach to define mainland sub-regions (MSRs) within which species composition could be determined from the IUCN range maps (Fig. 1.1). Each cookie-cutter's orientation and placement was randomly determined. Due to the larger land

area in South America, purely random placement could result in underrepresentation of more northern environments. To account for this, we first selected a random latitude. Longitude was then randomly chosen from the available land at the chosen latitude. This approach is similar to the spreading-dye method of [21] except that it preserved island shape as well as size. Each island in our study was represented by five MSRs, resulting in 230 non-overlapping MSRs for terraranans and 325 for anoles. Sampling was performed separately for anoles and terraranans and was limited to regions with at least one anole (or terraranan) species. Following [21], we excluded the disjunct distribution of *A. carolinensis* in the southeast United States as this region was colonized from the Caribbean [23], and whether it should be treated as part of the mainland or as a biogeographical island is unclear.
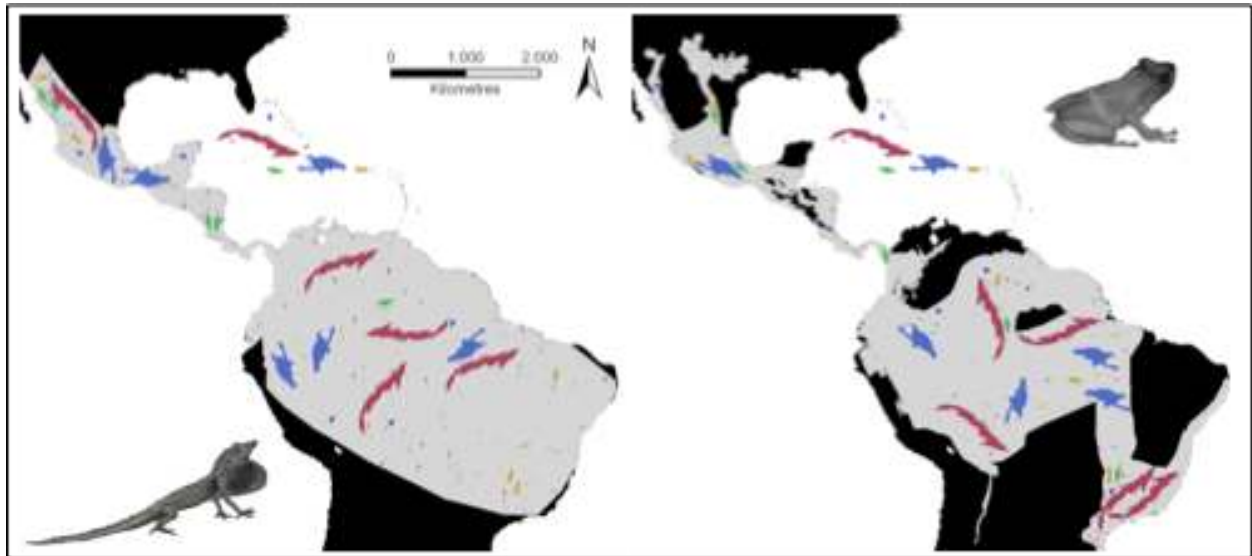

FIGURE 1.1. Island-shaped cookie cutter sampling scheme for anoles (a) and terraranans (b). Each island is represented five times on the mainland. The large, light-grey mainland areas depict the region where at least one species is present. Randomly sampled mainland areas are depicted within this region.

(c) *Quantifying Species Turnover*

We quantified species turnover between all island and MSR combinations using the Sørensen dissimilarity index, where zero indicates that two sites share the same species assemblage and one indicates no shared species. Turnover measured using Jaccard's index was nearly identical (Pearson's r > 0.98 for both clades). Sørensen and Jaccard's indices are commonly used and easily interpretable measures of species turnover. For islands, turnover from the IUCN ranges and from our manually assembled species lists was essentially indistinguishable (Pearson's r > 0.99), so only the latter was used as it included two additional islands.

(d) *Quantifying geographic, environmental, and area dissimilarity*

We measured geographic distance as the minimum straight-line distance among islands and MSRs. The straight-line distance between MSRs was highly correlated with minimum overland distance (Pearson's r = 0.99), so we used straight-line distance to maintain consistency with island comparisons. We measured environmental distance between two sampling areas using the Euclidean distance. Environment was quantified using the first five principal components from a PCA on 16 environmental variables derived from the Worldclim dataset (Table S1.2; [24]) and mean net primary productivity on an island from the MODIS satellite (productivity data from 2000-2010; https://lpdaac.usgs.gov/products/modis_products_table). The environmental variables quantified mean and extreme environmental conditions as well as spatial and seasonal variation in temperature, precipitation, and elevation. The five PCs accounted for 90% (terraranans) and 89% (anoles) of the environmental variation among islands and MSRs. We also calculated all pair-wise differences in area among MSRs and islands. Species turnover and geographic, environmental, and area dissimilarity data can be found in the Dryad depository: http://dx.doi.org/10.5061/dryad.gm2p8.

(e) *Model selection and prediction*

Treating anoles and terraranans separately, we used multiple regression on distance matrices [25] to determine the within-mainland (M–M) relationship between species turnover and geographic distance, environmental distance, and area difference. Because our turnover data were constrained between zero and one and were dominated by these values, we fit generalized linear models using a logit link. Following [21,26], we tested all possible models to identify the best fitting model(s), considering linear, quadratic, and all first-order interaction terms as potential predictors. We used Occam's Window [27] based on the Bayesian Information Criterion (BIC) to identify the best set of near-equivalent models for prediction. We evaluated model significance by randomly permuting island and MSR identities 1000 times and comparing observed BIC and deviance explained (D^2) to this null distribution.

We used Bayesian Model Averaging [27] using the best M–M model(s) chosen by model selection to predict species turnover between MSRs and islands (M–I) and between islands (I–I). Treating M–I and I–I separately, we evaluated predictive performance of the M-M models by regressing observed turnover on predicted turnover [28]. We generated a null distribution of 1000 slope and intercept coefficients by randomly permuting island and MSR identities, re-calculating turnover for M–I (or I–I), and refitting the observed vs. predicted regression. If the M–M model poorly predicts the M–I (or I–I) species turnover relationship, then the observed regression coefficients will differ from the null expectation. Importantly, the range of environmental and geographic distances for M-I and I-I comparisons fell within the range of M-M comparisons (Fig. S1.1), ensuring that we were not extrapolating beyond the range of the M-M data to predict M-I and I-I patterns.

RESULTS

(a) M–M Relationships

For terraranans, all-subsets regression using BIC and Occam's Window identified a single-best model that explained 65% of the deviance:

$$ST = -1.37 + 1.3 \times 10^{-3}(Dist) + 6.4 \times 10^{-7}(Dist)^2 + 0.23(Env) - 0.11(Area) - 9.6 \times 10^{-5}(Dist * Env) + \varepsilon,$$

where *ST* is species turnover, *Dist* is the geographic distance, *Env* is the environmental distance, and *Area* is the area difference between MSR sampling units. Turnover was more closely related to geographic and environmental distance than to area difference (Fig. 1.2). Observed model BIC and deviance explained were significant according to the permutation test ($D^2 = 0.65$, P< 0.001; BIC=7092.0, P<0.001).

For anoles, the relationship was similar to that for terraranans (Fig. 1.2). However, two models fit the data almost equally well. One of these models included the same predictors as the best terraranan model with a similar fit (D^2=0.62, BIC=36466.9):

$$ST = -2.16 + 6.0 \times 10^{-4}(Dist) + 1.2 \times 10^{-7}(Dist)^2 + 0.29(Env) - 0.096(Area) - 4.2 \times 10^{-5}(Dist * Env) + \varepsilon.$$

The second model included an additional interaction term between *Dist* and *Area* that slightly improved model fit (D^2=0.62, BIC=36462.5):

$$ST = -2.09 + 5.5 \times 10^{-4}(Dist) + 1.2 \times 10^{-7}(Dist)^2 + 0.30(Env) - 0.27(Area) - 4.6 \times 10^{-5}(Dist * Env) + 6.1 \times 10^{-5}(Dist * Area) + \varepsilon$$

Observed BIC and deviance explained were significant for both anole models according to the permutation test (P < 0.001 for both D^2 and BIC statistics).
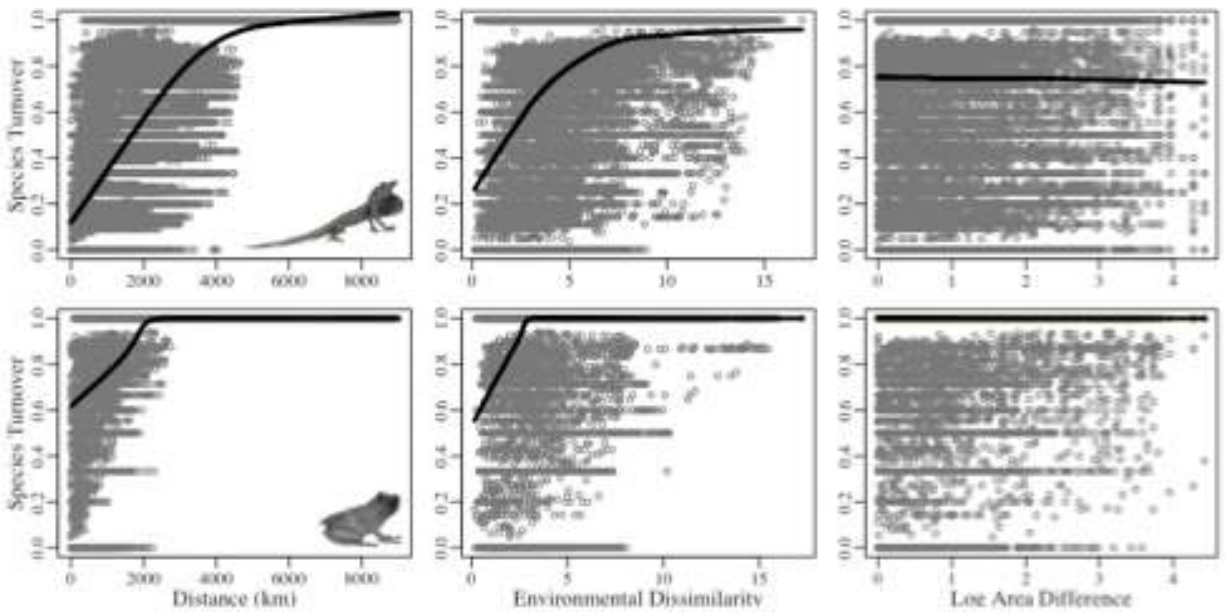
FIGURE 1.2. The relationship between species turnover (Sørensen dissimilarity) and geographic distance (a, d), environmental dissimilarity (b, e), and area difference (c, f) between mainland species assemblages for anoles (top row: a, b, c) and terraranans (bottom row: d, e, f). Lines are Lowess curves.

Species turnover between MSRs and islands was greater than predicted by the M–M model for both anoles and terraranans (Fig. 1.3). Slopes from the M–I observed vs. predicted regression were significantly shallower than the null expectation (anoles: $0.013 \pm 5.5e\text{-}4$ s.e.; terraranans: 0.0 ± 0.0 s.e ; $P<0.001$ for both clades), while intercepts were significantly greater than the null expectation (anoles: $0.99 \pm 4.4e\text{-}4$; terraranans: 1.0 ± 0.0; $P<0.001$ for both clades).

FIGURE 1.3. Observed vs. predicted species turnover for mainland–island (I–M) comparisons based on the mainland–mainland (M–M) relationship for anoles (a, c) and terraranans (b, d). In (a) and (c), M–M relationship are shown in grey and M–I in black. Note that the abundance of turnover values equal to 1.0 partially obscures the best-fit regression line in (a) and (b). (c) and (d) show the actual M–I regression line (black) relative to 1000 null observed vs. predicted relationships (grey).

(c) Predicting I–I relationships

Patterns of I–I turnover were more accurately predicted than M–I turnover, though accuracy was still low, especially for anoles (Fig. 1.4). The *Anolis* slope (0.48±0.03; P<0.001) and intercept (0.77±0.01, P<0.001) from the observed vs. predicted I–I regression remained significantly greater and shallower than the null expectation, respectively. For terraranans, the intercept was significantly greater than expected (0.30±0.02, P<0.03), but the slope did not differ significantly from null expectation (0.79±0.03, P>0.10).
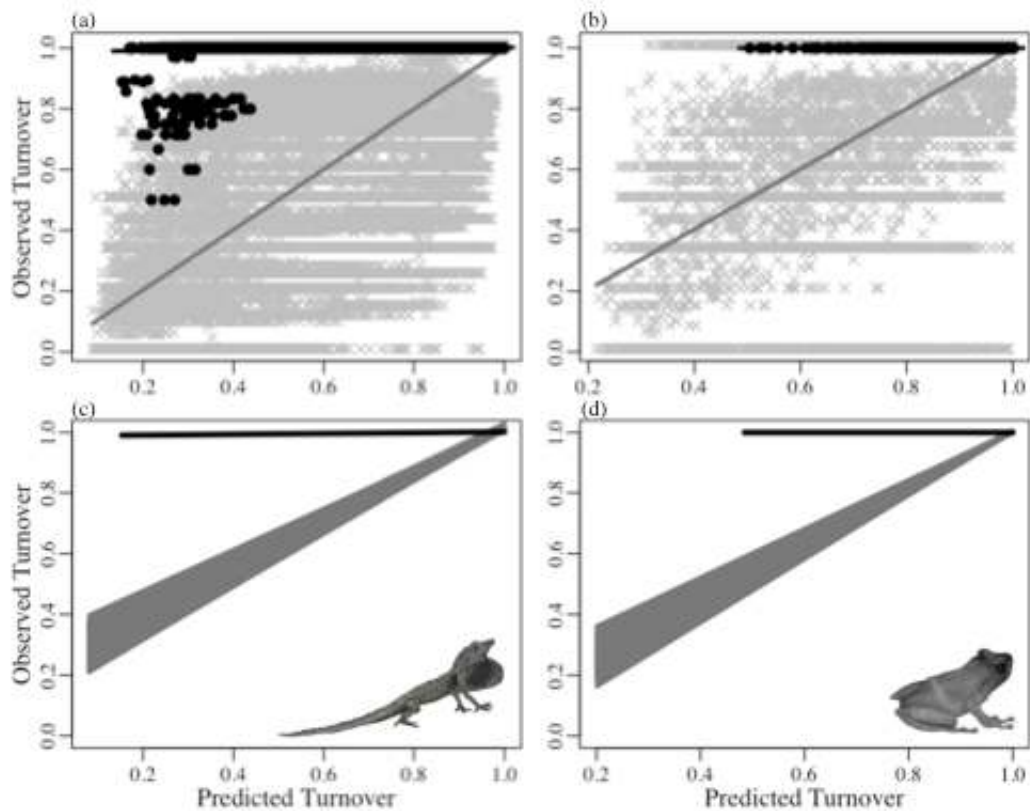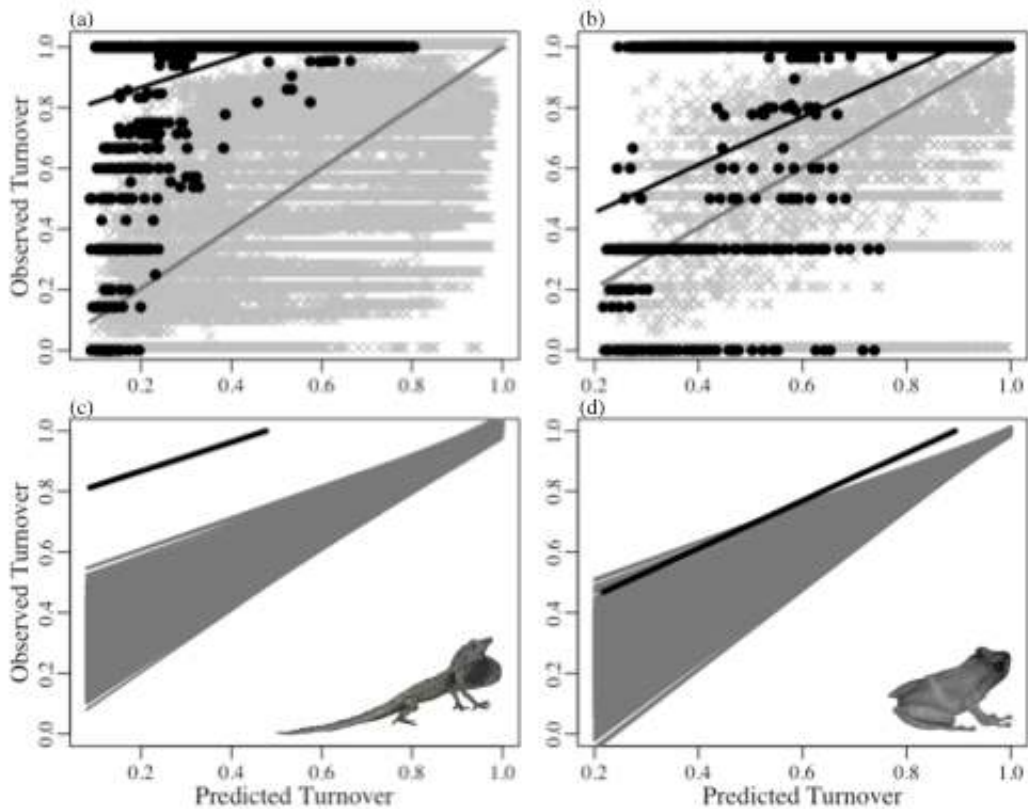

FIGURE 1.4. Observed vs. predicted species turnover for island–island (I–I) comparisons based on the mainland–mainland (M–M) relationship for anoles (a,c) and terraranans (b,d). In (a) and (c), M–M relationship are shown in grey and I–I in black. (c) and (d) show the actual I–I regression line (black) relative to 1000 null observed vs. predicted relationships (grey).

DISCUSSION

(a) *Mainland–Mainland Species Turnover*

Anolis and Terrarana species turnover among MSRs increased with geographic and environmental distance between assemblages (Fig. 1.2), a pattern consistent with turnover patterns of vertebrate and invertebrate taxa worldwide [10,11,29]. The best mainland model for terraranans shared the same predictors and interaction terms as one of the two best models for anoles, suggesting that the two clades accumulate biodiversity in similar ways along spatial and environmental gradients.

Mainland anole assemblages turned over more slowly through geographic and environmental space than terraranan assemblages. Buckley and Jetz [10] linked the rate of species turnover to average range size, with higher turnover in clades composed of smaller-ranged species. Our results are consistent with this pattern; the average range size of terraranans was ~6x smaller than that of anoles. An average range size–turnover relationship is expected on theoretical grounds [30]; however, rather than a direct causal relationship (e.g., small ranges cause high turnover), turnover pattern differences between anoles and terraranans likely reflect the interaction of dispersal ability, environmental adaptation [10], and biotic interactions, though differences in taxonomic splitting among clades may also play a role (see Caveats section below).

(b) *Mainland–Island Species Turnover*

M–I species turnover patterns were very similar for both anoles and terraranans (Fig 1.3 a, b). Species turnover was complete for every terraranan community and nearly every anole community. However, complete or high turnover alone is not sufficient to infer an island effect on species turnover. M–I turnover could still be consistent with the M–M relationship if islands

19

are sufficiently environmentally dissimilar or far away from mainland areas. Our data reject this possibility; for both clades, M–I species turnover was higher than predicted based on the M-M relationship, given M–I geographic and environmental distances (Fig. 1.3 c, d). This island effect on species turnover is likely the result of several related processes. The harshness of the intervening habitat matrix between islands and the mainland (i.e. salt water) likely renders M–I distances greater in terms of dispersal probability compared to equivalent distances on the mainland. Another key factor is the severe reduction of gene flow from mainland to island populations; without the opposing effects of gene flow [31], island colonizers are more likely to speciate [6]. Lastly, ecological opportunity on islands can promote adaptive radiation and diversification [32], leading to high rates of endemism and turnover. These same processes also contribute to differing M–I species richness patterns in anoles [21], and probably in terraranans (Y. Stuart, unpublished data), which may also influence rates of species turnover [33].

(c) *Island–Island Species Turnover*

Like M–I species turnover, I–I species turnover was significantly higher for each clade than predicted by the best M–M models (Fig. 1.4), again consistent with high speciation (anagenetic and cladogenetic) rates on islands. However, for both clades, the rate at which turnover varied with geographic and environmental distance was closer to the M–M relationship than for M–I comparisons. For terraranans, the slope of the observed vs. predicted regression was not significantly different from the null expectation, indicating a similar relationship as for mainland turnover. Similarly, while the *Anolis* observed vs. predicted species turnover regression slope differed significantly from the null expectation, it was closer to the null than M–I turnover (compare panels c and d from both Fig. 1.3 and Fig. 1.4).

Why would I–I species turnover be more influenced by geographic and environmental distance than M–I species turnover when the same overwater barriers and reductions in gene flow likely apply? Firstly, islands may have shared geological histories unique from the mainland that would not be accounted for in the M-M model. For example, many islands in our study belong to island banks that formed large, connected landmasses during periods of low sea level (e.g., the Great Bahama Bank, [34]). Resulting overland dispersal may have served to mix existing island assemblages, and increased gene flow between incipient species may have halted or reversed the speciation process and reset the speciation clock. Secondly, the relatively low average distance between islands (relative to M–I distances; Fig. S1.1), combined with the likelihood that a non-random subset of capable dispersers [35] colonized the Caribbean archipelago from the mainland could have combined to make dispersal among islands more common than dispersal from the mainland to the islands, thus recovering the relationship between species turnover and geographic and environmental distance.

The closer match of terraranan I–I turnover to the mainland-predicted slope is perhaps counterintuitive as amphibians are generally considered to be poor dispersers [36-38] and are expected to be more sensitive to salt water than reptiles. Thus, one might expect over-ocean dispersal limitation to have a greater effect on the terraranan I–I relationship. One possible explanation is that terraranans are also more limited by overland dispersal than anoles, perhaps because of susceptibility to desiccation. This is consistent with the smaller range sizes and higher rates of turnover among mainland terraranan assemblages compared to anoles. Thus, while terraranans may be poorer dispersers, the relative difference in overland and overwater dispersal may be lower for terraranans than anoles, leading to a smaller difference between M–M and I–I turnover relationships. In general, we predict that the greatest (or smallest) differences between

mainland and island turnover will not occur in clades that are uniformly poor (or strong) dispersers, but rather in those clades that have low overwater dispersal ability relative to overland dispersal ability.

(d) *Caveats*

An appreciable proportion of named anole and terraranan species are geographically restricted allopatric populations that are assumed but not known to be reproductively isolated from their sister species [16,17]. Because accurate estimates of species turnover depend on correct species assignment, taxonomic splitting of two populations that are quantifiably differentiated but not reproductively isolated could arbitrarily inflate estimates of species turnover, especially on islands where researchers may be more likely to delineate unique species. However, for anoles at least, a few recent studies have split mainland species (e.g., [39]), while several intraspecific genetic studies suggest that some island anoles may actually be under-split [40,41]. Furthermore, if the patterns we observed were due to an island taxonomic bias, then we would expect I–I turnover to be more elevated relative to the M–M relationship than M–I turnover; this was not the case. Last, closely related but allopatric populations and species of both clades are often substantially diverged at the molecular level (e.g., [16,40]), and anoles are often quite different in dewlap and body color (e.g., [42]), while terraranans may often differ in their calls (e.g., [43]), altogether suggesting that differential splitting is not likely to have biased our results.

Our random selection of mainland sub-regions may have missed individual centers of mainland species endemism (e.g. mountain tops). Sampling these areas specifically could have led to a small additional number of high M-M turnover measures. However, our goal was to determine whether M-I and I-I turnover patterns differed from the general, representative M-M

turnover patterns, rather than to focus on particular, possibly unrepresentative, areas. Additional comparison of the turnover patterns of mainland centers of endemism to islands remains an interesting topic for future enquiry.

CONCLUSIONS

Our results show that faunal assembly is, indeed, unique on oceanic islands relative to mainland assemblages. For a given geographic and environmental distance between two localities, mainland–island and island–island assemblages have higher turnover on average than mainland–mainland assemblages, indicating that island biotas are, in fact, exceptionally unique. Higher turnover likely stems from the interaction of reduced dispersal, reduced gene flow, higher ecological opportunity, and increased probability of speciation on islands generated by the inhospitable, overwater barrier. Mainland–island turnover is higher than island–island turnover on average, possibly because the connectivity of islands on the same island bank during glacial high-stands serves to homogenize communities and collapse incipient species. More work is needed to understand whether islands influence other aspects of beta-diversity, such as phenotypic or phylogenetic turnover, in similar ways.

REFERENCES

1. Kier, G. *et al.* (2009) A global assessment of endemism and species richness across island and mainland regions. *Proc. Nat. Acad. Sci. USA* 106, 9322-9327 (DOI 10.1073/pnas.0810306106)

2. Simpson, G. G. (1953) *The major features of evolution*. Columbia University Press

3. Schluter, D. (2000) *The ecology of adaptive radiation.* Oxford University Press

4. Losos, J. B. and Schluter, D. (2000) Analysis of an evolutionary species-area relationship. *Nature* 408, 847-850 (DOI 10.1038/35048558)

5. Losos, J. B. and Parent, C. E. (2009) The speciation-area relationship. In *The theory of island biogeography revisited* (eds. J. B. Losos & R. E. Ricklefs), pp. 415-438. Princeton University Press

6. Kisel, Y. and Barraclough, T.G. (2010) Speciation has a spatial scale that depends on levels of gene flow. *Am. Nat.* 175, 316-334 (DOI 10.1086/650369)

7. Rosindell, J. and Phillimore, A. B. (2011) A unified model of island biogeography sheds light on the zone of radiation. *Ecol. Lett.* 14, 552-560 (DOI 10.1111/j.1461-0248.2011.01617.x)

8. Orme, C. D. L. *et al.* (2005) Global hotspots of species richness are not congruent with endemism or threat. *Nature* 436, 1016-1019 (DOI 10.1038/nature03850)

9. Ruggiero, A. and Hawkins, B. A. (2008) Why do mountains support so many species of birds? *Ecography* 31, 306-315 (DOI 10.1111/j.2008.0906-7590.05333.x)

10. Buckley, L. B. and Jetz, W. (2008) Linking global turnover of species and environments. *Proc. Nat. Acad. Sci. USA* 105: 17836-17841 (DOI 10.1073/pnas.0803524105)

11. Qian, H. and Ricklefs, R. E. (2012) Disentangling the effects of geographic distance and environmental dissimilarity on global patterns of species turnover. *Global Ecol. Biogeogr.* 21, 341-351 (DOI 10.1111/j.1466-8238.2011.00672.x)

12. MacArthur, R. H. and Wilson, E. O. (1967) *The theory of island biogeography*. Princeton University Press

13. Losos, J. B. and Ricklefs, R. E., Eds. (2009) *The theory of island biogeography revisited.* Princeton University Press.

14. Whittaker, R. J. *et al.* (2008) A general dynamic theory of oceanic island biogeography. *J. Biogeog.* 35, 977-994 (DOI 10.1111/j.1365-2699.2008.01892.x)

15. Fattorini, S. (2010) The influence of geographical and ecological factors on island beta diversity patterns. *J. Biogeog.* 37, 1061-1070 (DOI 10.1111/j.1365-2699.2009.02252.x)

16. Hedges, S. B. *et al.* (2008) New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. Zootaxa 1737, 1-182

17. Losos, J. B. (2009) *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles.* University of California Press

18. Hanken, J. (1992) Life history and morphological evolution. *J. Evol. Biol.* 5, 549-557 (DOI 10.1046/j.1420-9101.1992.5040549.x)

19. Nicholson, K. E. *et al.* (2005) Mainland colonization by island lizards. *J. Biogeog.* 32, 929-938

20. Heinicke, M. P. *et al.* (2007) Major Caribbean and Central American frog faunas originated by oceanic dispersal. *Proc. Nat. Acad. Sci. USA* 104: 10092-10097 (DOI 10.1073/pnas.0611051104)

21. Algar, A. C. and Losos, J. B. (2011) Evolutionary assembly of island faunas reverses the classic island-mainland richness difference in *Anolis* lizards. *J. Biogeog.* 38, 1125-1137 (DOI 10.1111/j.1356-2699.2010.02466.x)

22. Schwartz, A. and Henderson, R. W. (1991) *Amphibians and reptiles of the West Indies: descriptions, distributions, and natural history.* University of Florida Press

23. Glor, R. E. *et al.* (2005) Out of Cuba: overwater dispersal and speciation among lizards in the *Anolis carolinensis* subgroup. *Mol. Ecol.* 14, 2419-2432 (DOI 10.1111/j.1365-294X.2005.02550.x)

24. Hijmans, R. J. *et al.* (2005) Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatology* 25, 1965-1978 (DOI 10.1002/joc.1276)

25. Lichstein, J. W. (2007) Multiple regression on distance matrices: a multivariate spatial analysis tool. *Plant. Ecol.* 188, 117-131 (DOI 10.1007/s11258-006-9126-3)

26. Algar, A. C., *et al.* (2009) Predicting the future of species diversity: macroecological theory, climate change, and direct tests of alternative forecasting methods. *Ecography* 32, 22-33 (DOI 10.1111/j.1600-0587.2009.05832.x)

27. Raftery, A. E. *et al.* (1997) Bayesian model averaging for linear regression models. *J. Am. Stat. Assoc.* 92, 179-191 (stable URL: http://www.jstor.org/stable/2291462)

28. Piñeiro, G. *et al.* (2008) How to evaluate models: Observed vs. predicted or predicted vs. observed? *Ecol. Model.* 216, 316-322 (DOI 10.1016/j.ecolmodel.2008.05.006)

29. Kerr, J. T. *et al.* (2001) Remotely sensed habitat diversity predicts species richness and community similarity in Canada. *Proc. Nat. Acad. Sci. USA* 98, 11365-11370 (DOI 10.1073/pnas.201398398)

30. Arita, H. T. and Rodríguez, P. (2002) Geographic range, turnover rate and the scaling of species diversity. *Ecography* 25, 541-550 (stable URL: http://www.jstor.org/stable/3683659)

31. Mayr, E. (1963) *Animal species and evolution*. Harvard University Press.

32. Losos, J. B. and Mahler, D. L. (2010) Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. In *Evolution since Darwin: the first 150 years* (eds. M. A. Bell, *et al.*), pp. 381-420. Sinauer Associates

33. Chase, J. M. *et al.* (2011) Using null models to disentangle variation in community dissimilarity from variation in α-diversity. *Ecosphere* 2(2): art 24 (DOI 10.1890/ES10-00117.1)

34. Hearty, P. J. (2010) Chronostratigraphy and morphological changes in *Cerion* land snail shells over the past 130 ka on Long Island, Bahamas. *Quart. Geocrhon*. 5, 50-64 (DOI 10.1016/j.quageo.2009.09.005)

35. Williams, E. E. (1969) The ecology of colonization as seen in the zoogeography of Anoline lizards on small islands. *Quar. Rev. Biol.* 44, 345-389 (stable URL: http://www.jstor.org/stable/2819224)

36. Sinsch, U. (1990) Migration and orientation in anuran amphibians. *Ethol. Ecol. & Evol*. 2, 65-79 (DOI 10.1080/08927014.1990.9525494)

37. Blaustein, A. R. *et al.* (1994) Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Cons. Biol.* 8, 60-71 (DOI 10.1046/j.1523-1739.1994.08010060.x)

38. Barker, B. S. *et al.* (2011) Deep intra-island divergence of a montane forest endemic: phylogeography of the Puerto Rican frog *Eleutherodactylus portoricensis* (Anura: eleutherodactylidae). *J. Biogeogr*. 38, 2311-2325 (DOI 10.1111/j.1365-2699.2011.02578.x)

39. Köhler, J. and Sunyer, J. (2008) Two new species of anoles formerly referred to as *Anolis limifrons* (Squamata: Polychrotidae). *Herpetologica* 64, 92-108 (stable URL: http://www.jstor.org/stable/25209102)

40. Glor, R. E. and Laport, R. (2011) Are subspecies of *Anolis* lizards that differ in dewlap color and pattern also genetically distinct? A mitochondrial analysis. *Mol. Phyl. Evol.* 64, 255-260 (DOI: 10.1016/j.ympev.2010.11.004)

41. Jackman, T. R. *et al.* (2002) Molecular phylogenetic perspective on evolution of lizards of the *Anolis grahami* series. *J. Exp. Zool.* 294, 1-16 (DOI 10.1002/jez.10073)

42. Macedonia, J. M. (2008) Habitat light, colour variation, and ultraviolet reflectance in the Grand Cayman anole, *Anolis conspersus*. *Biol. J. Linn. Soc.* 73, 299-320 (DOI 10.1111/j.1095-8312.2001.tb01365.x)

43. Padial, J. M. *et al.* (2008) Assessing the taxonomic status of tropical frogs through bioacoustics: geographical variation in the advertisement calls in the *Eleutherodactylus discoidalis* species group (Anura). *Zoo. J. Linn. Soc.* 152, 353-365 (DOI 10.1111/j.1096-3642.2007.00341.x)

Chapter 2:

Ecological Character Displacement: Glass Half Full or Half Empty?

Yoel E. Stuart and Jonathan B. Losos

Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology
26 Oxford Street, Cambridge, MA 02138

ABSTRACT

Ecological character displacement (ECD), the evolutionary divergence of competing species, has oscillated wildly in scientific opinion. Thought to play a central role in community assembly and adaptive radiation, ECD recovered from a 1980s nadir to present-day prominence on the strength of many case studies compiled in several influential reviews. However, only nine of 144 cases are strong examples of ECD that have ruled out alternative explanations for an ECD-like pattern. We suggest that ECD's rise in esteem has outpaced available data and that more complete, rather than simply more, case studies are needed. Recent years have revealed that evolutionary change can be observed as it occurs, opening the door to experimental field studies as a new approach to studying ECD.

ECOLOGICAL CHARACTER DISPLACEMENT

The theory of Ecological Character Displacement (ECD) proposes that sympatric species that compete for the same set of limited resources should be favored by natural selection to diverge in resource use and phenotype (Box 2.1) [1]. ECD is thought to be a key driver of evolutionary diversification and adaptive radiation [2]. In a comprehensive and thought-provoking monograph devoted entirely to the evolutionary significance of character displacement, Pfennig and Pfennig [3] conclude in their final paragraph: "Character displacement … plays a key, and often decisive, role in generating and maintaining biodiversity." Moreover, ECD exemplifies eco-evolutionary dynamics, the rapid feedback cycle between evolution and ecology [4,5]. During its 56-year history, ECD has experienced a rollercoaster ride of support in the scientific community, first being embraced enthusiastically, then suffering withering criticism and rejection of its general importance, and most recently climbing back to prominence in evolutionary ecology. In this paper, we review the fall and rise of ECD and conclude that just as many prematurely discarded it in the 1970s, ECD is now being excessively embraced. We show that the data, although mostly consistent with ECD, have not advanced at the same rate as positive opinion during ECD's revival. To fill this knowledge gap, we suggest a research approach meant to complement existing data on ECD – the evolutionary experiment.

THE RISE, FALL, AND RESURRECTION OF ECOLOGICAL CHARACTER DISPLACEMENT

The rise of ECD during the 1950s and 1960s coincided with community ecology's newfound focus on interspecific competition as a major player governing species interactions and community assembly. At that time, MacArthur [6] argued that if not for differences in

feeding times, canopy heights occupied, and perches used, warbler species would have competitively excluded one another in Northeast US conifer forests. Connell [7] showed that interspecific competition structured intertidal barnacle communities, and Hutchinson [8] proposed that the regular beak size differences in sympatry between three species of Galápagos tree finch were differences "necessary to permit two [or more] species to co-occur in different niches but at the same level of a food web." These and other studies (reviewed in [9]) meshed with intuition gleaned from verbal and mathematical models of competitive exclusion [10,11], minimum size ratios [8], and limiting similarity [12,13]. Steeped in this competition-dominated, MacArthurian worldview, evolutionary ecologists found it straightforward to predict ECD: natural selection should favor two species that compete for limited resources to evolve differences that allow coexistence. ECD quickly became "nearly axiomatic in the ecological literature" [14] and was considered a major driver of evolutionary diversification, freely invoked in many cases solely on the basis of a difference in size or trophic traits between sympatric species [reviewed in 15].

However, the importance of interspecific resource competition came under attack during the 1970s and early 1980s (discussed in [16,17]). Various authors argued that the "ubiquitous role of competition" in nature [18] had little support from either observation or experiment [15,18-20]. Wiens [18] noted that the arguments for resource competition were often based on faulty logic, namely that: (i) competition theory predicts differences in resources use; (ii) empirical studies invariably find some difference in resource use, and (iii) these differences are cited as evidence of competition but without conducting the experiments necessary to establish that competition was actually responsible. Indeed, to many, the ECD hypothesis had become unfalsifiable [20] – if species differing in resource use were shown to compete, then competition

was considered to have caused the divergence, but if they were found not to compete, then the "Ghost of Competition Past" must have already caused them to diverge to such an extent that they no longer compete [21].

Concomitant with resource competition's perceived decline in importance, researchers re-examined the theoretical and evidentiary basis for ECD and found it wanting [15,18-20]. An influential theoretical model showed that ECD could evolve only under a restricted set of ecological conditions [22] while re-analysis of previously published data against statistical null models found that size differences among potential competitors were often no greater than that expected by chance [19,20]. Moreover, a slew of alternative processes were proposed that could create a pattern similar to ECD (Box 2.1) [2,15]. By the end of the 1970s, few examples of ECD had survived the gauntlet [18-20] and according to some "the notion of coevolutionary shaping of competitor's niches [had] little support" [21].

However, these critiques did not go uncontested, and some researchers quickly came to the defense of interspecific competition and ECD (e.g., [9,23-25]); the resulting spirited debates spawned a wealth of research over the following decade that helped turn the tide of scientific opinion back in favor of ECD. Specifically, experimental studies garnered stronger evidence for the prevalence of interspecific resource competition in nature [reviewed in 23, 26, 27] while theoretical models with more realistic assumptions about resource use functions and trait variances suggested that the conditions under which ECD could evolve were less restrictive than previously thought (e.g., [28,29]; reviewed in [2]). Moreover, Schluter and McPhail [30] standardized a rigorous approach to the study of ECD by proposing a set of six testable criteria that a strong case of ECD should be able to pass (Box 2.2), thus addressing the criticisms of the

1970s and early 1980s that workers too readily accepted case studies as evidence of ECD before adequately addressing alternative explanations (Box 2.1).

But perhaps most important for the revival of ECD were two reviews by Schluter [2] and Dayan and Simberloff [17] that synthesized an enormous number of studies from the final two decades of the 20th century. These reviews revealed a long list of case studies, some more comprehensive than others, but nearly all providing at least some support for an ECD hypothesis [2,17]; nearly every subsequent discussion of ECD has cited one or both of these reviews.

Thus, today, ECD is again widely considered an important agent of diversification in evolution, a view now regularly expressed without reservation or qualification. A quote from a recent paper by Rando et al. [31] exemplifies the common sentiment: "[ECD] provides a unifying framework for understanding the evolutionary mechanisms of species coexistence and how diversity is maintained." Similarly, Goldberg et al. [32] write: "Ecological character displacement is considered to be widespread in nature and an important determinant of morphological and ecological differences between widespread species."

In contrast to this widespread consensus, however, we argue that ECD's current near-paradigmatic status is not consistent with the available data. While the critiques of the 1970s and early 1980s might have been overly dismissive of ECD, we feel that current opinion is overly accepting. As we show in the next section, case studies of ECD put forth since the contentious debates of the 1970s and early 1980s have increased in quantity, but still few ironclad examples exist. For most cases, interspecific competition hasn't been documented and confounding mechanisms haven't been ruled out.

REVISITING THE EVIDENCE FOR CHARACTER DISPLACEMENT

A dearth of strong cases

To survey the current state of evidence concerning ECD and whether stronger evidence has accumulated over time, we re-examined the reviews of Schluter [2] and Dayan and Simberloff [17] and complemented them with our own survey of ECD studies published since 2005. We measured each putative case of ECD against Schluter and McPhail's criteria [30], which provide a rigorous, standardized, and quantitative way to evaluate the strength of evidence for ECD.

The first observation from these reviews is that there is no shortage of cases documenting a pattern consistent with ECD and satisfying at least one ECD criterion. Schluter [2] identified 64 such cases spanning the years 1964-2000 (Table S2.1; Text S2.1). Dayan and Simberloff [17] simply reported putative cases of ECD, so we went back and scored each study for the ECD criteria. To do this, we trained our scoring against Schluter's [2] for those studies that were shared by both reviews and then scored the studies unique to Dayan and Simberloff [17] published after 1992. This revealed another 40 cases of ECD, approximately 2/3 of which were published after 2000 (Table S1.1). Our survey of papers published since 2005 yielded another 40 cases that met at least one criterion (Table S2.2; Text S2.1). Clearly, there are plenty of putative cases of ECD in the literature, but how well supported are these cases?

Of Schluter's 64 cases, only 20 cases met at least four criteria, and only five cases satisfied all six criteria (Figure 2.1; *Anolis* lizards once, three-spine sticklebacks twice, and Darwin's finches twice. The sticklebacks and finches are counted twice because they each meet two distinct patterns of ECD – see Box 2.1). Schluter [2,33] concluded that the evidence overall was fairly supportive of an important role for ECD in evolutionary diversification, especially compared to earlier and less rigorous times. He did note, however, that "[the] study of character

displacement nevertheless has a long way to go. Key evidence is still lacking in most of the cases that have been described, particularly about the mechanisms" [33].

Our scoring of the additional cases reviewed by Dayan and Simberloff [17] provides a very similar story, suggesting that the strength of the evidence changed little relative to Schluter [2]. Of their 40 cases that met at least one criterion, only ten cases met at least four criteria, and only one, the three-spine stickleback, met all six (Figure 2.1; Table S2.1).

To determine whether the quality of the evidence has increased in recent years, we reviewed papers published from 2005-2012 (Table S2.2). From those papers, 40 cases emerged that satisfied at least one ECD criterion. Twelve of these cases met at least four criteria and two cases satisfied all six (*Escherichia coli* in a lab study and three-spine stickleback yet again) (Figure 2.1).

In sum, the evidence for ECD has not improved greatly over two decades since Schluter and McPhail's paper [30], even as the number of purported examples continues to rise. For the 144 cases examined in the three reviews, the average number of criteria met per case is only 3.3 while just over 5% of cases meet all six criteria. Moreover, those cases that provide strong support for ECD are restricted to only a few groups (i.e., anoles, sticklebacks, Darwin's finches, spadefoot toads, and carnivore and rodent guilds; see [2], Tables S2.1, S2.2). While the number of cases consistent with ECD has grown in the last 20 years, the depth of the data underlying those cases has not.
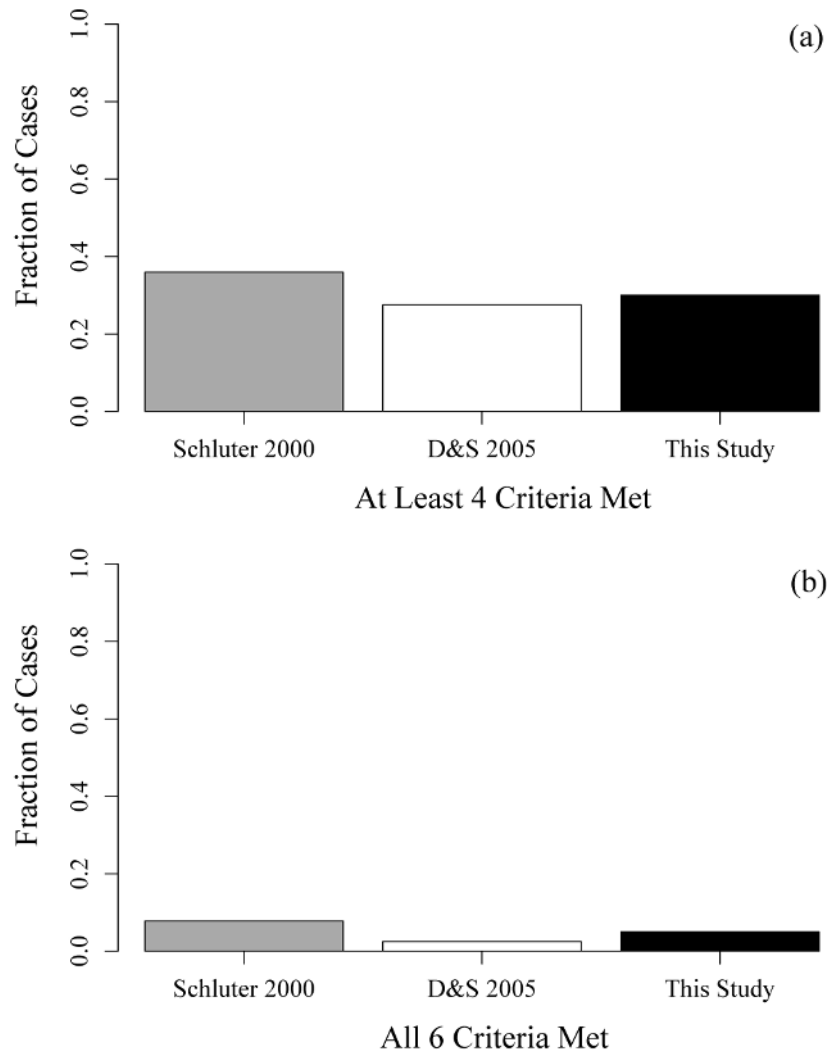
FIGURE 2.1. The fraction of cases in Schluter [2], Dayan and Simberloff (D&S) [17], and this study for which at least (a) four or (b) all six criteria were met. We considered only those cases that met at least one criterion and thus excluded 10 additional cases discussed by Dayan and Simberloff [17]. In some instances, the same case is considered in more than one review (e.g., stickleback, compare [2], Tables S2.1, S2.2). However, nearly every case was evaluated using only studies from within a given review; only one case was strengthened by combining data across reviews (*Canis* spp.; see Table S2.1). Thus, cases can be considered independent of one another across reviews.

Assessing individual criteria

How do the individual criteria fare and what can they tell us about the ecological and evolutionary processes generating patterns of ECD (Box 2.1; Figure 2.2)? Across all three reviews, for cases in which at least one criterion was met, criterion 2 (chance disproved) and criterion 4 (trait change matching resource use) were met most often (70% and 63% of cases, respectively), suggesting that the observed pattern of phenotypic divergence is often greater than expected if species evolved independently of one another, and that such divergence is usually consistent with changes in resource use.

The mechanism underlying the pattern of divergence, however, is often unclear. Resource competition (criterion 6) is the biotic interaction thought to drive ECD; yet, it is documented in only 17% of cases (Figure 2.2). Thus, it is possible that displacement patterns might instead have evolved from other interspecific interactions such as intraguild predation or apparent competition (Box 2.1). It is also possible, however, that in many cases the existence of an ECD-like displacement pattern may have nothing to do with an evolved response to interspecific interactions. For example, only 41% of cases showed that environmental conditions were similar among sites (criterion 5; Figure 2.2), so many putative patterns of ECD could be the result of local adaptation to environmental heterogeneity, irrespective of the presence of an interacting competitor. Similarly, *in situ* diversification (criterion 3) was documented in just over half of the cases (Figure 2.2), so many patterns of ECD might not be an evolutionary outcome of interspecific interactions, but instead an ecological outcome of processes like dispersal, environmental filtering, and competitive exclusion (Box 1.1). In sum, the majority of putative cases of ECD published in the last 20 years are also consistent with a host of evolutionary and ecological mechanisms other than interspecific resource competition (Box 2.1).
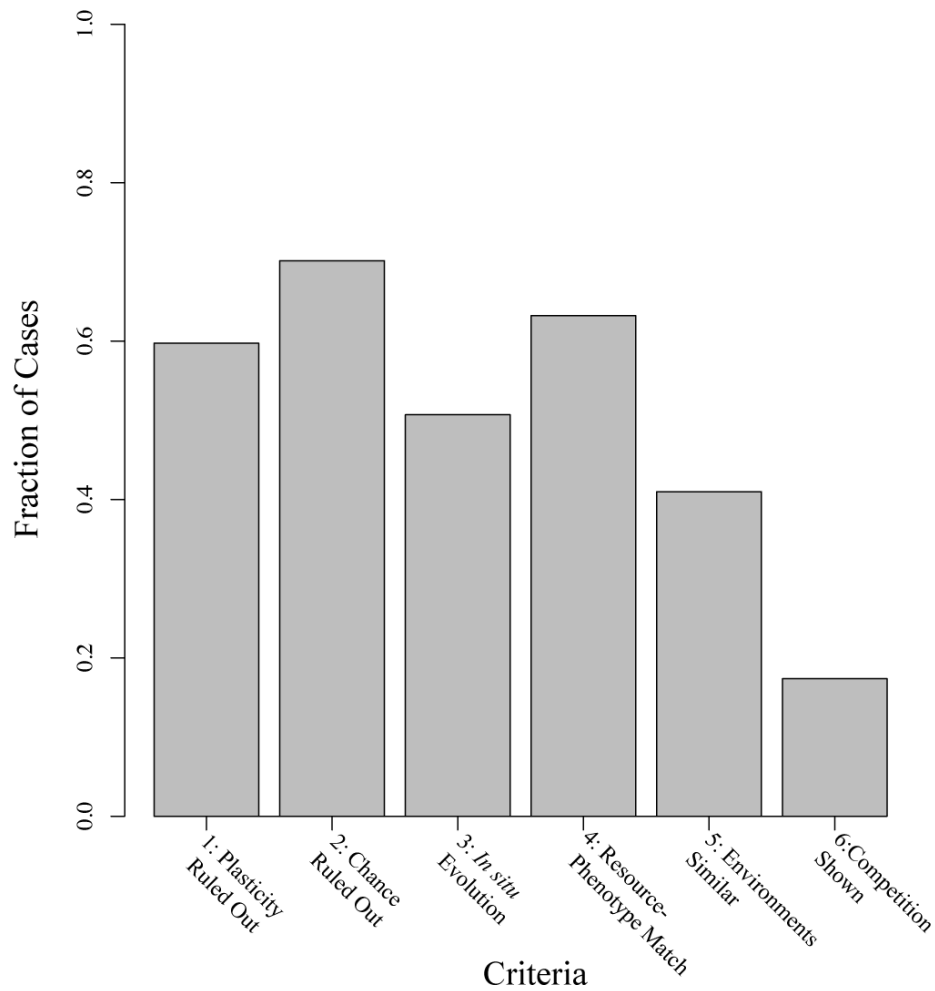
FIGURE 2.2. The fraction of cases for which each criterion was met. We pooled the cases from all reviews ([2,17], this study) for which at least one criterion was met. We note that, following Schluter [2], we assumed a genetic basis for any case of trait over-dispersion (Box 2.1) as these patterns are based on species in sympatry, whose differences likely have a genetic component.

Glass half full or half empty?

Taken together, the state of the evidence for ECD can be viewed in one of two ways. Under the glass half full view, Schluter [2,33] was certainly justified in noting that there are many reasonably well supported cases, especially in the context of the backlash of the 1970s-80s. Most criteria are individually satisfied many times across all cases (Figure 2.2), and the most recent treatment of character displacement [3] does not place undue emphasis on meeting all the ECD criteria in each case: "an overly rigorous application of [the six] criteria can be as problematic as not applying the criteria in the first place" (pp. 16). Thus perhaps it is sufficient to have many reasonably supported examples of ECD.

However, we take the glass half empty view. Despite more than a quarter century's emphasis on rigorous examination of ECD hypotheses, we still have only nine cases that meet the gold standard by satisfying all six criteria, and as noted above, these nine cases come from a taxonomically limited portion of the biological world. Moreover, it is difficult to determine the frequency and importance of ECD as positive cases are likely published more often than negative ones. ECD might indeed be a pervasive process driving evolutionary diversification, and more and more potential examples are being put forward, but the evidence in support of this proposition is still not overwhelming. At this point, the community needs not more cases of ECD, but better documented ones.

RAPID EVOLUTION AND THE EXPERIMENTAL STUDY OF EVOLUTION IN NATURE

One of the major developments in evolutionary biology in the last quarter century is the recognition that when directional selection is strong, evolution proceeds rapidly enough to be measurable over ecological time – a realization that spawned the new field of eco-evolutionary

dynamics [4-5,34-36]. Neither Schluter [2] nor Dayan and Simberloff [17] noted any studies

documenting the evolution of ECD in real time, perhaps not surprising given how recently it was

realized that evolution can act rapidly. Nonetheless, since 2005, one such study has appeared: a

long-term observational study of Darwin's finches by Grant and Grant [37] documented a shift in

beak size by *Geospiza fortis* in response to competition with a recent colonist, *G. magnirostris,*

for drought-limited seed resources (Box 2.3). Observing the evolution of ECD in action provides

the benefit of immediately confirming that divergence has occurred *in situ* (criterion 3). If such

evolution is repeated in multiple localities, then chance can be ruled out as well (criterion 2).

Thus we expect that additional long-term studies like that of Grant and Grant [37] will provide

stronger evidence for ECD in a variety of organisms. This might be especially true as climate

change and species introductions continue to bring together novel sets of potentially interacting

species in quasi-experimental settings.

Perhaps even more importantly, however, the reality of rapid evolutionary change creates

the possibility of actually conducting experiments in nature to directly test evolutionary

hypotheses, which the work of Endler, Reznick and colleagues has done for guppy life-history

traits responding to different predator conditions (e.g., [38]). Direct experimental studies of ECD

in the field would present the opportunity to observe ECD as it happens while simultaneously

controlling for many factors that have confounded observational studies of ECD (Box 1.3).

Laboratory biologists, of course, have known for some time that ECD can be observed in

real-time; indeed, some of the best examples of ECD have come from lab experiments with

microbes (e.g., [39,40]) and bean weevils [41]. Several groups have used elegant field

experimental approaches to estimate the strength of past natural selection that could have led to

present-day pattern of ECD, but those studies focused on populations that had already diverged

[33,42]. The next step, then, is to pair two or more un-diverged competitor species to determine experimentally if and how ECD evolves. We envision a series of experiments carried out in settings that mimic nature as closely as possible (i.e. ponds, small islands, large enclosures), involving transplants of local native species or taking advantage of recent species invasions that have brought together putative competitors with no recent history of competition.

While no field experiment will perfectly control for all factors that affect evolution in nature, carefully designed experiments would address the factors that tend to confound observational studies by controlling variables like environmental heterogeneity (criterion 5) and pre-existing divergence (criterion 3), by providing replication (criterion 2), and by further clarifying any causal link between the presence of resource competition (criterion 6) and morphological divergence in resource related traits (criterion 4). Such experiments address these criteria directly and simultaneously. The evolutionary experiment, combined with studies that address phenotypic plasticity (criterion 1) and further explore the mechanistic nature of the species interactions (i.e., confirming or rejecting resource competition as the driving interspecific interaction), will help provide more conclusive evidence whether ecological character displacement truly is a major driver of evolutionary diversification and adaptive radiation.

CONCLUSION

Our findings suggest that most cases of ECD are also consistent with other evolutionary and ecological processes. Perhaps not coincidently, then, in the few cases where support for ECD is unequivocal, we have a good understanding of the underlying eco-evolutionary dynamics – how does resource depletion lead to competition, lead to natural selection, lead to evolutionary divergence, lead to resource partitioning (e.g., Darwin's finches; Box 2.3)? Understanding such

dynamics provides a clearer understanding of the ECD and such research can reveal new ECD patterns not currently predicted by theory (e.g., repeated bouts of competition and divergence [39]). In sum, despite nearly two decades of rigorous study, the jury has not yet rendered a verdict on the evolutionary importance of character displacement; however, with the advent of eco-evolutionary thinking and the application of evolutionary experiments in the field, the next two decades should bring the jury much closer to a verdict.

BOX 2.1: PROCESS AND PATTERN IN ECOLOGICAL CHARACTER DISPLACEMENT

The ECD process, as traditionally defined, has two steps: (i) interspecific competition for limited resources creates natural selection that favors those individuals most adept at partitioning resources, which (ii) drives populations to diverge adaptively, either by changing trait means or shrinking trait variance. Interspecific competition is the proximate cause of ECD, creating the resource-use partitioning that is the ultimate cause of phenotypic divergence. The process of ECD is thought to produce two different patterns of displacement: exaggerated divergence in sympatry and trait over-dispersion [2]. Exaggerated divergence in sympatry is when two species are similar in resource use and phenotype in allopatry but diverge in sympatry. Trait over-dispersion is when several sympatric species of the same ecological guild exhibit resource-use phenotypes that are more different from one another than expected by chance, also known as community-wide character displacement [19]. A third pattern, species-for-species matching, is occasionally cited: the replicated, independent evolution of guild structure [2].

Species interactions other than resource competition, however, could produce the same displacement patterns as ECD. One example is apparent competition [43,44]. In this scenario, species A brings its predator into sympatry with Species B. To avoid this new predator, species B shifts its resource use and diverges in phenotype – an apparent response to competition that is only indirectly related to the presence of species A; the two species do not actually compete. Similarly, the outcomes of other species interactions can mimic ECD, including agonistic interactions [45], intraguild predation [46], and reinforcement [47,48]. Except for reinforcement, the importance of these other interactions to evolutionary divergence between closely related species is not known [2].

Independently of species interactions, however, non-evolutionary events or processes can also create a displacement pattern. Take, for example, the phenomenon of ecological sorting. Due to competitive exclusion, only those species that are different enough from one another are able to coexist, so a pattern of trait over-dispersion might result not from *in situ* co-evolution (i.e., ECD), but from the inability of species that have not diverged sufficiently in allopatry to coexist. Similarly, local adaptation along an environmental gradient [e.g., 49], phenotypic plasticity that mitigates a resource-use shift and precludes an evolutionary response, or even random associations can create displacement patterns normally attributed to ECD.
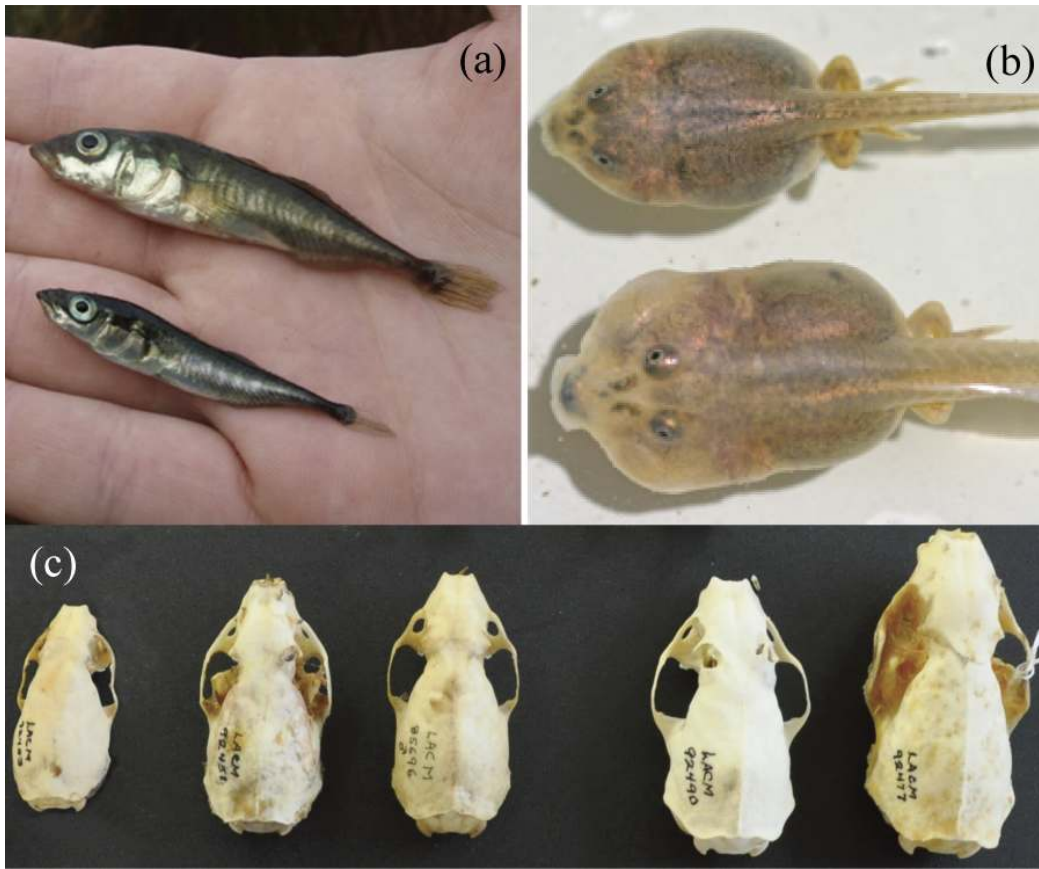
BOX 2.1 FIGURE. (a) Benthic (top) and limnetic (bottom) morphs of the threespine stickleback (*Gasterosteus aculeatus*) have diverged in body shape and body size in sympatry [30] (photo by L. Southcott). (b) In sympatric ponds, tadpoles of the Mexican spadefoot toad (*Spea multiplicata*) develop into omnivore morphs (top) more frequently and tadpoles of the Plains spadefoot toad (*Spea bombifrons*) develop into carnivore morphs (bottom) more frequently than either species does in allopatry [42] (photo by D. Pfennig). (c) Members of the North American weasel guild show equal size ratios in skull and canine size – community-wide character displacement [54, but see 49]. Pennsylvanian specimens of, left to right, a female Least Weasel (*Mustela nivela*), a female Ermine (*M. erminea*), a male Ermine, a male Long-tailed Weasel (*M. frenata*), and a female Long-tailed Weasel (photo by S. Meiri). Sexes are treated as separate "morphospecies" in many analyses of community-wide character displacement.

BOX 2.2: TESTING ECOLOGICAL CHARACTER DISPLACEMENT

The following six criteria are meant to rule out alternative processes that might lead to an ECD pattern [30]. Falsification of any of the six criteria indicates that observed differences did not result from ECD.

(1) *Phenotypic differences between species result from evolved genetic differences among populations in sympatry vs. allopatry.* Character displacement is an evolutionary hypothesis, yet the number of examples of adaptive phenotypic plasticity continues to rise [50, 51]. Thus, the potential for plastic responses to produce a pattern of ECD must be ruled out. Adaptive reaction norms evolved in response to competition (e.g., spadefoot toads [52]), however, are consistent with ECD.

(2) *The character displacement pattern could not arise by chance.* Sympatric species might differ in phenotype solely as a result of random processes; as a result, appropriate statistical tests must be used to demonstrate that differences observed are greater than expected by chance.

(3) *The character displacement pattern results from an evolutionary shift rather than species sorting.* Interspecific competition may allow only species that are phenotypically divergent to coexist, but such divergence among sympatric species need not have resulted from *in situ* displacement. Instead, species might have diverged in allopatry for other reasons, only then becoming capable of successfully colonizing and coexisting in regions of sympatry. Such allopatric divergence scenarios must be ruled out as a cause of an ECD pattern.

(4) *Changes in phenotype (i.e., character displacement) match ecological shifts in resource use.* The theory of ecological character displacement suggests that two species diverge in

phenotype in response to competitively mediated shifts in resource use. Thus, there should be a clear functional link between the displaced trait and the partitioned resource.

(5) *Sites of allopatry and sympatry do not differ appreciably in environment.* Species adapt to many biotic and abiotic components of their environment. Adaptation to undetected differences in resource-availability between sympatry and allopatry may create an ECD pattern (e.g., [49]). Thus, the possibility that an ECD pattern could be driven by variation in environmental factors other than the presence of competitors should be ruled out.

(6) *Evidence shows that similar phenotypes actually compete for limited resources.* The process of ecological character displacement is predicated on the occurrence of interspecific resource competition driving phenotypic divergence. However, a number of other interspecific interactions could cause the evolution of phenotypic differences (Box 2.1). Demonstration of ECD thus requires ruling out other processes by demonstrating competition for resources.

BOX 2.3: OBSERVING ECD IN DARWIN'S FINCHES

In a study of Darwin's finches, Grant and Grant [33] documented the evolution of ECD across one generational boundary. In 2004, depletion of seed resources during a major drought on Daphne Major island brought the medium ground finch, *Geospiza fortis*, into competition with the large ground finch, *G. magnirostris*, for large seeds. *Geospiza fortis* altered its resource use towards smaller seeds available in the seed bank and natural selection drove evolution toward smaller beaks that were more adept at using such seeds.

The change in phenotype matched the change in resource use (criterion 4) as the shift to smaller seeds was matched by a shift to a functionally more efficient smaller beak size. This was an evolved response (criterion 1; beak size has high heritability in this population and should respond to strong directional selection) that occurred *in situ* (criterion 3). Three decades of data prior to the 2004 drought served as a control, suggesting that there were no other environmental factors that could have driven the beak size shift (criterion 5). Three lines of evidence directly implicate resource competition for seeds (criterion 6): (i) *G. fortis* relied on large seeds when small seeds were depleted during a 1977 drought before the colonization of Daphne Major by *G. magnirostris*, indicating that the species can overlap in resource use; (ii) behavioral observations in 2004 suggested that *G. magnirostris* fed on a much higher fraction of large seeds than *G. fortis*; and, (iii) during the 2004 drought, the *G. fortis* population declined to a level lower than during any past drought. Of course, because this was a singular event, Grant and Grant [33] could not rule out the possibility that the decrease in beak size by *G. fortis* during the drought in the presence of *G. magnirostris* was a coincidence (criterion 2) [53], although the wealth of long-term data in this study suggests that that possibility is unlikely.

What advantages did the evolution-in-action approach provide? First, the population was observed evolving in real time, leaving no question of *in situ* phenotypic change (criterion 3). Second, the change was observed to occur during the peak of resource limitation, thus ruling out alternative environmental factors that could drive such a pattern (criterion 5) and directly implicating interspecific competition (criterion 6). These three criteria are the most difficult to meet with observational data alone (Figure 2.2).

small beaked phenotype
adept at handling small seeds

Geospiza fortis

large beaked phenotype
adept at handling large seeds

G. magnirostris
specialist on large seeds

BOX 2.3 FIGURE. Large beaked *Geospiza fortis* (middle) were inferior competitors to *G. magnirostris* (bottom) for large seeds during the 2004 drought. As large seeds became depleted in the seed bank, natural selection favored smaller beaked *G. fortis* (top) that were better able to exploit small seeds [37]. (Photos by P. and R. Grant.)

GLOSSARY

Apparent competition – An indirect interaction in which one species negatively affects the other species by increasing predation by a shared predator

Competitive exclusion – A principle stating that two species that compete for the same set of resources cannot coexist, all other ecological factors equal

Guild – Any group of species that exploits similar resources in similar ways

Intraguild predation – At least one member of a guild of competing species preys upon one or more other members

Limiting similarity – A corollary of competitive exclusion, the maximum level of resource use overlap between two given species for which coexistence is still possible

REFERENCES

1. Brown, W.L., and Wilson, E.O. (1956) Character displacement. *Syst. Zool.* 5, 49-64

2. Schluter, D. (2000) *The ecology of adaptive radiation,* Oxford University Press

3. Pfennig, D.W. and Pfennig, K.S. (2012) *Evolution's wedge: competition and the origins of diversity,* University of California Press

4. Hairston, N.G., Jr. *et al.* (2005) Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett*. 8, 1114-1127

5. Schoener, T.W. (2011) The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* 331, 426-429

6. MacArthur, R.H. (1958) Population ecology of some warblers of Northeastern coniferous forests. *Ecology* 39, 599-619

7. Connell, J.H. (1961) The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology* 42, 710-723

8. Hutchinson, G.E. (1959) Homage to Santa Rosalia, or why are there so many kinds of animals? *Am. Nat.* 93, 145-159

9. Schoener, T.W. (1982) The controversy over interspecific competition: despite spirited criticism, competition continues to occupy a major domain in ecological thought. *Am. Sci.* 70, 586-595

10. Gause, G.F. (1932) Experimental studies on the struggle or existence: I. Mixed population of two species of yeast. *J. Exp. Biol.* 9, 389-402

11. Hardin, G. (1960) The competitive exclusion principle. *Science* 131, 1292-1297

12. MacArthur, R. and Levins, R. (1964) Competition, habitat selection, and character displacement in a patchy environment. *Proc. Nat. Acad. Sci. USA* 51, 1207-1210

13. MacArthur, R. and Levins, R. (1967) The limiting similarity, convergence, and divergence of coexisting species. *Am. Nat.* 101, 377-385

14. Case, T.J. (1979) Character displacement and coevolution in some *Cnemidophorus* lizards. *Fortschr. Zool.* 25, 235-282

15. Grant, P.R. (1972) Convergent and divergent character displacement. *Biol. J. Linn. Soc*. 4, 39-68

16. Lewin, R. (1983) Santa Rosalia was a goat. *Science* 221, 636-639

17. Dayan, T. and Simberloff, D. (2005) Ecological and community-wide character displacement: the next generation. *Ecol. Lett.* 8, 875-894

18. Wiens, J.A. (1977) On competition and variable environments: populations may experience "ecological crunches" in variable climates, nullifying the assumptions of competition theory and limiting the usefulness of short-term studies of population patterns. *Am. Sci.* 65, 590-597

19. Strong, D.R. et al. (1979) Tests of community-wide character displacement against null hypotheses. *Evolution* 33, 897-913

20. Simberloff, D. and Boecklen, W. (1981) Santa Rosalia reconsidered: size ratios and competition. *Evolution* 35, 1206-1228

21. Connell, J.H. (1980) Diversity and the coevolution of competitors, or the Ghost of Competition Past. *Oikos* 35, 131-138

22. Slatkin, M. (1980) Ecological character displacement. *Ecology* 61, 163-177

23. Schoener, T.W. (1983) Field experiments of interspecific competition. *Am. Nat.* 122, 240-285

24. Schoener, T.W. (1984) Size differences among sympatric, bird-eating hawks: a worldwide survey. In *Ecological communities: conceptual issues and the evidence*. (Strong D.R., Jr. *et al.* eds.) pp. 254-281, Princeton University Press

25. Gilpin, M.E. and Diamond, J.M. (1984) Are species co-occurrences on island non-random, and are null hypotheses useful in community ecology. In *Ecological communities: conceptual issues and the evidence*. (Strong D.R., Jr. *et al.* eds.) pp. 297-315, Princeton University Press

26. Goldberg, D.E. and Barton, A.M. (1992) Patterns and consequences of interspecific competition in natural communities; a review of field experiments with plants. Am. Nat. 139, 771-801

27. Gurevitch, J. *et al.* (1992) A meta-analysis of competition in field experiments. *Am. Nat.* 140, 539-572

28. Taper, M.L. and Case, T.J. (1985) Quantitative genetic models for the coevolution of character displacement. *Ecology* 66, 355-371

29. Abrams, P.A. (1986) Character displacement and niche shift analysed using consumer-resource models of competition. *Theor. Pop. Biol.* 29, 107-160

30. Schluter, D. and McPhail, J.D. (1992) Ecological character displacement and speciation in sticklebacks. *Am. Nat.* 140, 85-108

31. Rando, J.C., *et al.* (2010) Disentangling ancient interactions: a new extinct passerine provides insights on character displacement among extinct and extant island finches. *PLoS ONE* 5, e12956

32. Goldberg, E.E. *et al.* 2012. Population regulation and character displacement in a seasonal environment. *Am. Nat.* 179, 693-705

33. Schluter, D. (2000) Ecological character displacement in adaptive radiation. *Am. Nat.* 156, S4-S16

34. Hendry, A.P. and Kinnison, M.T. (1999) Perspective: The pace of modern life: measuring rates of contemporary microevolution. *Evolution* 53, 1637-1653

35. Kinnison, M.T. and Hendry, A.P. (2001) The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* 112-113: 145-164

36. Rose, M.R. and Garland, T., Jr. (2009) Darwin's other mistake. In *Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments* (Garland, T., Jr. and Rose, M.R., eds), pp. 3-13, University of California Press

37. Grant, P.R. and Grant, B.R. (2006) Evolution of character displacement in Darwin's finches. *Science* 313, 224-226

38. Reznick, D.N., and C.K. Ghalambor. (2005) Selection in nature: experimental manipulations of natural populations. *Int. Comp. Biol.* 45, 456-462

39. Le Gac, M. *et al.* (2012) Ecological and evolutionary dynamics of coexisting lineages during a long-term experiment with *Escherichia coli*. *Proc. Nat. Acad. Sci. USA* 109, 9487-9492

40. Bailey, S.F. and Kassen, R. (2012) Spatial structure of ecological opportunity drives adaptation in a bacterium. *Am. Nat.* 180, 270-283

41. Taper, M.L. (1990) Experimental character displacement in the Adzuki Bean Weevil, *Callosobruchus chinensis*. In *Bruchids and legumes: economics, ecology, and coevolution* (Fujii, K. *et al.*, eds), pp. 289-301, Kluwer Academic Publishers

42. Martin, R.A. and Pfennig, D.W. (2011) Evaluating the targets of selection during character displacement. *Evolution* 65, 2946-2958

43. Holt, R.D. (1977) Predation, apparent competition, and the structure of prey communities. *Theor. Pop. Biol.* 12, 197-229

44. Blitzer, E.J. and S.C. Welter. (2011) Emergence asynchrony between herbivores leads to apparent competition in the field. *Ecology* 92, 2020-2026

45. Grether, G.F. *et al.* (2009) The role of interspecific interference competition in character displacement and the evolution of competitor recognition. *Biol. Rev.* 84, 617-635

46. Polis, G.A. *et al.* (1989) The ecology and evolution of intraguild predation: potential competitors that eat each other. *Ann. Rev. Ecol. Syst.* 20, 297-330

47. Pfennig, K.W. and Pfennig, D.W. (2009) Character displacement: ecological and reproductive responses to a common evolutionary problem. *Quart. Rev. Biol.* 84, 253-276

48. Pfennig, D.W. and Pfennig, K.S. (2010) Character displacement and the origins of diversity. *Am. Nat.* 176, S22-S44

49. Meiri, S. *et al.* (2011) Community-wide character displacement in the presence of clines: a test of Holarctic weasel guilds. *J. Anim. Ecol.* 80, 824-834

50. Pfennig, D.W. *et al.* (2010) Phenotypic plasticity's impacts on diversification and speciation. *Trends Ecol. Evol.* 25, 459-467

51. Ghalambor, C.K., *et al.* (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* 21, 394-407

52. Pfennig, D.W. *et al.* (2006) Ecological opportunity and phenotypic plasticity interact to promote character displacement and species coexistence. *Ecology* 87, 769-779

53. Grant, B.R. and Grant, P.R. (2010) Songs of Darwin's finches diverge when a new species enters a community. *Proc. Nat. Acad. Sci. USA* 107, 20156-20163

54. Dayan, T. *et al.* (1989) Inter- and intraspecific character displacement in Mustelids. *Ecology* 70, 1526-1539

Chapter 3:

Rapid evolution of character displacement in the lizard *Anolis carolinensis* following invasion by a congener

Yoel E. Stuart[1], Todd S. Campbell[2], R. Graham Reynolds[3], Liam J. Revell[3], and Jonathan B. Losos[1]

[1] Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138

[2] Department of Biology, University of Tampa, 401 W. Kennedy Blvd., Tampa, FL 33606

[3] Department of Biology, University of Massachusetts Boston, 100 Morrisey Blvd., Boston, MA 02125

(As prepared for *Science*)

ABSTRACT

Species divergence driven by interspecific ecological interactions, or character displacement (CD), is widely thought to play a key role in evolution. Yet very few conclusive case studies of CD exist, primarily because of the difficulty of ruling out alternative causes of a CD pattern. Here, we rule out such alternative causes by documenting the rapid, replicated evolution of CD in small-island populations of the lizard *Anolis carolinensis,* following invasion by an ecologically similar species, *Anolis sagrei.* We find that *A. carolinensis* perch higher on invaded islands and, in response, have evolved better-developed toepads in ~20 generations. Documenting the real-time evolution of this common adaptation to increased arboreality in lizards provides robust evidence for CD, a process considered crucial to evolution.

ONE SENTENCE SUMMARY

In only 20 generations, island populations of the lizard *Anolis carolinensis* have adapted morphologically to habitat-use shifts driven by an invasive and ecologically-similar congener.

Character displacement (CD) – in which phenotypic divergence is driven by interspecific interactions, (e.g., resource competition, traditionally) [1] – has ridden a roller coaster ride of scientific opinion in the six decades since it was proposed. In the 1950s and 1960s, CD was championed as a major driver of community structure, evolutionary diversification, and adaptive radiation, but was then dismissed as an unimportant phenomenon during the ecological-null-model backlash of the 1970s and early 1980s [2,3]. Most recently, in the last twenty years, CD's standing reversed again, gaining wide acceptance in the evolutionary ecology community [4-12]. Yet despite the current consensus that CD is a common and important evolutionary process, the underlying data are not nearly as strong as many might believe—despite a plethora of plausible case studies, very few cases conclusively demonstrate CD's occurrence [13]. Specifically, the recent rise in support for CD has been fueled by many studies that are consistent with but do not conclusively support CD, largely because it is difficult to rule out processes and events other than those arising from interspecific interactions that can generate similar phenotypic patterns to CD (e.g., local adaptation to environmental gradients, ecological sorting, phenotypic plasticity, or even chance; reviewed in [2,13]). Such difficulty arises from using present-day ecological pattern to distinguish among alternative evolutionary processes that acted in the past.

Documenting the evolution of CD over ecological time, however, may help remove the masking effects of evolutionary time and has great potential to provide strong tests of a CD hypothesis against its alternatives (e.g., [13,14]). Recent years have seen a flourish of studies demonstrating rapid evolution [15,16], which suggests that evolutionary hypotheses like CD should be testable in nature [17], whether such tests observe the real-time evolution of CD directly through manipulative experiment or opportunistically following environmental change. One such opportunistic study of the evolution of CD was conducted by Grant and Grant [14],

61

who documented diet and morphological shifts in a single population of the Galápagos finch *Geospiza fortis*, following invasion by a congeneric competitor. Because this was a singular occurrence, however, it is difficult to rule out the possibility that CD did not arise by chance or in response to some unmeasured factor. To our knowledge, the replicated, real-time evolution of CD in nature has not been reported.

Here, we document the replicated evolution of CD in the US-native Green Anole lizard, *Anolis carolinensis*, following contact with the invasive Cuban Brown Anole, *A. sagrei,* which colonized southern Florida from Cuba in the 1940s and subsequently spread north [18-20]. We studied lizard populations on a set of small man-made islands, approximately one hectare in size, that were created in the 1950s by the US Army Corps of Engineers during the dredging of the Intracoastal Waterway in Mosquito Lagoon (Fig. 3.1). Shortly after their creation, these islands were colonized by nearby mainland flora and fauna. In 1994, T.S.C. surveyed for the presence of *A. carolinensis* and *A. sagrei* on each of 26 islands along the western edge of the lagoon, finding that each island maintained populations of *A. carolinensis* and that 2 islands had already been invaded by *A. sagrei* [21]*,* which had arrived to the nearby mainland in the late 1980s [18,20]. Y.E.S. resurveyed these islands in 2010 and found that 19 more had been invaded by *A. sagrei* in the 16 years between surveys. Thus, given the 1994 survey, the maximum time that these 19 *A. carolinensis* populations could have been interacting with *A. sagrei* at the time of this study is 16 years (or ~20 generations) [19].

To test whether the presence of *A. sagrei*, an ecologically similar species, has driven CD in *A. carolinensis*, we compared *A. carolinensis* habitat use and morphology on six islands invaded by *A. sagrei* (hereafter "invaded" islands) to the habitat use and morphology of *A. carolinensis* on the last five islands not invaded by *A. sagrei* (hereafter "un-invaded" islands)

(Fig. 3.1). The six invaded islands were chosen because they were similar in size, shape, and age to the un-invaded islands; invaded islands did not differ significantly from un-invaded islands in distance to the mainland, total area, vegetated area, vegetation species richness, or available tree heights (Table S3.1; see SM for details).
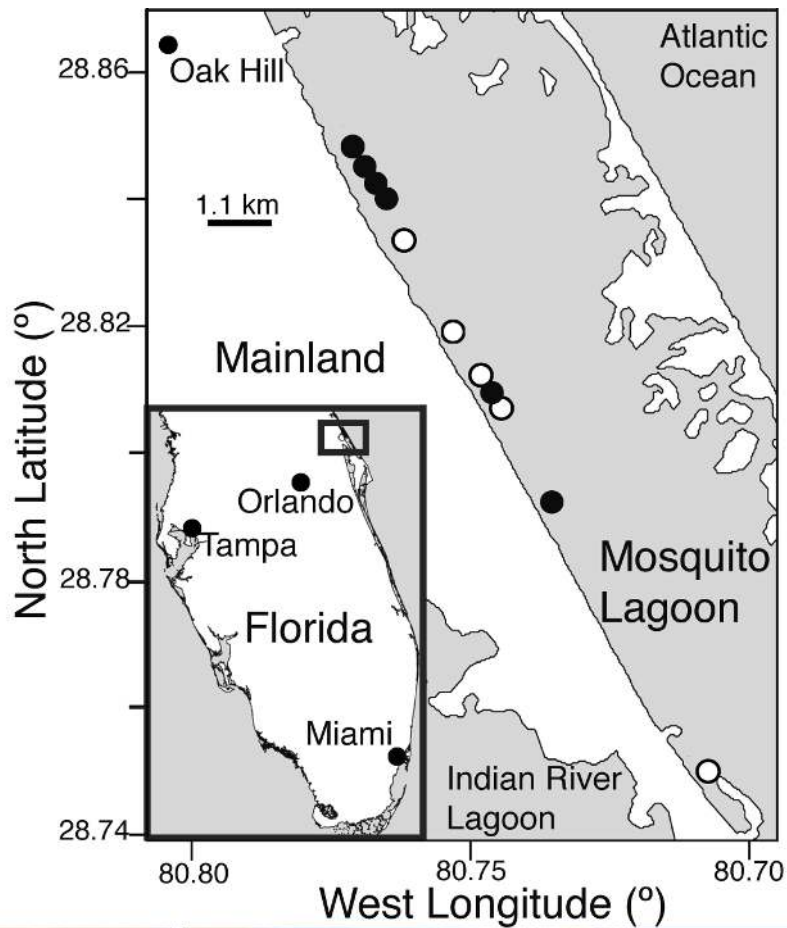
FIGURE 3.1. Top: Eleven study islands in Mosquito Lagoon, Florida. *Anolis carolinensis* inhabits all islands. We chose to study six islands invaded by *A. sagrei* (closed circles) and the five islands that remain un-invaded (open circles). Many other invaded islands line the western edge of the lagoon but are not shown for clarity. Bottom left: *A. carolinensis.* Bottom middle: *A. sagrei*. Bottom right: Pursuing lizards on Hook Island.

We developed two directional predictions for *A. carolinensis* ecology and morphology in this system. First, *A. carolinensis* should alter its habitat use to become more arboreal on invaded islands compared to un-invaded islands. Historically, as the only anole native to the United States, *A. carolinensis* used the entire habitat from ground to tree crown [22], a habit shown by most solitary anole species [23,24]. However, *A. sagrei* is aggressively territorial and prefers the ground and lower tree trunks up to approximately 1.5m [22]. Thus, on invaded islands, we predicted *A. carolinensis* would shift to higher perches [21,24,25], retreating to its ancestral niche exemplified today by its Cuban sister species *A. porcatus,* which partitions the vertical environment with *A. sagrei* in Cuba by favoring higher perches [22,26].

Second, such an arboreal shift by *A. carolinensis* should be accompanied by the evolution of larger toepads with more lamellae (setae-laden scales important for adhesion). Comparative studies across the *Anolis* genus have shown that size-corrected toepad area and lamella number correlate positively with perch height across species [22, 27-30]. Functionally, larger relative toepad area improves clinging ability [30], likely permitting anoles to better grasp unstable, narrow, and smooth arboreal perches like twigs and leaves during foraging, predator encounters, and territorial bouts with other lizards.

To test our first prediction of a shift in perch height, we conducted habitat use surveys during May-August 2010 and measured perch height for every undisturbed lizard observed (Table S3.2). We found that on invaded islands, *A. carolinensis* perches significantly higher than on un-invaded islands (Fig. 3.2A; island sample sizes: 57-111; Linear Mixed-Model; $\beta_{sagrei\ present}$ = 2.77, t_9 = 6.6, one-tailed $p < 0.001$; see SM for analytical details).

To test our second prediction of a morphological response to this perch height shift, we measured toepad area and counted lamella number for every lizard captured (Table S3.2).

Consistent with our prediction, we found that *A. carolinensis* populations on invaded islands had larger toepads and more lamellae (Fig. 3.2B; island sample sizes: 41-61; Linear Mixed Models; Toepad Area: $\beta_{\text{sagrei present}} = 0.15$, $t_9 = 2.7$, one-tailed $p = 0.012$; Lamella Number: $\beta_{\text{sagrei present}} = 0.57$, $t_9 = 3.3$, one-tailed $p = 0.005$; see SM for analytical details).
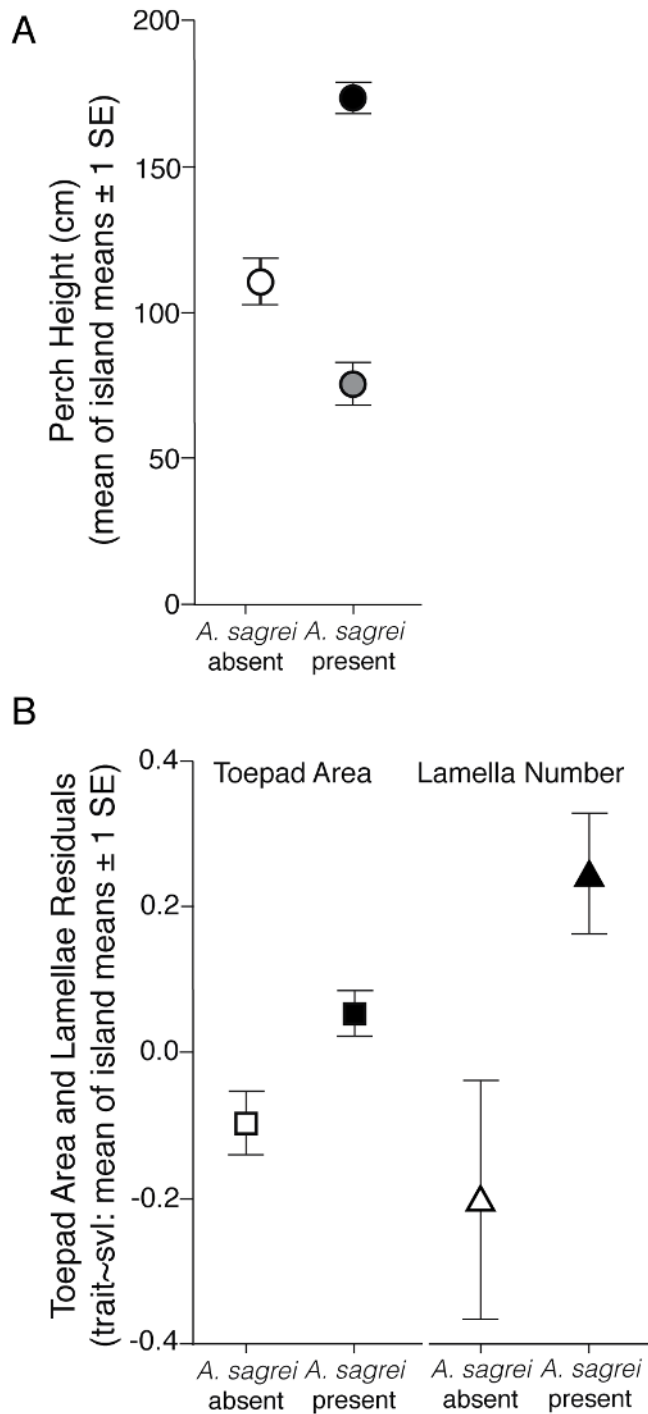
FIGURE 3.2. Habitat use shift and morphological response by *A. carolinensis*. A) Mean of island means (± 1 s.e.) for perch height by *A. carolinensis* in the absence (open circle; n = 5) and presence (closed circle; n = 6) of *A. sagrei*. Perch height of *A. sagrei* shown for comparison (grey circle; n = 6). The presence of *A. sagrei* corresponds with a significant increase in perch height by *A. carolinensis* (Linear Mixed Model: $\beta_{sagrei\ present}$ = 2.77, t_9 = 6.6, one-tailed $p <$ 0.001 – see text and SM for details). B) Mean of island means (± 1 s.e.) for *A. carolinensis* toepad area (squares) and lamella number (triangles) in the absence (open symbols; n = 5) and presence (closed symbols; n = 6) of *A. sagrei.* The y-axis represents size-corrected residuals for each trait. The presence of *A. sagrei* corresponds to a significant increase in both traits (Linear Mixed Models: Toepad Area, $\beta_{sagrei\ present}$ = 0.15, t_9 = 2.7, one-tailed p = 0.012; Lamella Number, $\beta_{sagrei\ present}$ = 0.57, t_9 = 3.3, one-tailed p = 0.005 – see text and SM for details).

Given this morphological divergence, we estimated rates of evolution for toepad area and lamella number, assuming that populations of *A. carolinensis* on invaded and un-invaded islands were similar before the invasion of *A. sagrei* and taking a conservative estimate of 20 generations since the arrival of *A. sagrei* after 1994. We found that *A. carolinensis* populations have diverged from one another at rates of 0.086 *haldanes* (randomization test for difference from zero: one-tailed $p = 0.009$) and 0.078 *haldanes* (randomization test: one-tailed $p = 0.013$) for toepad area and lamella number, respectively (*haldanes* calculated following [15] – see SM for analytical details). These rates are comparable to other well-known examples of rapid evolution [15] such as soapberry bug beak length in response to novel food [31] or guppy life-history under different predator regimes [32].

An alternative explanation for this morphological divergence is that it represents a phenotypically plastic response to differences in the degree of arboreality; we tested this plasticity hypothesis with a common garden laboratory experiment. We collected wild, gravid females from four invaded and four un-invaded islands, hatched their eggs and raised the offspring. We measured lamella number and toepad area of these offspring and found that the positive effect of *A. sagrei* invasion on *A. carolinensis* toepad area and lamella number was still apparent under shared laboratory conditions (Fig. 3.3; Table S3.3; Linear Mixed-Models. Toepad Area: $\beta_{sagrei\ present} = 0.14$, $t_6 = 2.1$, one-tailed $p = 0.043$; Lamella Number: $\beta_{sagrei\ present} = 1.45$, $t_6 = 3.6$, one-tailed $p = 0.006$; see SM for details). This result confirms that the observed morphological shift does indeed have an evolved, genetic component.
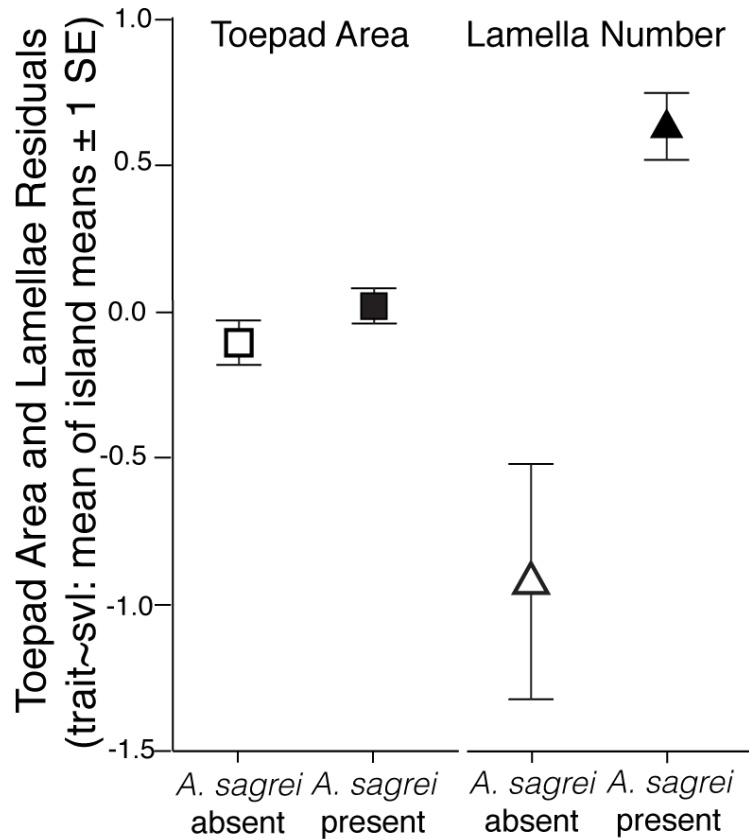
FIGURE 3.3. Morphological response maintained in the common garden. Mean of island means for *A. carolinensis* hatchling toepad area (squares) and lamella number (triangles) in the absence (open symbols; n = 4) and presence (closed symbols; n = 4) of *A. sagrei.* The y-axis represents size-corrected residuals for each trait. The presence of *A. sagrei* corresponds to a significant increase in both traits (Linear Mixed Models: Toepad Area, $\beta_{\text{sagrei present}}$ = 0.14, t_6 = 2.1, one-tailed p = 0.043; Lamella Number, $\beta_{\text{sagrei present}}$ = 1.45, t_6 = 3.6, one-tailed p = 0.006 – see text and SM for details).

We rule out several alternative processes and events that could create the observed CD-like pattern without relying on the hallmark interspecific interactions of CD [2,13,33,34]. First, the habitat-use and morphological patterns we observed in *A. carolinensis* could have been driven by environmental heterogeneity that is confounded with the presence of *A. sagrei*, but as noted above, invaded and un-invaded islands do not differ significantly from one another in age, distance to the mainland, total area, vegetated area, vegetation species richness, or available tree heights (Table S3.1; see SM for details).

Second, the observed morphological pattern could have risen through ecological sorting, wherein *A. sagrei* was only able to colonize those islands on which the *A. carolinensis* population is sufficiently different in morphology. However, *A. sagrei* was purposefully introduced to eleven islands elsewhere in the lagoon and thrived in each case [21], and *A. sagrei* has opportunistically colonized almost every other island in the entire lagoon (~70 total) suggesting that *A. sagrei* is able to invade any island regardless of any potential pre-existing variation among *A. carolinensis* populations.

Last, the inference that *A. sagrei* drives an increase in perch height in *A. carolinensis* could be spurious if the haphazard spread of *A. sagrei* missed, by chance, only those islands where *A. carolinensis* perches low. Indeed, of the eleven islands in this study, the five islands without *A. sagrei* were the five islands with the lowest perch height by *A. carolinensis*. However, this pattern would happen by chance only one time in 462; moreover, invaded and un-invaded islands did not differ in available perch heights (Table S3.1). The perch height shift we detected is consistent with other studies of this interaction [21,24,25].

In sum, our data support the hypothesis that ecological interaction with *A. sagrei* has driven the evolution of CD in *A. carolinensis*. As to exactly what type of ecological interaction,

however, we remain agnostic. Interspecific resource competition is known to be strong across *Anolis* [23,24,35,36], and *A. carolinensis* and *A. sagrei* do overlap in resource use in other parts of their range [21], suggesting that they may diverge in habitat to partition food resources. However, we have not ruled out other, non-mutually-exclusive interactions like intraguild predation [37], agonistic interactions [34], and apparent competition [38]. Reproductive character displacement [39], on the other hand, seems an unlikely explanation as the species differ in species-recognition mechanisms and have never been reported to hybridize.

Regardless of which interaction(s) is at play, we have shown that *A. carolinensis* has adapted morphologically to a perch-height shift driven by the invasion of *A. sagrei*. The replicated, controlled, well-documented nature of our study allowed us to rule out confounding factors that have plagued other observational studies of CD [13] while directly demonstrating rapid evolution of CD by *A. carolinensis*. Demonstrating such rapid evolution in this natural system provides further impetus to test CD and other evolutionary hypotheses directly with manipulative field experiments [32,40].

REFERENCES AND NOTES

1. Brown, W.L., and Wilson, E.O. (1956) Character displacement. *Syst. Zool.* 5, 49-64

2. Schluter, D. (2000) *The ecology of adaptive radiation,* Oxford University Press

3. Dayan, T. and Simberloff, D. (2005) Ecological and community-wide character displacement: the next generation. *Ecol. Lett.* 8, 875-894

4. Reznick, D.N. and Ricklefs, R.E. (2009) Darwin's bridge between microevolution and macroevolution. *Nature* 457, 837-842

5. Jang, Y. *et al.* (2009) Convergent and divergent patterns of morphological differentiation provide more evidence for reproductive character displacement in a wood cricket *Gryllus fultoni*. *BMC Evol. Biol.* 9, 27

6. Adams, D.C. (2010) Parallel evolution of character displacement by competitive selection in terrestrial salamanders. *BMC Evol. Biol.* 10, 72

7. Rando, J.C., *et al.* (2010) Disentangling ancient interactions: a new extinct passerine provides insights on character displacement among extinct and extant island finches. *PLoS ONE* 5, e12956

8. Levy, O. *et al.* (2011) Interspecific competition and torpor in Golden Spiny Mice: two sides of the energy-acquisition coin. *Int. Comp. Biol.* 51, 441-448

9. Martin, R.A. and Pfennig, D.W. (2011) Evaluating the targets of selection during character displacement. *Evolution* 65, 2946-2958

10. Thierry, D. *et al.* (2011) Ecological character displacement in competing common green lacewings in Europe: a route to speciation. *Biol. J. Linn. Soc.* 102, 292-300

11. Goldberg, E.E. *et al.* 2012. Population regulation and character displacement in a seasonal environment. *Am. Nat.* 179, 693-705

12. Pfennig, D.W. and Pfennig, K.S. (2012) *Evolution's wedge: competition and the origins of diversity,* University of California Press

13. Stuart Y.E. and Losos, J.B. (2013) Ecological character displacement: glass half full or empty? In Press, *Trends. Ecol. Evol.*

14. Grant, P.R. and Grant, B.R. (2006) Evolution of character displacement in Darwin's finches. *Science* 313, 224-226

15. Hendry, A.P. and Kinnison, M.T. (1999) Perspective: The pace of modern life: measuring rates of contemporary microevolution. *Evolution* 53, 1637-1653

16. Kinnison, M.T. and Hendry, A.P. (2001) The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* 112-113: 145-164

17. D. J. Irschick, D. Reznick. In *Experimental evolution: concepts, methods, and applications of selection experiments*, p 173, (Eds. T. Garland Jr., M. R. Rose. Univ. California Press, Berkeley, USA 2009)

18. Campbell, T.S. (1996) Northern range expansion of the brown anole (*Anolis sagrei*) in Florida and Georgia. *Herpet. Rev.* 27, 155-157.

19. Campbell, T.S. and Echternacht, A.C. (2003) Introduced species as moving targets: changes in body sizes of introduced lizards following experimental introductions and historical invasions. *Biol. Invas.* 5, 193-212

20. Kolbe, J.J. *et al.* (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature* 431, 177-181

21. Campbell, T. S. (2000) Analyses of the effects of an exotic lizard (*Anolis sagrei*) on a native lizard (*Anolis carolinensis*) in Florida, using islands as experimental units. *Ph.D. Dissertation*, University of Tennessee Knoxville

22. Collette, B.B. (1961) Correlations between ecology and morphology in Anoline lizards from Havana, Cuba and Southern Florida. *Bull. Mus. Comp. Zoo.* 125, 137-162

23. Lister, B.C. (1976) The nature of niche expansion in West Indian *Anolis* lizards I: Ecological consequences of reduced competition. *Evolution* 30 659-676

24. Schoener, T.W. (1975) Presence and absence of habitat shift in some widespread lizard species. *Ecol. Monog.* 45, 233-258

25. Edwards, J.R. and Lailvaux, S.P. (2012) Display behavior and habitat use in single and mixed populations of *Anolis carolinensis* and *Anolis sagrei* lizards. *Ethology* 118, 494-502

26. Schettino, L.R. *et al.* (2010) The anoles of Soroa: aspects of their ecological relationships. *Breviora* 520, 1-22

27. Lister, B.C. (1976) The nature of niche expansion in West Indian *Anolis* lizards II: evolutionary components. *Evolution* 30, 677-692

28. Glossip, D. and Losos, J.B. (1997) Ecological correlates of number of subdigital lamellae in anoles. *Herpetologica* 53, 192-199

29. Macrini, T.E. *et al.* (2003) Ecomorphological differences in toepad characteristics between mainland and island anoles. *J. Herpetology* 37, 52-58

30. Elstrott, J. and Irschick, D.J. (2004) Evolutionary correlations among morphology, habitat use, and clinging performance in Caribbean *Anolis* lizards. *Biol. J. Linn. Soc.* 83, 389-398

31. Carroll, S.P. and Boyd, C. (1992) Host race radiation in the Soapberry Bug: natural history with the history. *Evolution* 46, 1053-1069

32. Reznick, D.N. *et al.* (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* 275, 1934-1937

33. Schluter, D. and McPhail, J.D. (1992) Ecological character displacement and speciation in sticklebacks. *Am. Nat.* 140, 85-108

34. Grether, G.F. *et al.* (2009) The role of interspecific interference competition in character displacement and the evolution of competitor recognition. *Biol. Rev.* 84, 617-635

35. J.D. Rummel, J. Roughgarden. *Ecology* **66**, 430 (1985)

36. J. Roughgarden, J. Rummel, S. Pacala. In *Advances in herpetology and evolutionary biology: essays in honor of Ernest E. Williams*, p 499, (Eds. G.J. Rhodin, K. Miyata. Museum of Comp. Zool. Harvard Univ., Cambridge, USA, 1983)

37. Polis, G.A. *et al.* (1989) The ecology and evolution of intraguild predation: potential competitors that eat each other. *Ann. Rev. Ecol. Syst.* 20, 297-330

38. Holt, R.D. (1977) Predation, apparent competition, and the structure of prey communities. *Theor. Pop. Biol.* 12, 197-229

39. Pfennig, D.W. and Pfennig, K.S. (2010) Character displacement and the origins of diversity. *Am. Nat.* 176, S22-S44

40. Reznick, D.N., and C.K. Ghalambor. (2005) Selection in nature: experimental manipulations of natural populations. *Int. Comp. Biol.* 45, 456-462

Chapter 4:

The basis of toepad differentiation in island lizards: evolved or plastic?

Yoel E. Stuart[1], Todd S. Campbell[2], R. Graham Reynolds[3], Jonathan B. Losos[1], and Liam J. Revell[3]

[1] Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138

[2] Department of Biology, University of Tampa, 401 W. Kennedy Blvd., Tampa, FL 33606

[3] Department of Biology, University of Massachusetts Boston, 100 Morrisey Blvd., Boston, MA 02125

ABSTRACT

Traditionally, evolution is thought to proceed through natural selection on heritable phenotypic differences among individuals, resulting in genetic change in populations from one generation to the next. However, some workers argue that non-heritable phenotypic variation that arises through developmental plasticity may also play an adaptive role in evolution by allowing populations to persist in the face of environmental change, providing enough time for other traits, or even plasticity itself, to adapt to the environmental challenge. Here, we test whether phenotypic divergence in toepad area and lamella number in the lizard *Anolis carolinensis* has a genetic basis or is the result of phenotypic plasticity. Small-island populations of *A. carolinensis* that are sympatric with the recent invader, *Anolis sagrei*, have larger toepads with more lamellae compared to allopatric populations. We hatched and raised *A. carolinensis* offspring of mothers from islands invaded and un-invaded by *A. sagrei* and tested whether observed differences in toepad area and lamella number between populations in the field persisted in the laboratory. We found that the differences did persist in the laboratory, suggesting that toepad divergence in these populations has a genetic basis. We also found that lamella number had appreciable heritability although toepad area had much less. These data suggest that increased toepad area and lamella number in populations of *A. carolinensis* sympatric with *A. sagrei* are rapidly evolved adaptations to increased arboreality by *A. carolinensis* in the presence of the invader.

INTRODUCTION

The traditional perception of evolution that emerged from Modern Synthesis is that

evolution proceeds only through natural selection on heritable phenotypic differences among

individuals, resulting in genetic change in populations from one generation to the next [1]. As

such, phenotypic plasticity – or the ability of a single genotype to produce different phenotypes

in response to different environments – was viewed merely as a noise parameter unimportant to

adaptive evolution. Because plasticity shields genotypes from the environment (reviewed in [2]),

natural selection could not drive evolution [3].

However, a vociferous set of authors has argued that plasticity can indeed be adaptive

and thus play an important role in determining the trajectory of evolution (reviewed in [2,4-7].

Essentially, if environmentally induced plasticity allows a subset of individuals to persist in new

or changing environments, then directional selection will favor those individuals able to respond

and the degree of plasticity itself will evolve (e.g., the 'Baldwin Effect' [4]). Moreover, plasticity

may allow populations to persist long enough for the facultative plastic response to become

constitutively expressed (e.g., genetic assimilation [8,9]) or for other traits to evolve by

traditional evolution by natural selection and thus mediate the environmental challenge [2,4,6].

This broader picture of adaptive evolution suggests that we must understand how phenotypic

plasticity and genetic evolution combine to be able to reliably interpret the evolutionary history

of observed phenotypic variation in nature.

The *Anolis* lizard radiation is remarkable for the repeated evolution of a set of ecomorphs

across the four islands of the Greater Antilles [10,11,12]. Ecomorphs are habitat specialists with

morphologies functionally adapted to their specific lifestyles. The most obvious of these habitat-

morphology-function relationships is that of limb length to performance on perches of certain

diameters [11,12]. Specifically, those species adapted to broad perches have longer legs relative to their body size that make them more adept at running quickly, while species specialized to narrow perches have relatively shorter legs that make them better able to maneuver without stumbling [11,13].

To investigate how genetic evolution and phenotypic plasticity contribute to ecomorphological disparity in limbs, previous studies have tested the ability of *Anolis* hind-limb length to respond plastically to differences in growth environment during ontogeny [14,15]. Juveniles of both *A. sagrei* [14] and *A. carolinensis* [15] were grown on narrow (1cm) or wide (9cm) diameter perches to examine whether juveniles from each perch treatment would diverge in limb length, as predicted from observations of lizards in the field. The authors found that, for each species, lizard hind limb length changed in the expected direction given the perch diameter on which juveniles were raised, suggesting that hind-limb length has a plastic component and that plasticity may play an important role in the evolution of *Anolis* morphological disparity. However, the authors of these studies note that the degree of plasticity seen in their experiments is much less than phenotypic differences in hind-limb length seen between species, suggesting that there is an evolved, genetic component to the ecomorphological differences as well.

A less appreciated aspect of anole ecomorphology is the relationship between habitat use, toepad morphology, and clinging ability. Size-corrected toepad area and toepad scale, or lamella, number correlate positively with perch height across *Anolis* species [16-20]. Relative to body size, larger toepads with more lamellae translate functionally to better clinging ability in anoles [20,21]. This correlation of arboreal lifestyle with larger, better clinging toepads suggests that large toepads are an adaptation to arboreality. Recent research has shown that flexible perches like leaves and twigs make for treacherous movement through the habitat [22,23]. Because the

proportion of flexible perches is likely to increase with increased arboreality, better clinging ability afforded by large toepads may be more important for arresting falls from a poor jumps and correcting mistakes made on unstable, narrow, or waxy perches during foraging, predator encounters, or territorial bouts with other lizards.

Is phenotypic plasticity important for toepad traits? For lamella number, at least, plasticity seems an unlikely explanation, as scanning electron micrographs of *A. carolinensis* toepads suggest that they are fully established prior to hatching (T. Sanger, *pers. comm.*) and scale number may be fixed in lizards at birth [24]. Moreover, in a common garden experiment with *A. oculatus*, Thorpe et al. [25] tested for plasticity in the number of lamellae and other body scales for populations from mesic versus xeric environments. Thorpe et al. reasoned that if scale differences among populations of *A. oculatus* resulted from plastic responses to different environments, then those scale differences should disappear in common garden conditions. However, Thorpe et al. [25] found that lamella and body scale differences persisted in the common garden, suggesting that divergence in these traits has an evolved genetic basis. To our knowledge, such a study has not been carried out for toepad area.

In the study described here, we use a common garden experiment to test the contribution of phenotypic plasticity to variance in lamella number and toepad area for populations of *A. carolinensis*. As described in Chapter 3, Mosquito Lagoon spoil-island populations of *A. carolinensis* sympatric with the recent invader, *A. sagrei*, have diverged from allopatric *A. carolinensis* populations in toepad area and lamella number in response to an arboreal shift driven by *A. sagrei*. The invasion is recent [15, 26-27] and presence-absence surveys conducted in 1994 [28] and again in 2009 [Chapter 3] reveal that the observed divergence in toepad area and lamella number must have occurred ~16 years, or 20 generations [27]. If divergence in

toepad area and lamella number has a genetic basis, then the rates at which sympatric populations have diverged from allopatric populations are on par with well-known examples of rapid evolution in nature (Chapter 3).

METHODS

Lizard collection and care

In late July 2011, we collected gravid *A. carolinensis* females from four invaded and four un-invaded islands. These islands were a subset of those described in Chapter 3. We chose fewer islands for logistic reasons, as lab rearing of *Anolis* lizards is a laborious, costly, and space intensive. Although *A. carolinensis* toepad area and lamella number are significantly diverged between invaded and un-invaded islands, the effect is small. To give ourselves the most power to recover observed field differences in laboratory settings if there is indeed a genetic component to trait divergence, we collected females from the islands whose lamellae and toepad areas were most diverged. We targeted and returned the gravid females to common cage and lighting laboratory conditions within four days of collection. Snout-vent length (svl) and mass were measured at time of capture.

Females were housed individually in Critter Keeper® cages with bamboo dowels, cage carpet, and a potted plant for laying eggs. Lizards were misted twice daily and fed 2-3 times per week with crickets that had been gutloaded with Flukers® Orange Cubes and Flukers® High Calcium Cricket Diet. Directly before feeding the lizards crickets, the crickets were dusted with vitamin and calcium powders. Female cages were shuffled regularly to randomize any within room environmental variation.

We checked plant pots for eggs three times per week from August to November 2011. Females were measured for svl and mass when eggs were discovered. Eggs were collected and nested in 1:1 vermiculite:water nesting material, sealed in petri dishes to slow water loss, and incubated at 28°C [29]. We weighed petri dishes when first placed in the incubator and replaced water as it was lost. Upon hatching, we measured the svl and weight of each lizard. We raised offspring in individual cages and shuffled cages regularly to randomize any within room environmental variation. Offspring were fed and misted by the same regimen as adults, except that smaller cricket sizes were used as appropriate to the size of the lizard. We raised each offspring for six months and then measured toepad area and lamella number as described in Chapter 2. Not all females had offspring that survived to the measurement stage, while some females had multiple offspring survive. Island counts for number of successful females and surviving hatchlings are in Table 4.1.

TABLE 4.1. By island, *Anolis sagrei* invasion status, number of females collected in the field, number of females that successfully hatched offspring that survived to 6 months, and hatchling sample size. These hatchlings were used in linear mixed models to test whether observed toepad differences in the field were recovered under common growth conditions.

Island	*A. sagrei* invasion	Females collected	Successful Dams	Hatchling sample size
Hornet	No	10	3	6
Lizard	Yes	8	6	12
North Twin	Yes	10	8	10
Osprey	No	10	5	8
Pine	No	1	1	2
South Twin	No	9	5	7
Yang	Yes	11	6	10
Yin	Yes	10	5	6

Analysis

Testing for the persistence of divergence in the field

We tested whether lab-reared *A. carolinensis* with mothers from invaded islands showed the same pattern of increased toepad area and lamella number as *A. carolinensis* populations from invaded islands did in the field. We used linear mixed models with the *lme()* function implemented in the R package *nlme* to build, for toepad area and lamella number separately, a linear mixed model that included lizard svl as a random effect and island as a fixed effect: lme(trait ~ sagrei presence*svl, random = ~svl | island). The interaction term was not significant so we chose the following reduced model: lme(trait ~ sagrei presence + svl, random = ~svl | island). Because it can be difficult to confidently sex juvenile lizards, we did not include sex as a term in the model. Not being able to include a sex effect is unlikely to influence our results because our field data for adults demonstrate significant effects of the presence of *A. sagrei* on toepad traits regardless of whether sex is included in the model. Because of low hatchling and adult sample sizes, we did not include an indicator for each hatchling's dam. The rationale for including such an indicator is to help account for maternal effects that may influence offspring morphology; however, there were no differences among dams from invaded and un-invaded islands in field svl, mass, or body condition (see Results), suggesting that maternal effects should be minimal.

Heritability (h^2) estimates from mother-offspring regressions

We estimated heritability of toepad area and lamella number by regressing each hatchling's size-corrected trait value against the size-corrected trait value of its mother. There were too few individuals per island to make reliable within-island heritability estimates, so we pooled individuals from all the islands into one "Mosquito Lagoon" population. We corrected for

size by pooling females and offspring and regressing each toepad trait value against its corresponding. After checking that the interaction term between svl and age class was not significant, we saved the residuals from these trait~svl regressions for heritability estimation.

We estimated heritability using mother-offspring regression. Again, because of small sample sizes, we present one set of analyses where mother-offspring regressions were partially pseudo-replicated; ten dams were included in regressions more than once because they had more than one offspring. We present a second set of analyses where pseudoreplication was removed by taking sibling trait averages for siblings and running the same mother-offspring regressions. Both datasets are reported for comparison.

RESULTS

Testing for field patterns in the lab

A linear mixed model with svl as a random effect and island as a fixed effect revealed a significant positive effect of *A. sagrei* invasion on *A. carolinensis* toepad area and lamella number under shared laboratory conditions (Fig. 4.1; Table 4.1; Toepad Area: $\beta_{sagrei\ present} = 0.14$, $t_6 = 2.1$, one-tailed $p = 0.043$; Lamella Number: $\beta_{sagrei\ present} = 1.45$, $t_6 = 3.6$, one-tailed $p = 0.006$; see SM for details). This result suggests that the observed morphological shift does indeed have an evolved, genetic component that persists when raised in common laboratory conditions.

Common garden studies often run through an F2 generation to address concern about maternal effects influencing the result. We do no think maternal effects would confound our treatment effect (i.e., presence or absence of *A. sagrei*) because there were no differences among wild-caught dams from invaded and un-invaded islands in svl, mass, or body condition

(mass/svl) (Linear Mixed Models. svl: $\beta_{\text{sagrei present}}$ = -0.13, t_6 = -0.19, p = 0.86; mass: $\beta_{\text{sagrei present}}$

= 0.11, t_6 = 1.07, p = 0.33; body condition: $\beta_{\text{sagrei present}}$ = 0.002, t_6 = 1.34, p = 0.23).
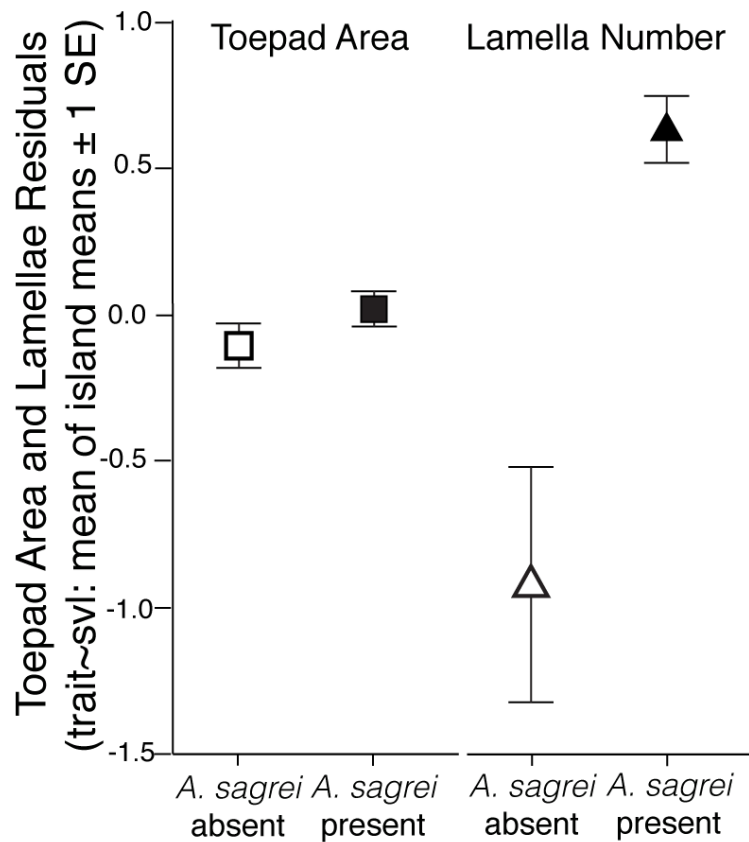

FIGURE 4.1. Morphological response maintained in the common garden. Mean of island means

± 1s.e. for *A. carolinensis* hatchling toepad area (squares) and lamella number (triangles) in the

absence (open symbols; n = 4) and presence (closed symbols; n = 4) of *A. sagrei.* The y-axis

represents size-corrected residuals for each trait.

Heritability (h²) estimates from mother-offspring regressions

Estimates of heritability for toepad area taken as the slope from mother-offspring regressions ranged from 0.13 (s.e. = 0.22) to 0.23 (s.e. = 0.17) for non-pseudo- and pseudo-replicated datasets, respectively (Figure 4.2A; Table 4.2); *P*-values were not significant for either case. Heritability estimates for lamella number ranged from 0.52 (s.e. = 0.16) to 0.61 (s.e. = 0.15) for non-pseudo- and pseudo-replicated datasets, respectively; *P*-values were significant at the 0.005 level for each case (Figure 4.2B; Table 4.2).

TABLE 4.2. Estimates of heritability for toepad area and lamella number taken from mother-offspring regressions.

Females pseudoreplicated[a]	Sample size	h^2 - Toepad Area (St. Error / p-value)	h^2 - Lamella Number (St. Error / p-value)
yes	43	0.23 (0.17 / 0.20)	0.61 (0.15, 0.0001)
no	31	0.13 (0.22 / 0.55)	0.52 (0.16 / 0.004)

a – Pseudoreplicated analyses did not account for females that were multiply represented because they had multiple offspring. Pseudoreplication was removed by averaging trait values for offspring, thereby including each female only once.

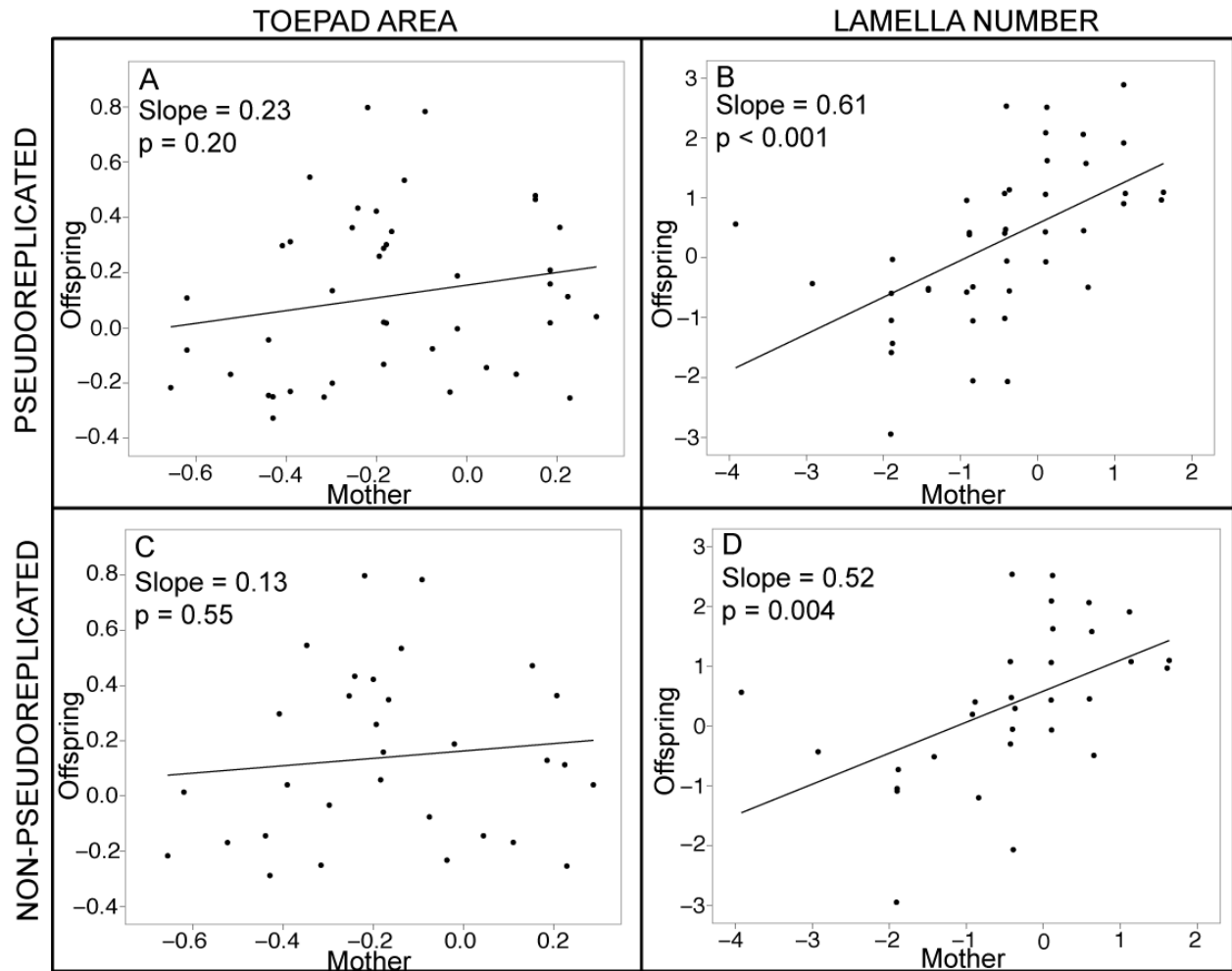FIGURE 4.2. Mother-offspring regressions for toepad area (A,C) and lamella number (B,D). Axes represent size-corrected residuals from a trait~svl linear regression. The top row includes all individuals so mothers that had more than one offspring are pseudoreplicated. The data in the bottom row are not pseudoreplicated, as offspring averages were taken for mothers that had more than one offspring.

DISCUSSION

The basis of toepad variation

In the field, we observed that island populations of *Anolis carolinensis* that were

sympatric with *A. sagrei* perched higher and showed larger toepads with more lamellae,

compared to allopatric populations (Chapter 3). We wished to know whether such morphological

divergence was the result of phenotypic plasticity or genetic evolution. We tested this question

by taking gravid *A. carolinensis* females from sympatric and allopatric populations and raising

their offspring under common growth conditions. If the field differences between sympatric and

allopatric populations were due to genetic evolution, then those differences should be maintained

in the lab. If plasticity was the source of the differences, then toepad morphologies should

converge in the lab. We found the former: that the differences seen in the field persisted in the

lab (Fig. 4.1). Thus, morphological divergence in these populations of *A. carolinensis* is likely

the result of evolved character displacement in response to novel interactions with *A. sagrei* over

a short period of time. At most, *A. carolinensis* populations have been evolving with *A. sagrei*

since 1994, which translates to approximately 20 generations if one assumes slightly more than

one generation per year [27]. Using the Haldane to estimate rates of divergence for toepad area

and lamella number reveals divergence rate estimates similar to other well known examples of

rapid evolution such as such as soapberry bug beak length responding to novel food [30] and

guppy life-history adapting to different predator regimes [31] (see Chapter 3).

Heritability in toepad area and lamella number

The mother-offspring regressions estimate narrow-sense heritability (h^2), or the

proportion of phenotypic variance that is due to additive genetic variance. Additive genetic

variance is the avenue through which populations are able to respond to natural selection because additive variance determines how closely offspring resemble parents [32].

For lamella number, h^2 estimated in the non-pseudoreplicated dataset is 0.52 (s.e. 0.16) (Figure 4.2A; Table 4.2). This value falls well within the range of heritabilities observed for artificially selected traits in agricultural and livestock species [33], so toepads should respond strongly to any selection for more lamellae in an arboreal environment. This result is consistent with our findings that arboreal *A. carolinensis* sympatric with *A. sagrei* maintained toepads with more lamellae in both the field (Chapter 3) and in the common garden experiment (Fig. 4.1).

For toepad area, h^2 in the non-pseudoreplicated dataset is 0.13 (s.e. 0.22), and the estimate is not significantly different from zero (Table 4.2). Thus, much of the phenotypic variance in toepad area must stem from non-additive sources of variance, like dominance variance, epistatic variance, or variance due to the environment [32]. Because offspring toepad area does not reliably reflect the phenotype of the mother in these populations, toepad area might not respond to selection in a predictable manner. Such low heritability likely explains why the differences we recovered for toepad area in the common garden experiment were not as strong as the differences for lamella number (Fig. 4.1).

Yet we do see toepad area differences persisting in the common garden, suggesting that the differences have evolved despite the lack of additive genetic variance. This result may be explained by our pooling of mother-offspring pairs across island populations to estimate heritability in a single Mosquito Lagoon population. Because narrow-sense heritability is defined as $h^2 = V_A/(V_P)$, where V_A is the additive genetic variance, V_P is the total phenotypic variance, and V_P contains variance due to dominance, epistasis, and the environment, estimates of h^2 are strictly valid only for the population and environment in which heritability was measured [32].

Thus, our lagoon-wide estimates for heritability may differ from the true values for individual islands because independently evolving island populations of *A. carolinensis* may differ in how much epistatic, dominance, and environmental variance contribute to heritability. In other words, within islands, toepad area may be more heritable than the estimate from pooled data, possibly explaining why we still found toepad area divergence in common garden conditions. Another possibility is that we are underestimating the true heritability because we do not have data from the sires. Parent-offspring regression approaches to estimating heritability often test the average trait value of both parents against the offspring value. Because *A. carolinensis* has appreciable sexual dimorphism, the relationship between male and female offspring to just female parents may not be as tight as if both parents were included in the regression. For heritability from a single-parent/offspring regression, some workers double the slope of the regression as an estimate of heritability (e.g., [34]).

Implications for the Anolis *radiation*

The common garden experiment described in this chapter suggests that toepad variation in these island populations of *Anolis carolinensis* is heritable (for lamella number at least) and that the observed toepad divergence from Chapter 3 has a genetic basis (for both toepad area and lamella number). Our findings are consistent with other results in anoles, especially with respect to lamella number. First, the lamellae appear to be fully developed prior to hatching (T. Sanger, *pers. comm*.). Second, Thorpe et al. [25] found evidence for a genetic basis of differentiation in scale characters, including lamellae, in *Anolis oculatus.* Third, Calsbeek and colleagues [34-36] report broad-sense heritabilities above 0.50 for scales (albeit without saying which scale type) in Bahamian populations of *A. sagrei*. Given that *A. carolinensis, A. sagrei* and *A. oculatus*

diverged roughly 16-18 million years ago [15,37,38], it may be safe to assume that most *Anolis* populations show heritability in lamella number and that diversity in lamella number among species and ecomorphs is the result of genetic evolution.

What of other ecomorphological traits? Thorpe et al. [25] also found evidence for a genetic basis of differentiation in limb traits, whereas Calsbeek and colleagues [34-36] report broad-sense heritabilities above 0.50 for body size, dorsal pattern, and hindlimb length in Bahamian populations of *A. sagrei*. Thus, while phenotypic plasticity may explain some variation in morphological traits among populations (e.g., limb length [14, 15]), much of the diversity among populations is likely to have an evolved genetic basis.

The ready ability of *Anolis* populations to respond to selection and rapidly evolve in ecomorphologically important traits ([39,40]; Chapter 3) suggests phenotypic plasticity during ontogeny may not have played a large role in the evolution of morphological disparity during *Anolis* evolution. To the extent that such intraspecific processes can be extended to interspecific evolution, it seems likely that ecomorphological diversity across the *Anolis* adaptive radiation evolved according to the traditional ideas of evolution that emerged from the Modern Synthesis.

REFERENCES

1. Williams, G.C. (1966) *Adaptation and natural selection.* Princeton University Press

2. Ghalambor, C.K. *et al.* (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Func. Ecol.* 21, 394-407

3. Endler, J.A. (1986) *Natural selection in the wild*. Princeton University Press

4. West-Eberhard, M.J. (2003) Developmental plasticity and evolution. Oxford University Press

5. Gotthard, K. and Nylin S. (1995) Adaptive plasticity and plasticity as an adaptation: a selective review of plasticity in animal morphology and life history. Oikos 74, 3-17

6. Pfennig, D.W. *et al.* (2010) Phenotypic plasticity's impacts on diversification and speciation. *Trends. Ecol. Evol.* 25, 459-467

7. Pfennig, D.W. and Pfennig, K.S. (2010) Character displacement and the origins of diversity. *Am. Nat.* 176, S26-S44

8. Waddington, C.H. (1942) Canalization of development and the inheritance of acquired characters. Nature 150, 563-565

9. Waddington, C.H. (1953) Genetic assimilation of an acquired character. Evolution 7, 118-126

10. Williams, E.E. (1983) Ecomorphs, faunas, island size, and diverse end points in island radiations of *Anolis*. pp. 326-370 *in* Huey, R.B. *et al.* (eds), *Lizard ecology: studies of a model organism*, Harvard University Press

11. Losos, J.B. (1990) Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol. Monog.* 60, 369-388

12. Losos, J.B. (2009) *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles*. University of California Press

13. Irschick, D.J. and Losos, J.B. (1998) A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean *Anolis* lizards. *Evolution* 52, 219-226

14. Losos, J.B. *et al.* (2000) Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. *Evolution* 54, 301-305

15. Kolbe, J.J. and Losos, J.B. (2005) Hind-limb length plasticity in *Anolis carolinensis*. *J. Herp.* 39, 674-678

16. Collette, B.B. (1961) Correlations between ecology and morphology in Anoline lizards from Havana, Cuba and Southern Florida. *Bull. Mus. Comp. Zoo.* 125, 137-162

17. Lister, B.C. (1976) The nature of niche expansion in West Indian *Anolis* lizards II: evolutionary components. *Evolution* 30, 677-692

18. Glossip, D. and Losos, J.B. (1997) Ecological correlates of number of subdigital lamellae in anoles. *Herpetologica* 53, 192-199

19. Macrini, T.E. *et al.* (2003) Ecomorphological differences in toepad characteristics between mainland and island anoles. *J. Herpetology* 37, 52-58

20. Elstrott, J. and Irschick, D.J. (2004) Evolutionary correlations among morphology, habitat use, and clinging performance in Caribbean *Anolis* lizards. *Biol. J. Linn. Soc.* 83, 389-398

21. Irschick, D.J. *et al.* (1996) A comparative analysis of clinging ability among pad-bearing lizards. *Biol. J. Lin. Soc.* 59, 21-35

22. Gilman, C. *et al.* (2012) Total recoil: perch compliance alters jumping performance and kinematics in green anole lizards. *J. Exp. Biol.* 215, 220-226

23. Gilman, C. and Irschick, D.J. (2013) Foils of flexion: the effects of perch compliance on lizard locomotion and perch choice in the wild. *Funct. Ecol.* Doi: 10.1111/1365-2435.12063

24. Hecht, M.K. (1952) Natural selection in the lizard genus *Aristelliger*. *Evolution* 6, 112-124

25. Thorpe, R.S. *et al.* (2005) Common garden and natural selection experiments support ecotypic differentiation in the Dominican Anole (*Anolis oculatus*)

26. Campbell, T.S. (1996) Northern range expansion of the brown anole (*Anolis sagrei*) in Florida and Georgia. *Herpet. Rev.* 27, 155-157.

27. Campbell, T.S. and Echternacht, A.C. (2003) Introduced species as moving targets: changes in body sizes of introduced lizards following experimental introductions and historical invasions. *Biol. Invas.* 5, 193-212

28. Campbell, T. S. (2000) Analyses of the effects of an exotic lizard (*Anolis sagrei*) on a native lizard (*Anolis carolinensis*) in Florida, using islands as experimental units. *Ph.D. Dissertation*, University of Tennessee Knoxville

29. Sanger, T.J. *et al.* (2008) Laboratory protocols for husbandry and embryo collection of *Anolis* lizards. *Herp. Rev.* 39, 58-63

30. Carroll, S.P. and Boyd, C. (1992) Host race radiation in the Soapberry Bug: natural history with the history. *Evolution* 46, 1053-1069

31. Reznick, D.N. *et al.* (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* 275, 1934-1937

32. Futuyma, D.J. (1998) *Evolutionary biology 3rd ed.* Sinauer Associates, Inc.

33. Hartl, D.L. and Clark, A.G. (2007) *Principles of population genetics 4th ed.* Sinauer Associates, Inc.

34. Calsbeek, R. and Smith, T.B. (2007) Probing the adaptive landscape using experimental islands: density-dependent natural selection on lizard body size. *Evolution* 61, 1052-1061

35. Calsbeek, R. *et al.* (2006) Variation in scale numbers is consistent with ecologically based natural selection acting within and between lizard species. *Evol. Ecol.* 20, 377-394

36. Calsbeek, R. *et al.* (2008) Differential fitness effects of immunocompetence and neighborhood density in alternative female lizard morphs. *J. Anim. Ecol.* 77, 103-109

37. Nicholson, K.E. *et al*. (2005) Mainland colonization by island lizards. *J. Biogeog.* 32, 929-938

38. Alföldi, J. *et al.* (2011) The genome of the green anole lizard and a comparative analysis with birds and mammals. *Nature* 477, 587-591

39. Losos, J.B. *et al.* (1997) Adaptive differentiation following experimental island colonization in *Anolis* lizards. *Nature* 387, 70-73

40. Losos, J.B. *et al.* (2006) Rapid temporal reversal in predator-driven natural selection. *Science* 314, 1111

Conclusion

Yoel E. Stuart

Museum of Comparative Zoology and Department of Organismic and Evolutionary Biology

26 Oxford Street, Cambridge, MA 02138

Community ecologists are interested in the large and small scale factors that influence how species in communities assemble, coexist, and change [1]. In Chapter 1, I investigated the large scale biotic and abiotic factors that govern species turnover patterns in mainland and island communities of *Anolis* lizards and Terrarana frogs.

Consistent with other studies [2-4], we found that species turnover among mainland communities increased with geographic distance and environmental dissimilarity for both anoles and Terraranan frogs. Turnover in Terraranans occurred more quickly than for anoles, probably because the frogs less capable dispersers and more sensitive to environmental changes between communities. We found that species turnover between mainland and island communities was much higher than predicted by the mainland model. In fact, turnover was nearly always complete; only the remarkably good colonizer *Anolis sagrei* was found on both islands and the mainland. Such severe turnover between island and mainland communities is likely driven by the inhospitable barrier (i.e., saltwater) that separates islands from the mainland. The saltwater barrier effectively increases the distance across which dispersers must travel and reduced mainland-to-island dispersal and gene flow will increase the probability of speciation islands. Moreover, increased ecological opportunity on islands promotes within island radiation, increasing levels of island endemism and species turnover from the mainland.

In island-island comparisons, we found that species turnover was on average higher than predicted by the mainland model. However, the rate of turnover with distance and environment was similar to the mainland. Why might the mainland model better predict island-island turnover? First, the set of island-to-island geographic and environmental distances are not as large as the set of mainland-to-island distances; shorter dispersal distances increase the odds of two communities sharing species. Second, island species are already likely to be good dispersers

because they colonized the islands in the first place. Combining good dispersal abilities with typically high population densities on islands increases the likelihood of successful colonization of more than one island. Third, many island systems are part of larger banks that are connected during periods of low sea-level. When islands are connected, they effectively create larger land masses and overland dispersal homogenizes communities. In this way, island-island turnover is higher than expected from the mainland model but not as severe as mainland-island turnover. Overall, these results suggest that islands do accumulate diversity uniquely from the mainland because of the water barrier that surrounds them.

Future work in this system should include a phylogenetic approach (e.g., [5]). For now, the phylogenies of *Anolis* lizards and Terrarana frogs do not have good enough coverage of mainland species to investigate the island effect on phylogenetic beta diversity. At present, however, the phylogenies for the two clades are sufficient to ask whether islands that accumulate diversity through *in situ* diversification have different patterns of phylogenetic turnover than islands that accumulate diversity through anagenetic change or dispersal.

In Chapters 2-4, I examined the evolutionary outcome of a novel species interaction, with implications for community assembly and species coexistence. In Chapter Two, I examined whether the current, positive scientific opinion for Ecological Character Displacement (ECD) is warranted by the available data. I reviewed 144 putative cases of ECD and found that the average case met only 3.3 ECD criteria. Only 5% of cases met all six criteria and these gold standard cases were limited to a small portion of the biological world. The most surprising result, given the weight that competition-driven evolutionary divergence is afforded in the literature (e.g., [6]), is that only 17% of cases showed evidence of resource competition. I recommended that more complete data could be gathered by measuring ECD in real time, either in species

responding to environmental change or species invasion, or directly through evolutionary experiment. Unlike cases that rely on observational data, such studies would directly test several of the ECD criteria that are most difficult to meet, thus providing more conclusive evidence for the importance of ECD in nature.

In Chapter Three, I presented a real-time test of character displacement in the lizard *Anolis carolinensis*. I found that *A. carolinensis* shifted its habitat use to become more arboreal in the presence of an invader, *A. sagrei*. As a result, *A. carolinensis* adaptively diverged in toepad area and lamella number to match the habitat shift and the rates of divergence in toepad area and lamella number rival other well-known examples of contemporary evolution [7,8]. This study addressed four of the six character displacement criteria: it ruled out chance, showed a trait/habitat-use relationship, documented *in situ* change, and showed that invaded and un-invaded island environments were not significantly different in any major habitat variables. Chapter Four addressed the fifth criterion: that observed morphological changes were the result of genetic evolution and not phenotypic plasticity. We found that the morphological differences observed in the field persisted in common garden conditions, suggesting that toepad divergence was an evolved response. The heritability estimate for toepad area was 0.13 (s.e. = 0.22); the estimate for lamella number was 0.52 (s.e. = 0.16).

Together, Chapters Three and Four showed that *A. carolinensis* has shifted its habitat use and adaptively evolved its toepads to match its new environment. These studies ruled out environmental heterogeneity, ecological sorting, phenotypic plasticity, and chance as possible explanations of the habitat and morphological shift, leaving novel interactions with *A. sagrei* as the most likely mechanism driving evolution in *A. carolinensis*. The last piece of the puzzle will be to sort out the nature of this interaction between *A. carolinensis* and *A. sagrei*. Interspecific

competition is well documented in anoles [9] and likely plays an important role in character displacement in our system. However, I have not ruled out alternative interactions that may also contribute, like apparent competition, agonistic interactions, and intraguild predation (reinforcement seems unlikely as the two species differ greatly in dewlap and have not been reported to hybridize). Discerning the type (or types) of species interaction that serves to drive initial habitat divergence by *A. carolinensis* will close the book on this story. Regardless of the outcome, however, this study stands as a strong case for the rapid evolution of character displacement driven by *A. carolinensis* in response to novel interactions with *A. sagrei.*

REFERENCES

1. Morin, P.J. (1999) *Community ecology*. Blackwell Science

2. Buckley, L. B. and Jetz, W. (2008) Linking global turnover of species and environments. *Proc. Nat. Acad. Sci. USA* 105: 17836-17841

3. Fattorini, S. (2010) The influence of geographical and ecological factors on island beta diversity patterns. *J. Biogeog.* 37, 1061-1070

4. Qian, H. and Ricklefs, R. E. (2012) Disentangling the effects of geographic distance and environmental dissimilarity on global patterns of species turnover. *Global Ecol. Biogeogr.* 21, 341-351

5. Fine, P.V.A. and Kembel, S.W. (2011) Phylogenetic community structure and phylogenetic turnover across space and edaphic gradients in western Amazonian tree communities. *Ecography* 34, 552-565

6. Goldberg, E.E. *et al.* 2012. Population regulation and character displacement in a seasonal environment. *Am. Nat.* 179, 693-705

7. Carroll, S.P. and Boyd, C. (1992) Host race radiation in the Soapberry Bug: natural history with the history. *Evolution* 46, 1053-1069

8. Reznick, D.N. *et al.* (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* 275, 1934-1937

9. Losos, J.B. (2009) Lizards in an evolutionary tree: ecology and adaptive radiation of anoles. University of California Press

APPENDIX

Supplementary Materials, Figures, and Tables for Chapters 1, 2, 3

CHAPTER 1 SUPPLEMENTARY MATERIAL

The island–mainland species turnover relationship

Yoel E. Stuart, Jonathan B. Losos & Adam C. Algar

(As published in *The Proceedings of the Royal Society of London B)*

Includes
Table S1.1 – Islands included for anoles and terraranans

Table S1.2 – Environmental variables used to quantify environmental dissimilarity

Figure S1.1 – Ranges of M-M, M-I, and I-I geographic and environmental distances

TABLE S1.1. Islands included for anoles and terraranans

Island	Anoles	Terraranans
Abaco	x	x
Acklins	x	
Andros	x	x
Anegada	x	
Anguilla	x	x
Antigua	x	x
Barbados	x	x
Barbuda	x	x
Beata	x	x
Berry	x	x
Cat	x	x
Catalina	x	
Cayman Brac	x	
Crooked	x	
Cuba	x	x
Culebra	x	x
Desirade		x
Dominica	x	x
Eleuthera	x	x

Exuma	x	x
Gonave	x	
Grand Bahama	x	x
Grand Cayman	x	
Grand Turk	x	
Great Inagua	x	
Grenada	x	x
Guadeloupe	x	x
Hispaniola	x	x
Isla de Juventud	x	x
Jamaica	x	x
Jost Van Dyke	x	x
Little Cayman	x	
Little Inagua	x	
Little San Salvador	x	x
Long	x	x
Marie-Galante	x	x
Martinique	x	x
Mayaguana	x	
Mona	x	x
Montserrat	x	x
Navassa	x	
Nevis	x	x
New Providence	x	x
Petit Cayemite	x	
Puerto Rico	x	x
Rum Cay	x	
Saba	x	x
Samana Cay	x	
San Salvador	x	x
Saona	x	
South Bimini	x	
St. Barthelmey	x	x
St. Christopher	x	x
St. Croix	x	x
St. Eustatius	x	x
St. John	x	x
St. Lucia	x	x

Table S1.1 (Continued)

St. Martin	x	x
St. Thomas	x	x
St. Vincent	x	x
Tortola	x	x
Tortue	x	x
Vache	x	
Vieques	x	x

TABLE S1.2. Fifteen environmental variables derived from Worldclim dataset for quantifying environmental dissimilarity (in addition to mean NPP from the MODIS satellite). The function column gives the function applied to all 0.008 dd cells within an island or mainland sub-region to derive the variables.

| Variable	Function	Related Bioclim variable
Elevation	Mean	ALT
Elevation	Range	ALT
Elevation	Standard deviation	ALT
Mean annual temperature	Mean	BIO1
Mean annual temperature	Range	BIO1
Mean annual temperature	Standard deviation	BIO1
Minimum temperature	Mean	BIO6
Maximum temperature	Mean	BIO5
Temperature seasonality	Mean	BIO4
Annual precipitation	Mean	BIO12
Annual precipitation	Range	BIO12
Annual precipitation	Standard deviation	BIO12
Minimum monthly precipitation	Mean	BIO14
Maximum monthly precipitation	Mean	BIO13
Precipitation seasonality	Mean	BIO15
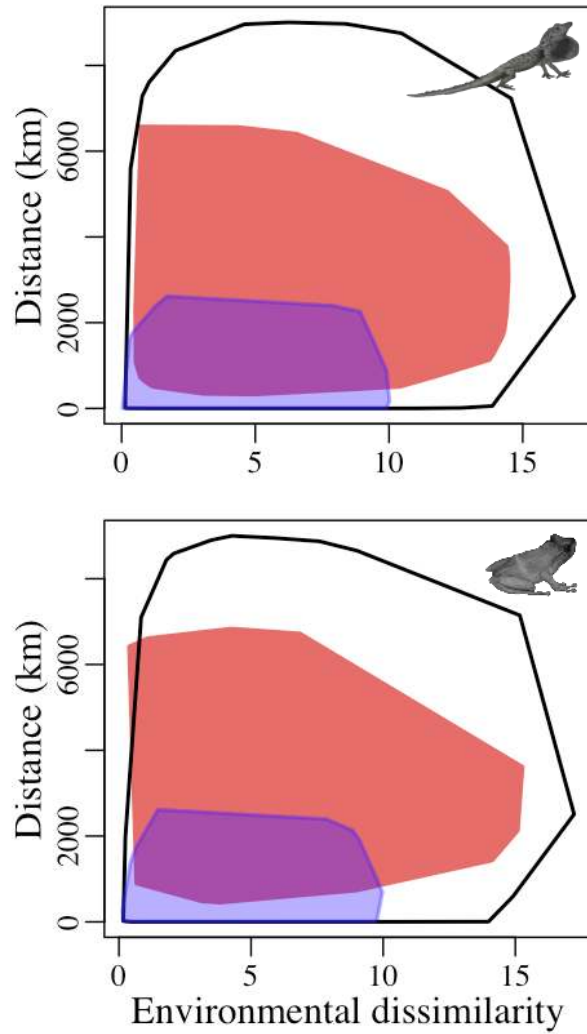
FIGURE S1.1. Minimum convex polygons showing the range of environmental and geographic distances encompassed by mainland-mainland (black line), mainland–island (red), and island–island (blue) comparisons.

CHAPTER 2 SUPPLEMENTARY MATERIAL

Ecological Character Displacement: Glass Half Full or Half Empty?

Yoel E. Stuart and Jonathan B. Losos

(Published in *Trends in Ecology and Evolution*)

(Available electronically from http://eresearch.lib.harvard.edu/V)

Includes

Table S2.1 – Scored criteria for references cited in Dayan and Simberloff 2005 [17]

Table S2.2 – Scored criteria for studies published 2005-2012

Text S2.1 – References cited in Table S1 and Table S2

Rapid evolution of character displacement in the lizard *Anolis carolinensis* following

invasion by a congener

Yoel E. Stuart, Todd S. Campbell, R. Graham Reynolds, Liam J. Revell, Jonathan B. Losos

(As prepared for *Science*)

<u>Includes</u>

Materials and Methods

Table S3.1 – Tests for environmental heterogeneity among islands

Table S3.2 – Habitat use and morphology sample sizes

Table S3.3 – Common garden experiment sample sizes

MATERIALS AND METHODS

Perch Height

We visited islands between 7am and 2pm from May-August 2010. The average number of visits across the summer per island was 8.3. We collected lizard perch height data using the "Rand survey" method (Rand 1964), whereby we walked through the habitat slowly until we observed an undisturbed adult lizard. We noted the perch at which the lizard was first observed and measured the height of the perch to the nearest cm with a tape measure. Sample sizes are in Table S2.

We used Linear Mixed Models to analyze our data because they incorporate within-island variation by nesting islands as a random effect within the fixed treatment effect (i.e., the presence of *Anolis sagrei*) (Gelman and Hill 2007). The untransformed perch height data were right-skewed, so we square-root transformed them to approximate normality. We conducted our analyses using the *lme()* function in the R package *nlme* and built a full model that includes sex as an explanatory variable as follows: lme(sqrt(lizard perch height) ~ sagrei presence * sex,

random = ~sex | island). The interaction term in the full model was not significant so we built the following reduced model: lme(sqrt(lizard perch height) ~ sagrei presence + sex, random = sex | island). Residuals from this model were exhibited no structure around zero. The presence of *A. sagrei* significantly predicts perch height in *A. carolinensis* populations (see main Text for statistics), even after significant perch differences by sex are taken into account (β_{male} = 1.94, t_{807} = 3.7, one-tailed p < 0.001).

Morphological Divergence

We measured toepad area and lamella number from flatbed digital scans of the fourth toe of the hindfoot for every adult lizard we caught. This toe is commonly used in studies of *Anolis* toepad functional morphology because it is the largest toe, likely most important, and because Glossip and Losos (1997) showed high correlation for lamella number among toes. We measured lamella number by counting all lamellae on the third and fourth phalanges of the toe and traced the area encompassed by those lamellae for toepad area. We measured right and left toes and averaged them for analysis. We also measured snout-to-vent length (svl) as a proxy for body-size correction during analysis. Distributions of toepad area and lamella number were approximately normal and were not transformed. Sample sizes are in Table S3.2.

As above, we used Linear Mixed Models to nest island random effects within our *A. sagrei*-presence fixed effect. For toepad area and lamella number, separately, we built full models that included lizard sex and svl as random effects: lme(trait ~ sagrei presence*sex*svl, random = ~sex + svl | island), where trait is either toepad area or lamella number. Neither the three-way interaction term nor any of the two way interaction terms were significant so we chose a reduced model that did not include interaction terms: lme(trait ~ sagrei presence + sex + svl, random = ~sex + svl | island). Residuals of this model also exhibited no structure around zero.

The presence of *A. sagrei* was a significant predictor for both toepad area and lamella number (see main text for significance values). Toepad area was also significantly predicted by sex ($\beta_{male} = 0.46$, $t_{551} = 4.4$, one-sided $p < 0.001$) and svl ($\beta_{svl} = 0.12$, $t_{551} = 12.8$, one-sided $p < 0.001$), as was lamella number ($\beta_{male} = 0.88$, $t_{551} = 4.5$, one-sided $p < 0.001$) and svl ($\beta_{svl} = 0.04$, $t_{551} = 2.4$, one-sided $p = 0.008$).

Rates of Divergence

We calculated rates of evolution using the *haldane (h),* a measure of the proportional change per generation in standard deviation units (Kinnison and Hendry 2001). We used the equation

$$h = \left((x_s / s_p) - (x_a / s_p)\right) / g.$$

x is the mean of island trait-means for either size-corrected toepad area or size-corrected lamella number. Subscript *s* represents islands where *A. carolinensis* is sympatric with *A. sagrei* (i.e., invaded islands) while subscript *a* represents islands where *A. carolinensis* is allopatric to *A. sagrei* (i.e., un-invaded islands). *g* is the number of generations since divergence began, which we conservatively take to be twenty generations as *A. carolinensis* likely has slightly more than one generation per year and *A. sagrei* began colonizing the islands during or after 1994. s_p is the pooled standard deviation of the island means across *a* and *s* islands; this value was calculated as the square root of the within mean-squared error taken from a linear regression of size-corrected trait mean against *A. sagrei* presence or absence. *p*-values were calculated using a randomization test, whereby *a* and *s* were assigned to island means in every permutation and *h* was recalculated in each case to provide a distribution of possible *h* values. We compared our observed *h* values to this distribution. R scripts are available from the authors.

Common Garden Experiment

In late July 2011, we collected gravid *A. carolinensis* females from four invaded and four un-invaded islands. We returned these gravid females to common cage and lighting laboratory conditions. Females were housed individually in Critter Keepers with bamboo dowels, cage carpet, and a potted plant for laying eggs. Lizards were misted twice daily and fed 2-3 times per week with crickets that had been fed Flukers Orange Cubes and Flukers High Calcium Cricket Diet. Directly before feeding to lizards, crickets were also dusted with vitamin and calcium powders.

We checked plant pots three times per week for eggs from August-November 2011. We collected, incubated, and hatched laid eggs. We raised the offspring in individual cages and shuffled cages regularly to randomize any within room environmental variation. Offspring were fed and misted by the same regimen as adults, except that smaller cricket sizes were used as appropriate to the size of the lizard.

We raised the offspring for six months and then measured toepad area and lamella number, as described above. Because of low sample sizes (Table S3.3), we did not differentiate by sex in our models as our field data demonstrate significant effects of the presence of *A. sagrei* regardless of whether sex is included in the model. We did not include an indicator for each hatchling's dam, as there were no differences among dams from invaded and un-invaded islands in svl, mass, or body condition (mass/svl) (Linear Mixed Models. svl: $\beta_{sagrei\ present}$ = -0.13, t_6 = -0.19, p = 0.86; mass: $\beta_{sagrei\ present}$ = 0.11, t_6 = 1.07, p = 0.33; body condition: $\beta_{sagrei\ present}$ = 0.002, t_6 = 1.34, p = 0.23).

For toepad area and lamella number, individually, we built a full model that included lizard svl as random effects: lme(trait ~ sagrei presence*svl, random = ~svl | island). The

interaction term was not significant so we chose the following reduced model: lme(trait ~ sagrei presence + svl, random = ~svl | island).

Environmental Heterogeneity

We tested for environmental heterogeneity between invaded and un-invaded islands. We used Google Earth to estimate distance to the mainland, island area, and vegetated area for each island in our study. Because the distributions were not normal, we used Wilcoxon Rank Sum Tests to test for differences between these variables with island invasion status as the explanatory variable (Table S3.1).

To test for differences in available tree heights and vegetation species richness, we conducted point-quarter habitat surveys of island vegetation. Islands have two distinct habitat types: a forested edge and an open center. Within the forested edge, we used Google Earth to haphazardly choose survey points in an outer circle close to the forest/water edge and an inner circle near the forest/center edge. For the open center, we surveyed three to four points along three to four regularly placed north-south transects, the number of each depending on island size. At each point, we noted the species identity for the four closest trees and then measured their heights. We used Wilcoxon Rank Sum Tests to test for differences in invaded vs. un-invaded islands in both tree height and species richness. Species richness was calculated using both the Shannon and Simpson diversity indices using the *diversity()* function in the R package Vegan. Results are shown in Table S3.1.

Line of Cedars Island

We studied one additional island, Line of Cedars, which we did not include in the main manuscript because its colonization history for *A. sagrei* is not as well documented as our main study islands. When T.C.S. conducted his island surveys in 1994, he found this island already

colonized by *A. sagrei,* meaning that it arrived sometime in the previous seven to ten years. Including this island does not change our finding that the invaded and un-invaded islands are similar in macro-ecological characteristics. Similarly, there is no qualitative change to our perch height, toepad area, or lamella number findings if this island is included.

Anolis sagrei still has a significant effect on *A. carolinensis* perch height ($\beta_{\text{sagrei present}}$ = 2.71, t_{10} = 6.9, one-tailed $p < 0.001$; sex effect: β_{male} = 1.88, t_{10} = 3.9, one-tailed $p < 0.001$), toepad area ($\beta_{\text{sagrei present}}$ = 0.15, t_{10} = 3.0, one-tailed $p = 0.007$; sex effect: β_{male} = 0.47, t_{605} = 4.9, one-sided $p < 0.001$; size effect: β_{svl} = 0.12, t_{605} = 13.8, one-sided $p < 0.001$), and lamella number ($\beta_{\text{sagrei present}}$ = 0.43, t_{10} = 2.3, one-tailed $p = 0.022$; sex effect: (β_{male} = 0.86, t_{605} = 4.4, one-sided $p < 0.001$; size effect: β_{svl} = 0.05, t_{605} = 2.5, one-sided $p = 0.007$). Estimated rates of evolution are similar and remain significant (toepad area: $h = 0.094$, one-sided $p = 0.006$; lamella number: $h = 0.056$, one-sided $p = 0.043$).

Chapter 3 Supplementary Materials References Cited

Glossip, D. and Losos, J.B. (1997) Ecological correlates of number of subdigital lamellae in anoles. *Herpetologica* 53, 192-199

Kinnison, M.T. and Hendry, A.P. (2001) The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* 112-113: 145-164

Rand, A.S. (1964) Ecological distribution in Anoline lizards of Puerto Rico. *Ecology* 45, 745-752

Gelman, A. and Hill, J. (2007) *Data analysis using regression and multilevel/hierarchical models*, Cambridge University Press

TABLE S3.1. Tests for environmental heterogeneity between un-invaded (n=5) and invaded (n=6) islands. W is the test statistic for Wilcoxon Ranked Sum tests.

Variable	W Statistic	p-value (two-sided)
Distance to Mainland (m)	12	0.66
Island Area (m^2)	8	0.25
Vegetated Area (m^2)	16	0.93
Available Tree Heights (cm)	16.5	0.85
Shannon Diversity Index	13	0.79
Simpson Diversity Index	12	0.66

TABLE S3.2. *Anolis sagrei* invasion status, *A. carolinensis* perch height sample size, and *A. carolinensis* morphology sample size by island for the 2010 field experiment. For sample sizes, males are listed before the "/" and females after.

Island	*A. sagrei* invasion	Perch height sample size (M/F)	Morphology sample size (M/F)
Channel	Yes	51 / 15	38 / 15
Crescent	No	50 / 12	38 / 10
Hook	Yes	53 / 22	42 / 16
Hornet	No	60 / 27	44 / 15
Lizard	Yes	71 / 40	42 / 19
North Twin	Yes	48 / 21	32 / 11
Osprey	No	52 / 15	33 / 10
Pine	No	38 / 19	27 / 14
South Twin	No	60 / 38	34 / 24
Yang	Yes	57 / 14	41 / 16
Yin	Yes	48 / 12	27 / 16

TABLE S3.3. *Anolis sagrei* invasion status, dam and hatchling sample size by island for the common garden experiment.

Island	*A. sagrei* invasion	Dam sample size	Hatchling sample size
Hornet	No	3	6
Lizard	Yes	6	12
North Twin	Yes	8	10
Osprey	No	5	8
Pine	No	1	2
South Twin	No	5	7
Yang	Yes	6	10
Yin	Yes	5	6