Author for correspondence:
Julián A. Velasco
e-mail: juvelas@gmail.com

THE ROYAL SOCIETY
PUBLISHING

Solitary ecology as a phenomenon extending beyond insular systems: exaptive evolution in *Anolis* lizards

Julián A. Velasco[1,2], Steven Poe[4], Constantino González-Salazar[3,5] and Oscar Flores-Villela[1]

[1]Museo de Zoología 'Alfonso L. Herrera', Facultad de Ciencias, [2]Centro de Ciencias de la Atmósfera, and [3]Centro de Ciencias de la Complejidad C3, Universidad Nacional Autónoma de México, Mexico City, Mexico
[4]Department of Biology, University of New Mexico, Albuquerque, NM, USA
[5]Departamento de Ciencias Ambientales, CBS Universidad Autónoma Metropolitana, Unidad Lerma, Estado de México 52006, Mexico

JAV, 0000-0002-2183-5758; CG-S, 0000-0001-7347-714X

The mechanisms driving phenotypic evolution have been of interest to biologists since Darwin. Ecological release—wherein adaptive evolution occurs following relaxation of constraining selective pressures—and environmental filtering—wherein exaptive traits allow colonization of a new area—have been studied in several insular cases. *Anolis* lizards, which may exist in solitude or sympatry with multiple congeners, are an excellent system for evaluating whether ecological release and environmental filtering are associated with phenotypic shifts across phylogenetic and geographical scales. Insular solitary *Anolis* exhibit phenotypic differentiation in body size and sexual size dimorphism—SSD—through exaptive and adaptive evolution, respectively. But, the generality of these effects has not yet been addressed. Here, we analyse the evolution of body size and SSD relative to sympatry in mainland *Anolis*. We found that mainland species co-occurring with few congeners exhibit uniform body size and greater SSD relative to other random mainland assemblages, consistent with the insular solitary pattern. The locations of evolutionary shifts for both traits do not coincide with evolutionary transitions to decreased levels of sympatry. These results are consistent with exaptive environmental filtering but not adaptive ecological release. Future studies should be conducted at local scales to evaluate the role of these factors in the evolution of solitary existence in mainland and island species.

1. Background

The interplay between mechanisms of species co-occurrence and the evolution of phenotypic traits is a fundamental topic in evolutionary biology [1,2]. The avenues by which species evolve ecological niche differences at regional and local scales have been studied extensively in insular cases [3,4]. For instance, relaxed competition for resources at regional scales is thought to promote trait evolution [1,2]. By contrast, environmental filtering mechanisms may be implicated when functional species traits evolved shortly after colonization of a new area [5,6].

Assembly processes can be studied at large spatial scales by combining information about species' ranges, phylogenies and traits [1,7,8]. Patterns of co-occurrence are useful for elucidating which mechanisms are driving trait evolution at large scales [7,8]. The novel concept of a diversity field [9,10]—a measure of the degree of species co-occurrence for a species across its range—may be used to test whether sympatry can predict elements of

phenotypic evolution at regional scales. For example, if competition at regional scales promotes phenotypic shifts in co-occurring species [1,11], we might predict that species co-occurring with many congeners will be habitat specialists displaying extensive phenotypic differentiation [10,12,13]. Conversely, species that tend to co-occur with few or no close relatives (e.g. solitary insular species living in geographical isolation) might be habitat generalists with little phenotypic differentiation from ancestral species [10,14,15].

Anolis lizards are ideal organisms for studying how competitive interactions promote phenotypic differentiation at regional scales. Studies of Anolis species on islands have shown that lineages that disperse from areas of broad sympatry to areas with few close relatives may display unusual traits (e.g. uniform body size and greater sexual size dimorphism, SSD; [15,16]). These traits may evolve according to the disparate processes of adaptation via ecological release or exaptation (sensu [17]) via environmental filtering. Poe et al. [15] found that an intermediate (i.e., approx. 65 mm) body size is a prerequisite (i.e. an exaptation) that facilitates the colonization of islands uninhabited by close relatives. Additionally, they found that solitary species evolved independently to a greater SSD, apparently to reduce intraspecific (intersexual) competition [16]. In other words, solitary species shown strong conservatism in body size and extensive evolution in SSD. These two processes are only known for insular solitary species; it is unknown whether the same processes can be inferred for mainland species co-occurring with few congeners and potentially relaxing competition across large spatial scales.

Here, we take advantage of recently developed comparative methods and new large datasets of traits, phylogeny and distribution for Anolis lizards to rigorously test hypotheses of ecological release and environmental filtering on an unprecedented geographical scale including both mainland and island environments. First, we identify mainland species co-occurring with few congeners (mainland 'species-poor' forms) based on range overlap and test whether they show non-random patterns of uniform body size and greater SSD (i.e. unusual morphologies). We then test whether rates of body size and SSD evolution are different for forms co-occurring with few congeners. Finally, we evaluate whether evolutionary transitions to reduced sympatry are associated with phenotypic shifts across the phylogeny. These sequential tests are designed to discern whether solitariness is a general ecological phenomenon, with manifestation outside insular systems [14–16].

2. Material and methods

We compiled distributional, phenotypic and phylogenetic data for almost all known species of Anolis (377 spp.) from several sources [18,19]. Body size was measured from specimens and SSD was calculated as the ratio of maximum female SVL to maximum male SVL. SSD values below one indicate male-biased dimorphism, whereas values above one indicate female-biased dimorphism (see electronic supplementary material for details). We calculated the maximum number of species co-occurring within the range of each Anolis species (i.e. within-range richness; see electronic supplementary material). This approach allows us to estimate the maximum number of species that co-occur within the range of each species at large scales (i.e. the diversity field concept; [9,10]). This approach has been used extensively in local [1,2] and regional scale ecology [10,20] to understand

potential competitive interactions emerging at regional scales. These co-occurrence patterns vary across localities and species can occur with few or no species at alternative sites (electronic supplementary material, figure S1).
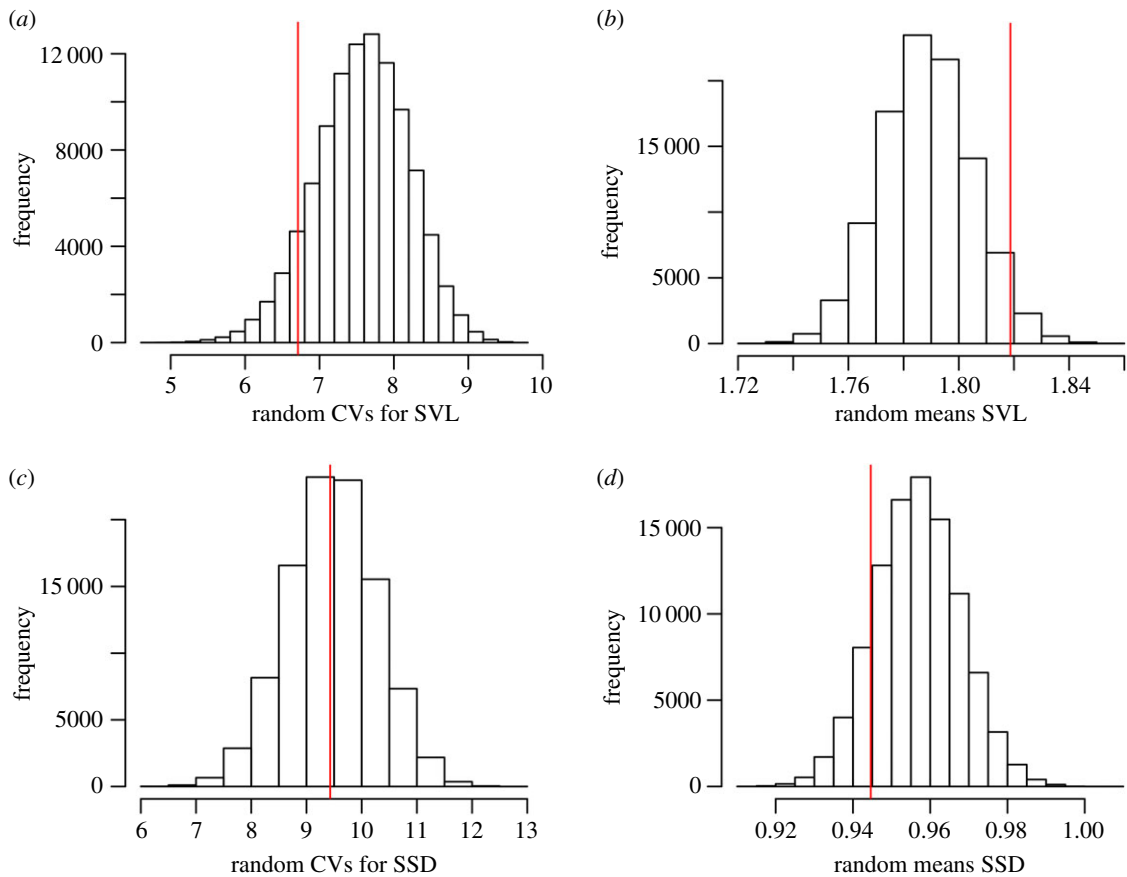
We used two approaches to analyse whether mainland species that tend to co-occur with fewer species are similar in body size and SSD to solitary species (i.e. those inhabiting islands without other congeners). In the first one, we classified each species according to its within-range richness and used the first quantile of the distribution to classify mainland species according to their degree of co-occurrence with other congeners (electronic supplementary material, figure S2). Although the choice of the first quantile as threshold is arbitrary, this choice has been used extensively in macroecological studies exploring biotic interactions from range overlap at large scales [21–24]. Each mainland species with fewer than 12 co-occurring species across its range was coded as a species that co-occurs with few congeners (i.e. mainland 'species-poor' anoles; a total of 47 species; see electronic supplementary material, table S1). We evaluated whether these mainland 'species-poor' anoles differ non-randomly in body size and SSD from other mainland anole species (electronic supplementary material, figure S3). We selected 100 000 random mainland pools of similar size (47 species) and tested whether mean and coefficient of variation in body size and SSD from mainland 'species-poor' anoles differ from these random pools (see [15] for details). We also used sextiles to classify mainland species to thereby explore the sensitivity of our results to threshold values (electronic supplementary material, figure S4). In the second one, we tested whether body size and SSD increase with increasing within-range richness values (i.e. from solitary species to species co-occurring with many congeners) using phylogenetic generalized least-squares regressions (electronic supplementary material, figure S5).

Using the discrete categories based on within-range richness, we inferred evolutionary transitions to decreased levels of sympatry in Anolis using 100 histories of stochastic character mapping [25] between four states as follows: insular, solitary, mainland 'species-poor' and mainland (electronic supplementary material, figure S6). Insular refers to species inhabiting islands with more than one congener, solitary refers to species inhabiting islands without other congeners, mainland 'species-poor' refers to mainland species co-occurring with fewer than 12 species and mainland refer to mainland species co-occurring with 12 or more species. We fitted seven different models of body size and SSD evolution for each ecological state using the anole phylogeny [18] with the OUwie package [26]. We selected the most informative model with Akaike information criteria (AIC). We explored the effect of topological uncertainty on these estimates (see electronic supplementary material, figures S7 and S8) using a set of 100 trees from a Bayesian posterior distribution of trees. We calculated the evolutionary rates for each trait from the best selected model for each ecological category.

We estimated the location and magnitude of phenotypic rate- and regime shifts for body size and SSD across the anole phylogeny using a reversible-jump Bayesian method [27]. This method allows us to discover the placement and magnitude of these shifts based on a multi-optima Ornstein-Uhlenbeck process. We ran a Markov chain for SVL and SSD for 5×10^6 generations with a burnin-in the first 30% of samples. All analyses were conducted in R and data and R scripts were deposited in the Dryad Digital Repository [28].

3. Results

We found that mainland Anolis species in sympatry with few congeners (mainland 'species-poor' forms) show non-random patterns of body size and SSD in comparison with other mainland species (figure 1). Variance in body size in these mainland

Figure 1. Distributions of means and coefficients of variation (CV) for body size (snout to vent length, SVL) and sexual size dimorphism (SSD) for 100 000 random mainland assemblages. The vertical red line corresponds to the observed value of CVs for the mainland species that co-occur with few congeners (i.e. mainland '*species-poor*' forms; see main text). Body size in mainland species-form forms was greater than expected, whereas SSD was lower than expected (*p*-values < 0.001). Note that low SSD values indicate higher SSD (female/male ratio). Body size but not SSD was more uniform (i.e., had lower CV) in mainland '*species-poor*' species (*p* < 0.001).

forms was lower than variance observed in random assemblages (figure 1*a*; *p* < 0.001) suggesting that these species display uniform body sizes, as in insular solitary anoles. The mean body size was larger in these mainland forms than expected from random assemblages (figure 1*b*; *p* < 0.001). Variance in SSD in these mainland forms was not different from random assemblages (figure 1*c*; *p* > 0.5). SSD in these mainland forms was greater than expected from random assemblages (figure 1*d*; *p* < 0.001); i.e. the insular pattern of SSD also holds on the mainland (SSD values close to zero indicate that males are greater than females). Similar patterns were recovered when we used sextiles to classify species (electronic supplementary material, figure S4). We also found that body size increases as the number of potential competitors increases but SSD decreases (electronic supplementary material, figure S5). Body size and SSD fitted very well to a model with multiple α per selective regimes with similar evolutionary rates between species categories of degrees of sympatry (electronic supplementary material, table S2). Rates of body size and SSD evolution were similar across all groups and not affected substantially by topological uncertainty (electronic supplementary material, figure S8).
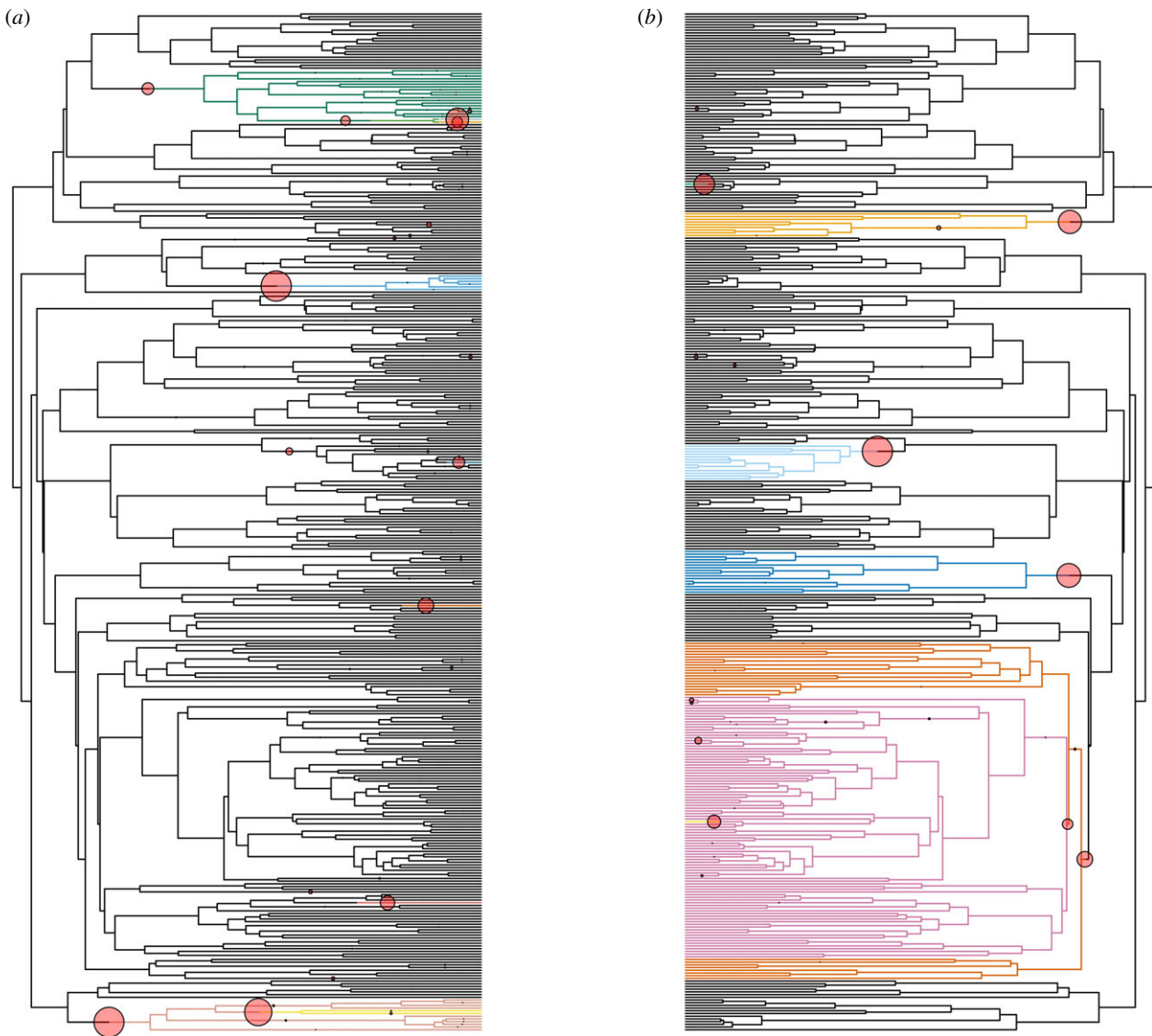
The location of rate- and regime-shifts for SVL and SSD did not coincide across the entire anole radiation (figure 2). In addition, these shifts in trait evolution did not coincide with transitions to solitary existence in most mainland '*species-poor*' anoles (electronic supplementary material, figures S9 and S10). In the branch descending to *Anolis forbesi*, there was a small shift in body size evolution which seems to coincide with a transition to solitary existence in the

mainland (figure 2; electronic supplementary material, figure S9). Similarly, the branch to insular *A. villai* exhibited a shift in SSD associated with a transition to solitary existence (figure 2; electronic supplementary material, figure S10).

4. Discussion

Mainland '*species-poor*' forms exhibit non-randomly uniform body sizes as has been found for insular solitary species (i.e. species occurring in geographical isolation from other congeners). Poe *et al.* [15] showed that uniform body size evolves by exaptation [17] in insular solitary species, possibly through environmental filtering mechanisms. For these insular forms, Poe *et al.* [15,29] showed that a certain body size may be a prerequisite for successful colonization of empty islands. Given the lack of correlation of body size changes with the evolution of solitary existence (electronic supplementary material, figure S9), it is plausible that exaptation also is playing a role in body size evolution in these mainland forms co-occurring with few congeners. That is, perhaps only a narrow range of body sizes in mainland forms allows invasion of areas with few congeners.

Although mainland '*species-poor*' forms exhibited greater SSD in comparison with species in larger mainland assemblages (figure 1*c*), the observed SSD was very different between insular solitary and non-solitary species (electronic supplementary material, figure S3). This result and the lack of correlation between changes in SSD and decreased sympatry (electronic supplementary material, figure S10) suggest

Figure 2. Location of phenotypic rate (circles) and regime-shifts (colours) across the entire *Anolis* radiation using a reversible-jump Bayesian approach. (*a*) Body size (snout–vent length, SVL) and (*b*) SSD. The size of the circle represents the magnitude of rate shifts across the tree. (Online version in colour.)

that the evolution of SSD in mainland forms may have followed a different evolutionary trajectory relative to insular counterparts. The non-random SSD of mainland 'species-poor' forms coupled with the lack of correlative evolutionary change accompanying the transition to geographical isolation is consistent with exaptation in SSD. Thus, although shifts in the timing of SSD and body size evolution are uncoupled (figure 2), a shared process for these traits (i.e. exaptation) may be occurring in mainland species.

We were unable to detect a large-scale association between trait changes and shifts in the number of congeners; we detected only a few cases where this correlation occurred. This result suggests that environmental filtering might be occurring with no evolutionary change via escape from a competitive assemblage. Moreover, there is evidence that competitive interactions drive evolution in body size and SSD in insular *Anolis* [30,31]. However, the role of other factors such as climate and geography in the evolution of body size has not been evaluated comprehensively. We suggest that both climate and competitive interactions might modulate trait evolution in *Anolis* lizards.

Instances of rapid evolution in phenotype and habitat use have been detected in anole species at local scales [32,33]. It is

possible that ecological release exerts a role at regional scales in mainland species. As our regional approach likely does not capture phenotypic differentiation at local scales, more work at local scales is necessary to evaluate when and how ecological release and intraspecific competition drove phenotypic and habitat use shifts in mainland forms. Recent studies have explored evolutionary and ecological scenarios in individual species [34] and new methods to test character displacement at local scales are available [35]. These approaches should prove useful in identifying local selective factors driving the evolution of body size and SSD in anoles and other lineages.

royalsocietypublishing.org/journal/rsbl   *Biol. Lett.* **15**: 20190056

5

References

1. Davies JT, Meiri S, Barraclough TG, Gittleman JL. 2007 Species co-existence and character divergence across carnivores. *Ecol. Lett.* **10**, 146–152. (doi:10.1111/j.1461-0248.2006.01005.x)

2. Sanderson JG, Pimm SL. 2015 *Patterns in nature: the analysis of species co-occurrences*. Chicago, IL: University of Chicago Press.

3. Losos JB, Jackman TR, Larson A, de Queiroz K, Rodríguez-Schettino L. 1998 Contingency and determinism in replicated adaptive radiations of island lizards. *Science* **279**, 2115–2118. (doi:10.1126/science.279.5359.2115)

4. Cadotte M, Albert CH, Walker SC. 2013 The ecology of differences: assessing community assembly with trait and evolutionary distances. *Ecol. Lett.* **16**, 1234–1244. (doi:10.1111/ele.12161)

5. Harvey PH, Pagel MD. 1991 *The comparative method in evolutionary biology*. Oxford, UK: Oxford University Press.

6. Kraft NJ, Adler PB, Godoy O, James EC, Fuller S, Levine JM. 2015 Community assembly, coexistence and the environmental filtering metaphor. *Funct. Ecol.* **29**, 592–599. (doi:10.1111/1365-2435.12345)

7. Barnagaud JY, Kissling DW, Sandel B, Eiserhardt WL, Şekercioğlu ÇH, Enquist BJ, Tsirogiannis C, Svenning JC. 2014 Ecological traits influence the phylogenetic structure of bird species co-occurrences worldwide. *Ecol. Lett.* **17**, 811–820. (doi:10.1111/ele.12285)

8. Villalobos F, Olalla-Tárraga MÁ, Cianciaruso MV, Rangel TF, Diniz-Filho JAF. 2017 Global patterns of mammalian co-occurrence: phylogenetic and body size structure within species ranges. *J. Biogeogr.* **44**, 136–146. (doi:10.1111/jbi.12826)

9. Arita HT, Christen JA, Rodríguez P, Soberón J. 2008 Species diversity and distribution in presence–absence matrices: mathematical relationships and biological implications. *Am. Nat.* **172**, 519–532. (doi:10.1086/590954)

10. Villalobos F, Arita HT. 2010 The diversity field of New World leaf-nosed bats (Phyllostomidae). *Glob. Ecol. Biogeogr.* **19**, 200–211. (doi:10.1111/j.1466-8238.2009.00503.x)

11. Dayan T, Simberloff D. 2005 Ecological and community-wide character displacement: the next generation. *Ecol. Lett.* **8**, 875–894. (doi:10.1111/j.1461-0248.2005.00791.x)

12. Williams EE. 1983 Ecomorphs, faunas, island size, and diverse end points in island radiations of *Anolis*. In *Lizard ecology: studies of a model organism* (eds RB Huey, ER Pianka, TW Schoener), pp. 326–370. Cambridge, MA: Harvard University Press.

13. Bar-Massada A, Belmaker J. 2017 Non-stationarity in the co-occurrence patterns of species across environmental gradients. *J. Ecol.* **105**, 391–399. (doi:10.1111/1365-2745.12713)

14. Losos JB, Queiroz KD. 1997 Evolutionary consequences of ecological release in Caribbean *Anolis* lizards. *Biol. J. Linnean Soc.* **61**, 459–483. (doi:10.1111/j.1095-8312.1997.tb01802.x)

15. Poe S, Goheen JR, Hulebak EP. 2007 Convergent exaptation and adaptation in solitary island lizards. *Proc. R. Soc. B* **274**, 2231–2237. (doi:10.1098/rspb.2007.0569)

16. Schoener TW. 1969 Size patterns in West Indian *Anolis* lizards: I. Size and species diversity. *Syst. Zool.* **18**, 386–401. (doi:10.2307/2412183)

17. Gould SJ, Vrba ES. 1982 Exaptation—a missing term in the science of form. *Paleobiology* **8**, 4–15. (doi:10.1017/S0094837300004310)

18. Poe S *et al.* 2017 A phylogenetic, biogeographic, and taxonomic study of all extant species of *Anolis* (Squamata; Iguanidae). *Syst. Biol.* **66**, 663–697. (doi:10.1093/sysbio/syx029)

19. Velasco JA, Villalobos F, Diniz-Filho JA, Algar AC, Flores-Villela O, Köhler G, Poe S, Martinez-Meyer E. 2018 Climatic and evolutionary factors shaping geographical gradients of species richness in *Anolis* lizards. *Biol. J. Linnean Soc.* **123**, 615–627. (doi:10.1093/biolinnean/blx160)

20. Borregaard MK, Rahbek C. 2010 Dispersion fields, diversity fields and null models: uniting range sizes and species richness. *Ecography* **33**, 402–407. (doi:10.1111/j.1600-0587.2010.06323.x)

21. Jetz W, Rahbek C. 2002 Geographic range size and determinants of avian species richness. *Science* **297**, 1548–1551. (doi:10.1126/science.1072779)

22. Rahbek C, Gotelli NJ, Colwell RK, Entstninger GL, Rangel TFL, Graves GR. 2007 Predicting continental-scale patterns of bird species richness with spatially explicit models. *Proc. R. Soc. B* **274**, 165–174. (doi:10.1098/rspb.2006.3700)

23. González-Salazar C, Stephens CR, Marquet PA. 2013 Comparing the relative contributions of biotic and abiotic factors as mediators of species' distributions. *Ecol. Modell* **248**, 57–70. (doi:10.1016/j.ecolmodel.2012.10.007)

24. Rengifo-Correa L, Stephens CR, Morrone JJ, Téllez-Rendón JL, González-Salazar C. 2017 Understanding transmissibility patterns of Chagas disease through complex vector–host networks. *Parasitology* **144**, 760–772. (doi:10.1017/S0031182016002468)

25. Huelsenbeck JP, Nielsen R, Bollback JP. 2003 Stochastic mapping of morphological characters. *Syst. Biol.* **52**, 131–158. (doi:10.1080/10635150390192780)

26. Beaulieu JM, O'Meara B. 2014 OUwie: analysis of evolutionary rates in an OU framework. R package version, 1.

27. Uyeda JC, Harmon LJ. 2014 A novel Bayesian method for inferring and interpreting the dynamics of adaptive landscapes from phylogenetic comparative data. *Syst. Biol.* **63**, 902–918. (doi:10.1093/sysbio/syu057)

28. Velasco JA, Poe S, González-Salazar C, Flores-Villela O. 2018 Data from: The evolution of isolation in mainland lizards: solitary ecology as a phenomenon extending beyond insular systems. Dryad Digital Repository. (https://doi.org/10.5061/dryad.h234b36)

29. Poe S, Giermakowski JT, Latella IM, Schaad EW, Hulebak EP, Ryan MJ. 2011 Ancient colonization predicts recent naturalization in *Anolis* lizards. *Evolution* **65**, 1195–1202. (doi:10.1111/j.1558-5646.2011.01238.x)

30. Losos JB. 1994 Integrative approaches to evolutionary ecology: *Anolis* lizards as model systems. *Annu. Rev. Ecol. Syst.* **25**, 467–493. (doi:10.1146/annurev.es.25.110194.002343)

31. Mahler DL, Revell LJ, Glor RE, Losos JB. 2010 Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. *Evolution* **64**, 2731–2745. (doi:10.1111/j.1558-5646.2010.01026.x)

32. Losos JB, Warheitt KI, Schoener TW. 1997 Adaptive differentiation following experimental island colonization in *Anolis* lizards. *Nature* **387**, 70–73. (doi:10.1038/387070a0)

33. Stuart YE, Campbell TS, Hohenlohe PA, Reynolds RG, Revell LJ, Losos JB. 2014 Rapid evolution of a native species following invasion by a congener. *Science* **346**, 463–466. (doi:10.1126/science.1257008)

34. Diniz-Filho JAF, Raia P. 2017 Island Rule, quantitative genetics and brain–body size evolution in *Homo floresiensis*. *Proc. R. Soc. B* **284**, 20171065. (doi:10.1098/rspb.2017.1065)

35. Davies TJ, Cooper N, Diniz-Filho JAF, Thomas GH, Meiri S. 2012 Using phylogenetic trees to test for character displacement: a model and an example from a desert mammal community. *Ecology* **93**, S44–S51. (doi:10.1890/11-0400.1)