JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12935

Climate-mediated adaptation after mainland colonization of an ancestrally subtropical island lizard, *Anolis carolinensis*

S. C. CAMPBELL-STATON*, S. V. EDWARDS†‡ & J. B. LOSOS†‡

*School of Integrative Biology, University of Illinois, Champaign-Urbana, IL, USA
†Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA
‡Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA

Keywords:

adaptation;
ecological genetics;
evolutionary physiology.

Abstract

Climate-mediated evolution plays an integral role in species migration and range expansion. Gaining a clearer understanding of how climate affects demographic history and adaptation provides fundamental insight into the generation of intra- and interspecific diversity. In this study, we used the natural colonization of the green anole (*Anolis carolinensis*) from the island of Cuba to mainland North America to investigate the role of evolution at the niche, phenotypic and genetic levels after long-term establishment in a novel environment. The North American green anole occupies a broader range of thermal habitats than its Cuban sister species. We documented niche expansion in the mainland green anole, mediated primarily through adaptation to winter temperatures. Common garden experiments strongly suggest a genetic component to differences in thermal performance found between populations in different temperature regimes. Analysis of geographic variation in population structure based on 53 486 single nucleotide variants from RAD loci revealed increased genetic isolation between populations in different vs. similar thermal environments. Selection scans for environment-allele correlations reveal 19 genomic loci of known function that may have played a role in the physiological adaptation of *A. carolinensis* to temperate environments on the mainland.

Introduction

Niche conservatism is the tendency of species to retain aspects of their ancestral niche over time (Wiens & Graham, 2005). Interpreted strictly, if niche conservatism prevails, migrating species should be limited to environments similar to their ancestral ranges. However, the prevalence of niche conservatism has been strongly debated, with some studies finding evidence for niche similarity among closely related species (Ricklefs & Latham, 1992; Peterson *et al.*, 1999; Prinzing *et al.*, 2001), whereas others have documented niche lability (Bohning-Gaese *et al.*, 2003; Losos *et al.*, 2003; Graham *et al.*, 2004). Evolutionary adaptation to the abiotic environment has been identified as an important contributor to the establishment of species in novel ranges (Hendry *et al.*, 2000; Huey *et al.*, 2000; Sakai *et al.*, 2001; Lee, 2002; Storz *et al.*, 2007; Eales *et al.*, 2010; Hohenlohe *et al.*, 2010; Sultan *et al.*, 2013; Flores-Moreno *et al.*, 2015). Adaptation to different environments can result in phenotypic and genetic divergence between populations and ultimately result in ecological speciation (Nosil, 2012). In this study, we present an example of phenotypic and genetic divergence within a widespread species after colonization of novel habitats using the North American green anole, *Anolis carolinensis*.

Anolis carolinensis is phylogenetically nested within the Cuban green anole (*A. porcatus*) clade; its current distribution in North America is the result of overwater dispersal leading to establishment in peninsular Florida during the Miocene-Pliocene (Glor *et al.*, 2005; Campbell-Staton *et al.*, 2012; Tollis *et al.*, 2012). The only anole native to the continental United States, *A. carolinensis* has spread from Florida throughout the

Correspondence: Shane C. Campbell-Staton, Department of Animal Biology, University of Illinois at Urbana-Champaign, 233 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801, USA.
Tel.: +217 244 4298; fax: +217 244 4565;
e-mail: shane.campbellstaton@gmail.com

south-eastern United States to occupy a range of environments as far north as Tennessee and North Carolina and west into Texas and Oklahoma.

Winter temperatures likely limit the northern edge of the species' distribution (Williams, 1969). In contrast to most reptiles at these latitudes, northern populations do not hibernate; despite regular ambient temperatures below freezing, this species endures winter by retreating to sheltered sites and basking on rock faces during periods of sun exposure (Bishop & Echternacht, 2004). Populations also display geographic variation in acute cold tolerance (CT_{min}) (Wilson & Echternacht, 1987, 1990).

The recently published genome of *A. carolinensis* (Alfoldi *et al.*, 2011) provides an invaluable resource for understanding the influence of climatic variation on population structure and natural selection at the molecular level. In this study, we combine niche modelling, thermal physiology and genomic techniques to explore climate-mediated evolution associated with the colonization of this ancestrally subtropical Cuban lizard species into more temperate habitats in mainland North America. Towards this aim, we ask three central questions: (i) Is the climatic niche of the mainland green anole different from its subtropical sister species and, if so, along which climatic axes? (ii) Is there evidence of ecological divergence within the species along the identified axes? (iii) Which genomic regions may have been targets of climate-mediated natural selection during mainland range expansion? Our results point to an important role of adaptation to winter temperatures in the long-term survival and spread of species into temperate mainland environments.

Materials and methods

Climatic differences between mainland and island green anole species

Climate is known to be an important contributor to intraspecific (Morgenstern, 1996; Savolainen *et al.*, 2007; Olson *et al.*, 2013) and interspecific (Rundle & Nosil, 2005; Schluter, 2009; Schluter & Conte, 2009; Keller & Seehausen, 2012; Nosil, 2012) divergence via local adaptation and ecological speciation, respectively. Identifying climatic differences that distinguish lineages is integral to developing hypotheses concerning climate-mediated evolution. Abiotic environmental factors that differ most between divergent lineages may be causal agents of natural selection, shaping patterns of adaptation and population dynamics across geographic space. Once identified, these climatic features may be used to develop hypotheses regarding niche shift, phenotypic selection and patterns of genetic structure and selection across geographic space.

To identify climatic conditions that differ most between mainland and island green anole species, we gathered georeferenced locality data for *A. carolinensis* and its Cuban relatives, *A. allisoni* and *A. porcatus*. We combined data from Herpnet (accessed April 2013) and our own collections. Because *A. porcatus* contains two genetically and geographically distinct clades (Glor *et al.*, 2004; Campbell-Staton *et al.*, 2012), we treated localities from the eastern and western sides of the island separately. Duplicate sites and those clearly in error (e.g. not corresponding to land areas) were removed from the data set. We downloaded contemporary climate layers for 19 bioclimatic variables from the Worldclim database (Hijmans *et al.*, 2005) and extracted climate data for each variable at each occurrence point using the raster package in R (version 3.1.2, R Core Team, 2014).

We quantified environmental variation within and between each species with a principal components analysis of bioclimatic variables from all sample localities using prcomp function in R. We used variable loadings of principal components to determine the relative importance of temperature and precipitation in differentiating climate between clades.

Environmental niche differences between mainland and island species

We estimated the degree of niche shift of *A. carolinensis* by comparing its niche breadth with that of its closet Cuban relatives. We created a climate raster from PC1 across a geographic range including Cuba and south-eastern North America with Arcmap software (ESRI 2011.) for use in environmental niche modelling. Environmental niche models for each clade were made using maximum entropy modelling implemented in Maxent (version 3.3.3, Phillips *et al.* 2004, 2006). We generated each model using 10 000 random background points from the native range of each lineage. Each model was run 10 times, each time using independently generated background points. The 10 models were then averaged to generate the final model using the logistic output option of the programme. To avoid potential biases in niche breadth estimation due to the physical isolation of the Cuban lineages, we projected each model onto a common environmental range including both south-eastern North America and Cuba for the comparisons of environmental suitability between lineages.

To identify geographic regions that are the most diverged between *A. carolinensis* and its closest island lineage, we used the niche projection of the western clade of *A. porcatus*, the sister lineage of *A. carolinensis*, to generate a multivariate similarity surface (MESS) map in Maxent. MESS maps highlight the similarity of environmental variation within a given geographic space to that experienced during model training. This map was used to determine where geographically the *A. carolinensis* range is most dissimilar to the western *A. porcatus* model.

We compared niche breadths between *A. carolinensis* and its Cuban relatives, using the ENMtools package

(Warren *et al.*, 2010). Habitat suitability scores from projected models were standardized and imported into the niche breadth calculator within the ENMtools toolkit. The programme was used to generate a measure of niche breadth, inverse concentration (Levins, 1968), for comparison across each green anole lineage.

Genomic data collection and variant calling

Liver, tail or toe tissue samples were collected for 186 individuals across 22 populations within the native range of *A. carolinensis* from collections in 2006–2007 and 2012. Tissues were flash frozen in liquid nitrogen or preserved in ethanol or RNAlater, then stored at −80 °C before tissue extraction. Genomic DNA was extracted using Qiagen DNA extraction kits. Double digests were performed using EcoR1 and Sph1 restriction enzymes to fragment DNA. Genomic libraries were prepared using a modified version of the protocol described in Peterson *et al.* (2012). Details of the modified protocol are available in the supplementary materials. Sequencing was performed using 100-bp single-end and 150-bp paired-end sequencing on the Illumina Hiseq platform.

We used Geneious (version 6.1.8, Biomatters, Auckland, New Zealand) to separate the resulting sequence fragments by individual barcode. Trimmomatic (Bolger *et al.*, 2014) was used to quality filter reads for each individual. The quality of each read was examined in a 4-bp sliding window, and regions of sequence were discarded once the average read quality in this window fell below a Phred33 score of 15. An end-to-end alignment to the *A. carolinensis* genome was performed on single-end and forward reads of each individual using bowtie2 (Langmead & Salzberg, 2012). Variants were called using the Stacks 1.06 pipeline (Catchen *et al.*, 2011, 2013). Using the *populations* pipeline contained therein, we filtered the resulting variants for use in two downstream analyses. We first filtered for variants with at least 5× coverage and with a minor allele frequency of 0.05 or higher, shared between at least two populations in the data set and present in at least 40% of individuals within each population. This data set was used to calculate a pair-wise fixation index (F_{st}) matrix between populations with the STACKS software for use in mixed model and regression analyses. Second, we selected a subset of populations with at least four individuals with ≥ 500 k representative reads to minimize missing data for selection analyses. Allele counts from this data set were then used to identify variants within RADseq reads associated with geographic variation in environment.

Experimental test of phenotypic adaptation within mainland anole populations

Phenotype–environment correlations are among the oldest and most widely used criteria for identifying local adaptation in wild populations (Endler, 1986). Under a hypothesis of climate-mediated local adaptation, geographic variation in a selective force is expected to give rise to variation in phenotypes under selection (Endler, 1986). We tested for associations between thermal tolerance and temperature extremes across the entire native range of *A. carolinensis* using the critical thermal method (Cowles & Bogert, 1944). We collected critical thermal maximum (CT_{max}) and minimum (CT_{min}) data for 202 lizards from 16 sites across the entire native range of the species during the summer of 2012. Each lizard was acclimated to 10 °C for 24 h. After acclimation, we placed a thermocouple into the cloaca of each animal to monitor internal body temperature during testing. Each lizard was then placed in a 1.2-L plastic container, cooled at a rate of ~1 °C/min and periodically flipped onto its back and stimulated with forceps. The body temperature at which an animal could no longer right itself after 30 s was recorded as its CT_{min}. After CT_{min} trials, we acclimated each lizard to room temperature (~25 °C) for 24 h. After the second acclimation period, each animal was then set up as above and heated by ~1 °C/min and tested for righting response. The temperature at which an animal could not right itself after 30 s was recorded as its CT_{max}. Rates of temperature change during each trial were recorded for use in statistical analyses.

To identify correlations between thermal tolerance and thermal extremes, we performed linear regressions using R. The first linear regression analysis was performed testing the significance of the association between mean population CT_{max} and BIO10 (mean temperature of the warmest quarter of the year), using rate of heating as a covariate. The second linear regression was performed testing the significance of association between CT_{min} and BIO11 (mean temperature of the coldest quarter of the year), using rate of cooling as a covariate. BIO10 and BIO11 were chosen to represent temperatures experienced during the warmest and coldest parts of the year at each site, respectively.

The nonindependence of phenotypes due to shared ancestry and ongoing gene flow between populations can confound patterns of phenotypic variation due to local selection pressures (Stone *et al.*, 2011). To account for this, sampling phenotypes across a wide distribution (Endler, 1986) and controlling for phenotypic variation due to shared ancestry and migration between populations is necessary (Stone *et al.*, 2011). To account for phenotypic similarity between populations due to ancestry and ongoing gene flow, we fit a generalized linear mixed model (glmm) using Markov chain Monte Carlo techniques in the mcmcglmm package in R (Hadfield, 2010) as described by Stone *et al.* (2011). Using 12 populations for which we had both genetic and phenotypic data, we structured glmms to search for a statistically significant relationship between thermal tolerance and extremes of local temperature. We used standardized genomewide genetic distances between

populations, estimated with the fixation index (F_{st}), as a random variable in these analyses.

Testing the heritability of cold tolerance

Whereas phenotypic correlations with environmental variation can imply local adaptation, this pattern may also be the result of nonevolutionary phenotypic response to different post-hatching environments (phenotypic plasticity). Therefore, we performed a common garden experiment to test for population differences in cold tolerance in animals raised under identical environmental conditions. Gravid females from three populations were brought into a common laboratory setting. Females were kept under a 12-h light : dark cycle at ~30 °C and fed three times/week. Eggs laid in the laboratory were incubated until hatching, and the offspring were raised under identical laboratory conditions for 7 months. CT_{min} trials were performed as described in the previous section. We performed a linear regression using CT_{min} as the dependent variable, population of origin and generation (parent or offspring) as independent categorical variables, and mean rate of cooling per group as a covariate.

Testing for genetic isolation by environment

The genetic consequences of range expansion into different environments can have significant effects on the resulting patterns of genetic interchange across geographic space. The restriction of genetic exchanges between climatically differentiated populations can produce an association between genetic distance and environmental dissimilarity, a pattern known as isolation by environment (IBE). IBE may be a step along the way to divergence into distinct species (Nosil, 2012), although this may not always be the outcome. Under IBE, climate acts as a barrier to gene flow, leading to associations between genetic distance and environmental dissimilarity across a species' range (Nosil, 2004, Nosil *et al.*, 2005; Freedman *et al.*, 2010, Wang, 2013; Wang *et al.*, 2013).

To estimate the effects of IBE and geographic distance, we performed a multiple matrix regression with randomization analysis (MMRR, Wang, 2013). A pairwise genetic distance matrix was calculated using genome averaged F_{st} values estimated in Stacks (Catchen *et al.*, 2011, 2013). A geographic distance matrix was calculated using geographic coordinates in ArcMap. Linear geographic distances were calculated between each pair of coordinates. When the linear distances traversed a major body of water, the shortest overland distance was calculated using least cost path analyses.

To create environmental distance matrices, we focused on three environmental variables that together describe variation in low temperature extremes and variability. We used BIO6 (Minimum temperature of the coldest month of the year) as a measure of winter severity at each site. BIO2 (mean diurnal range of temperatures) and BIO4 (temperature seasonality) were used as metrics of daily and annual fluctuations in temperature, respectively. We ran principal components analysis on these three variables and calculated environmental distance between populations as the Euclidean distance in the first principal component, using the prcomp function in R. To test for isolation by distance and IBE, we ran a MMRR analysis using genetic distance as a dependent variable. Geographic distance and environmental distance were used as predictor variables.

Allelic associations with environmental variation

To search for regions of the genome under the influence of natural selection, we identified changes in allele frequencies associated with spatial variation in climate (Haldane, 1948; Slatkin, 1973; Nagylaki, 1975; Lenormand, 2002; Rank & Dahlhoff, 2002; Coop *et al.*, 2010). Allelic associations with environmental variation have been used to identify putatively adaptive loci in several other species (Dobzhansky, 1948; Berry & Kreitman, 1993; Hoekstra *et al.*, 2004; Storz *et al.*, 2007; Schmidt *et al.*, 2008; McCracken *et al.*, 2009). We searched for regions of the *A. carolinensis* genome associated with variation in temperature and precipitation extremes by calculating Bayes factors (BF) using bayenv2 (Gunther & Coop, 2013).

The programme was run in two steps. First, we estimated a population covariance matrix over 200 000 MCMC iterations to control for the effects of population structure using all genomic variants. Next, we detected environmental correlations between each allele and estimates of the upper and lower extremes of temperature (BIO10 and BIO11) and precipitation (BIO16 and BIO17). Because BF estimates can be sensitive to outliers, we also calculated nonparametric Spearman's rank correlation coefficients (ρ). Previous work has shown that the results of this analysis can be variable between runs (Blair *et al.*, 2014). Therefore, we ran the programme independently five times for 500 000 MCMC iterations and averaged estimates across all runs before identifying candidates. Candidate variants were identified as those lying within the top 99% of both BF and ρ distributions. The VariantAnnotation package in BioConductor was used to identify genes containing candidate SNPs. The bioMart package was used to identify names and ontology descriptions for all candidate genes.

Results

Climatic differentiation between mainland and Cuban green anole lineages

We collected climate data for 56 localities for *A. allisoni*, 146 for *A. carolinensis*, 56 for western *A. porcatus* and 72

for eastern *A. porcatus*. Principal components analysis reveals divergent patterns of climate availability across the *A. carolinensis* range when compared to its Cuban relatives (Fig. 1a). The first two principal components explain 73.9% of variation within the data set. Principal components 1 and 2 represented the majority of variation in temperature and precipitation profiles, respectively, at each site. The top five variables contributing to the first principal component (53.2% of variation) all represent aspects of environmental temperature, based on variable loadings (BIO1 = 0.31, BIO4 = −0.30, BIO6 = 0.31, BIO7 = −0.30, and BIO11 = 0.31). *Anolis carolinensis* localities show high variation across this axis, whereas *A. porcatus* and *A. allisoni* localities cluster in principal component space indicative of warmer temperature with low annual thermal variability.

In contrast, the second principal component (20.7% of variation) loads most heavily on precipitation related variables. Four of the top five contributing variables to this component are associated with aspects of environmental precipitation (BIO5 = 0.30, BIO12 = 0.45, BIO13 = 0.43, BIO16 = 0.47 and BIO18 = 0.42). *Anolis carolinensis* localities show less differentiation along this axis when compared to Cuban lineages, clustering in principal component space characterized by relatively dry climate with low precipitation seasonality (Fig. 1a).

Environmental niche expansion associated with mainland colonization

The mainland species of green anole has a greater environmental breadth compared to Cuban populations, as indicated by inverse concentration scores (*A. carolinensis* = 0.41, western *A. porcatus* = 0.06, *A. allisoni* = 0.07, eastern *A. porcatus* = 0.04) (Fig. 1b). Projection of the western *A. porcatus* niche onto the *A. carolinensis* range reveals increasingly negative similarity values with increasing latitude (Fig. 1c). Higher latitudes within the *A. carolinensis* range contain environmental variation outside that currently experienced in the species' ancestral range on Cuba. A map of all niche models can be found in Fig. S2.

Phenotypic signatures of climate-mediated local adaptation

Multiple linear modelling reveals no increase in CT_{max} in relation to mean temperature of the warmest quarter of the year in either the linear regression (adjusted $R^2 = 0.02$, $P = 0.38$, $\beta_{Bio10} = -0.024$, $P = 0.58$) or MCMCglmm (pMCMC = 0.53) (Fig. 2b). Conversely, CT_{min} shows a significant association with the mean temperature of the coldest quarter of the year in linear regression (adjusted $R^2 = 0.16$, $P = 0.02$, $\beta_{Bio11} = 0.14$, $P = 0.03$) (Fig. 2a). This association remains significant after accounting for genetic relatedness among populations in MCMCglmm (pMCMC = 0.02). Therefore, geographic variation in cold tolerance seems to be driven by differences in local environment across the species' mainland range.

Evidence for heritability of putatively adaptive phenotypes

Eggs laid and hatched in the common garden produced sixteen total offspring (Lousiana: $N = 4$, North Carolina: $N = 4$, Tennessee: $N = 8$). Generation (wild-caught parents vs. common garden offspring) has no significant influence on variation in CT_{min} ($F_{1,36} = 0.12$, $P = 0.73$),

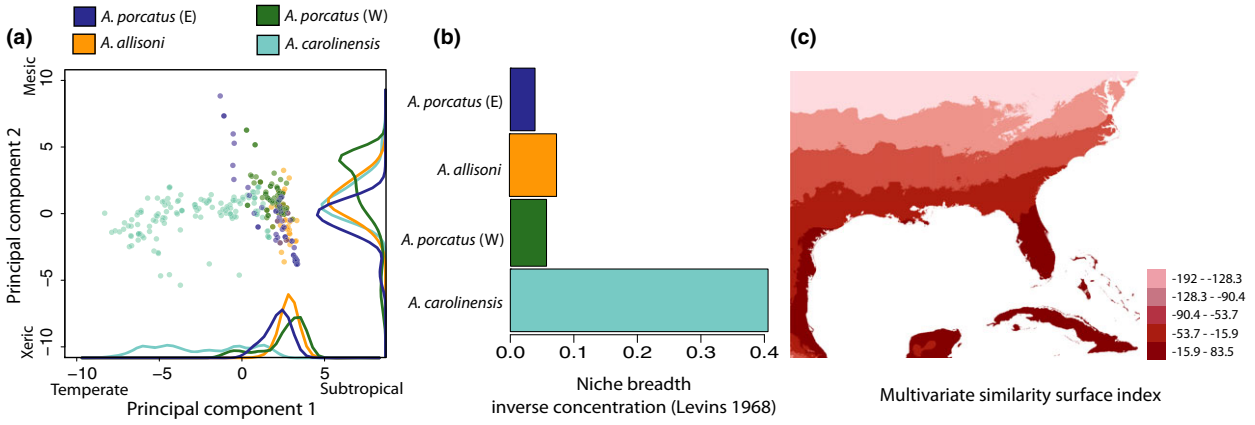

Fig. 1 (a) Principal components analysis of climatic variables for known sites of occurrence of *Anolis carolinensis* (teal), *A. allisoni* (yellow), and the eastern (purple) and western (green) clades of *A. porcatus*. (b) Comparison on niche breadth, as measured by inverse concentration (Levins, 1968), between the four clades of the Cuban green anole lineage. (c) Multivariate similarity surface (MESS) map depicting the degree of similarity of the climate of the western *A. porcatus* clade and the available climate within the *A. carolinensis* range. Lighter colours indicate more dissimilar environments, whereas darker colours indicate similar environments.

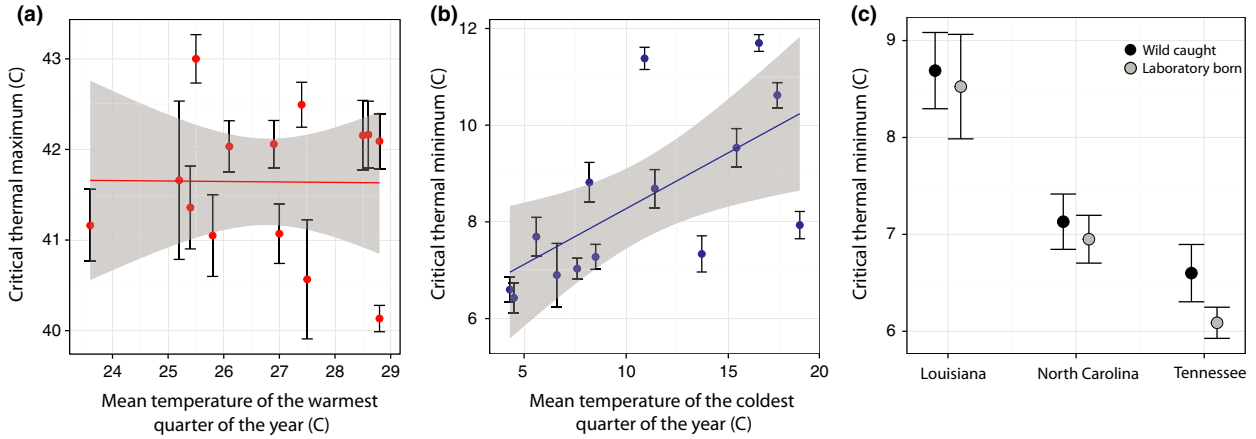

Fig. 2 Geographic association of (a) critical thermal maximum (CT_{max}) with BIO10 (mean temperature of the warmest quarter of the year and (b) critical thermal minimum (CT_{min}) with BIO11 (mean temperature of the coldest quarter of the year) for populations on *Anolis carolinensis* across its native range. (c) Comparisons of cold tolerance between wild-caught (black) and laboratory born and raised (grey) lizards from three populations across the *A. carolinensis* range. All points represent group means, and error bars indicate ± standard error.

suggesting that lizards born in the laboratory retain population-specific signatures of cold tolerance. Population of origin has a significant influence on variation in cold tolerance ($F_{2,36} = 0.55$, $P < 0.001$) (Fig. 2c). This result suggests that genetic differences between populations have a significant effect on the observed variation in cold tolerance.

Evidence for genetic isolation by environment

Filtering for genomic variants resulted in a total of 53 486 single nucleotide variants available to estimate genomewide genetic distance between populations. The linear model including geographic and environmental distance between populations ($R^2 = 0.116$, $F = 8.69$, $P = 0.034$) significantly explains the observed patterns of genetic distance within *A. carolinensis* (Fig. 3c). Within this model, the effect of genetic isolation by distance is not significant ($\beta_{\text{geographic distance}} = 0.07$, $t = 0.84$, $P = 0.53$). Conversely, differences in environment between collection localities contribute significantly to the model ($\beta_{\text{environment}} = 0.32$, $t = 3.86$, $P = 0.033$). Associations between environmental variables and latitude as well as loadings of each variable on PC1 used in the environmental dissimilarity analysis can be found in Fig. 3a, b, respectively.

Allelic associations with environmental variation

We recovered 20 282 SNPs representing 28 individuals from six populations across the *A. carolinensis* range for the identification of selection candidates. Bayesian analyses of allele–environment associations resulted in 81 total candidate SNPs associated with the mean temperature of the warmest quarter of the year, BIO10 (41 intergenic, two coding, 37 intronic, one 3′ UTR; Table S1). Forty-four of these candidate polymorphisms are unique to this environmental variable. Seventy-two candidates SNPs (48 intergenic and 24 intragenic; 58 unique) were identified for the mean temperature of the coldest quarter of the year (BIO 11); 62 SNPs (40 intergenic, 21 intronic, one promoter; 41 unique) for mean precipitation of the wettest quarter (BIO 16); and 25 SNPs (nine intergenic, 16 intronic; 16 unique) for mean precipitation of the driest quarter (BIO17) (see Table S1). Temperature-related variables (BIO 10 and BIO 11) shared 12 candidate SNPs, whereas precipitation-related variables shared two. A diagram of SNP overlap between environmental variables and chromosomal positions of outliers can be found in Fig. 4a and b, respectively. Bayes factor distributions can be found in Fig. S2.

Discussion

The major goal of this study was to understand the effect of novel environmental conditions on the niche, phenotypic and molecular evolution of an ancestrally subtropical terrestrial ectotherm. We aimed to (i) determine along which niche axes mainland populations of this species have diverged from their island relatives; (ii) quantify the effects of environment on gene flow between populations; (iii) determine whether adaptation to a novel ecological realm has a genetic basis; and (iv) gain preliminary insight through RADseq of the genetic targets of natural selection in a novel habitat.

Overall, we found evidence of a significantly expanded niche in the colonizing populations of the Cuban green anole, determined that there is likely a genetic component to differences in thermal

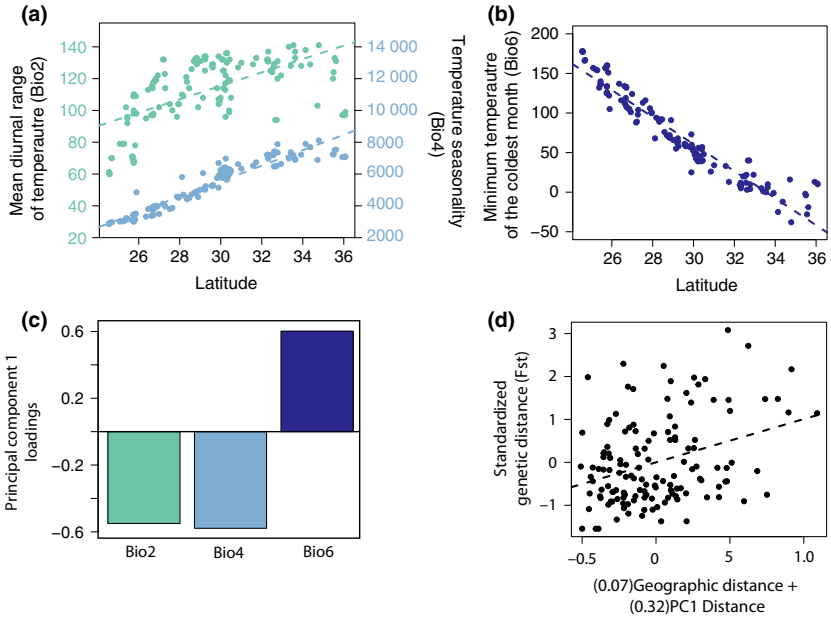

Fig. 3 (a) Geographic association of BIO2 (mean diurnal range of temperature) (teal) and BIO4 (temperature seasonality) with latitude for known sites of occurrence across the *Anolis carolinensis* range (b) Geographic association of BIO6 (minimum temperature of the coldest month) (dark blue) with latitude for known sites of occurrence across the *A. carolinensis* range. (c) magnitude and direction of loadings of BIO2, BIO4 and BIO6 on principal component 1, which was used to estimate environmental distance between populations for multiple matrix regression analysis (d) Association between genetic distance (genomewide average F_{st}) and a multiple matrix regression model including geographic distance and environmental distance (Euclidean PC1 distance between populations) across the *A. carolinensis* range.

performance found between populations in different temperature regimes and revealed several loci that may have played a role in the physiological adaptation of *A. carolinensis* to its novel mainland niche. Our results suggest that the mainland lineage has significantly expanded its niche and that detectable genetic differentiation associated with this expanded niche may have facilitated or accompanied colonization. These data support recent findings in other anole species that the physiological performance and thermal niche of reptile species can be labile (Munoz *et al.*, 2014) and evolve rapidly in the face of novel thermal regimes (Kolbe *et al.*, 2012; Leal & Gunderson, 2012). Taken together, this body of work complicates the classical view that reptilian thermal physiology is highly conserved (Bogert, 1949; Hertz *et al.*, 1983; Vandamme *et al.*, 1990), and constrained evolutionarily, highlighting the importance of continued study to gain a clearer understanding of how reptilian species respond to novel environments.

Novel environments and climatic niche shift

We have identified increased daily and seasonal thermal variability and decreased winter temperatures associated with the migration of this subtropical island clade into higher latitude environments in mainland North America. These differences provide the potential for climate-mediated selection post-colonization. Niche modelling results support this finding, revealing niche expansion (increased niche breadth) of *A. carolinensis* beyond that of its Cuban relatives.

Evolution of cold tolerance on the mainland

We might expect that the cold temperatures at high latitudes would restrict the northward expansion of the Cuban green anole on the mainland. Therefore, adaptive evolutionary response to cold-mediated selection may have been necessary for *A. carolinensis* to obtain its current distribution. Analyses of geographic variation in thermal tolerance support this hypothesis. Animals occurring in colder winter environments at higher latitudes are able to maintain function at lower temperatures than their southern counterparts, supporting the findings of Wilson & Echternacht (1987, 1990) on a wider geographic scale; these results are robust even after controlling for shared phylogenetic history and ongoing migration between populations. Additionally, lizards from climatically differentiated populations maintain population-specific signatures of cold tolerance, despite being raised in identical thermal environments, suggesting a genetic basis of the trait. However, trans-generational plasticity (i.e. maternal effects) cannot be ruled out as a potential factor influencing the observed patterns of variation in thermal performance

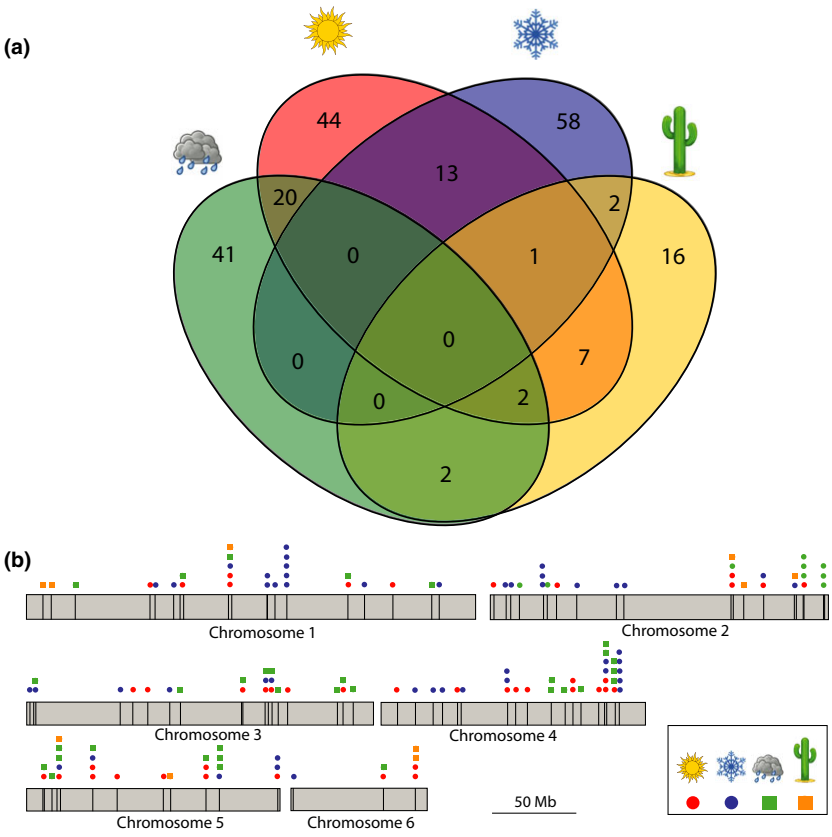

Fig. 4 (a) Patterns of overlap between outlier loci associated with the upper and low boundaries of precipitation (BIO 16 and BIO17, respectively) and temperature (BIO10 and BIO11, respectively). (b) Chromosomal position of identified outliers on the six annotated macrochromosomes of the *Anolis carolinensis* genome. Only outliers associated with a single environmental variable (BIO 10: red circle, BIO11: blue circle, BIO16: green square, BIO17: yellow square) are shown.

(Salinas & Munch, 2012). Quantitative genetic analyses of thermal tolerance would provide further insights into the heritability and genetic architecture of thermal tolerance in terrestrial ectotherms, an area significantly understudied to date.

Consequences of adaptation to different environments on genetic differentiation

Under the hypothesis of climate-mediated selection, we expect the effects of the putative selective pressure to be apparent at the genomic level. First, differences in local environment may act as a barrier to dispersal, resulting in climate-associated genetic isolation. Our landscape genetics analyses reveal support for climatic influence on the genetic structure. Geographic variation in low temperature extremes and variability was a significant predictor of gene flow across the *A. carolinensis* range after accounting for geographic distance between populations. Surprisingly, isolation by distance is not a significant contributor to genetic differentiation after accounting for environmental dissimilarity, despite the large geographic range of the species.

These findings are consistent with IBE (reviewed in Shafer & Wolf, 2013), in which local adaptation reduces overall gene flow between populations via selective mating in favour of locally adaptive phenotypes (Weissing *et al.*, 2011), matching habitat choice (Edelaar *et al.*, 2012) or selection against migrants from climatically differentiated source populations (Hendry, 2004; Thibert-Plante & Hendry, 2009). The relationship between ecological divergence and reproductive isolation across disparate taxa has been well established (Funk *et al.*, 2006), although IBE need not always give rise to a speciation event (Nosil, 2012).

Spatial structuring of populations across an environmental gradient is expected when the environmental change is steep and migration distances are short (Doebeli & Dieckmann, 2003). The temperature-mediated population structuring of the green anole likely reflects its limited dispersal ability (as a small, territorial species) and the degree of environmental change in winter conditions across geographic space. Although winter temperatures change gradually with latitude within the species' range, the physiological constraints imposed by temperate winter environments appear to be sufficiently intense to effect migration of this ancestrally subtropical lizard.

Studies of anole species suggest that environment may play an important role in genetic (Wang *et al.*, 2013) and morphological (Malhotra & Thorpe, 2000; Thorpe & Stenson, 2003; Glor *et al.*, 2003, Leal & Fleishman, 2004; Gunderson *et al.*, 2011; Ng *et al.*, 2013) differences between populations on the islands of

the Caribbean as well. This is one of the first studies, to our knowledge, to look at these phenomena in anoline lizards at a genomewide scale. Correlations between environmental and geographic distances may impose some limitations on our ability to precisely disentangle the contributions IBE and isolation by distance with multiple regression approaches (Manly, 1986; Smouse *et al.*, 1986; Legendre *et al.*, 1994). Additional studies are necessary to fully understand the effects of this covariation on a finer geographic scale.

Potential genetic targets of climate-mediated selection

Natural selection should also act on regions of the genome responsible for producing phenotypic adaptation to local climate. As a result, the frequency of an adaptive allele should display a higher than expected association with its putative selective pressure across a climatically heterogeneous range. Using RADseq data, we have identified genes within the *A. carolinensis* genome that show support as candidates for climate adaptation, based on the strength of their association with climatic variation across the range of the species.

Candidate genes associated with local winter temperatures show a diversity of functions. These candidates include 19 genes of known function and six uncharacterized proteins. Previously known genes containing candidate variants within this data set are associated with several biological processes that may have been important for colonization of temperate environments at higher latitudes. Several of these genes have been linked to aspects of neuromuscular development. AGRN is involved in the formation of neuromuscular junctions during embryogenesis (Rupp *et al.*, 1991), and TCF4 has been associated with involuntary neuromuscular function. Mutations in the latter gene have been linked to developmental and respiratory dysfunction (Zweier *et al.*, 2007). Two genes related to DNA repair also show correlations with geographic variation in cold temperature within *A. carolinensis*. MMS21 has been linked to recovery from DNA damage (Payne *et al.*, 2014), and KDM5B is a regulator of genomic stability (Li *et al.*, 2014).

Several candidate genes are also involved in aspects of physiology and metabolic efficiency. XIRP2 has been shown to play a role in cardiac remodelling (McCalmon *et al.*, 2010), whereas TIMAP is involved in the regulation of barrier function in pulmonary endothelial cells (Poirier *et al.*, 2011) and promotes angiogenesis (Obeidat *et al.*, 2014). ROCK2 is associated with variation in blood pressure and cardiovascular physiology (Riento & Ridley, 2003; Noma *et al.*, 2006; Seasholtz *et al.*, 2006; Rankinen *et al.*, 2008), and MOGAT2 plays an important role in metabolic efficiency and regulation of energy metabolism (Gao *et al.*, 2013). Lastly, USP13 plays a role in dermal UV signalling pathways (Chen *et al.*, 2014) and is a regulator of the interferon pathway, which mounts host response to pathogen infection (Yeh *et al.*, 2013). A full list of candidate SNPs and associated data from baynev2 and annotation analyses can be found in the Table S1. Edwards *et al.* (2015) present a preliminary analysis of RADseq SNPs associated with BIO 11 (mean temperature of coldest quarter).

These results suggest genomic adaptation to climate across the green anole range involves genetic variants spanning multiple biological pathways and may reflect selection along several different biotic and abiotic axes (i.e. temperature-induced constraints on developmental and physiological processes and resistance to novel pathogens). These data provide a foundation for further genomic studies utilizing higher density genomic sampling. Although we focus our description of outlier loci to SNPs within the boundaries of known genes, further studies utilizing whole genome resequencing, exome sequencing or transcriptome variation may provide a clearer picture of genomic targets of natural section in continental populations (Edwards *et al.*, 2015).

Conclusion

In this study, we investigate the long-term evolutionary outcome of migration to mainland Florida and subsequent range expansion of a subtropical lizard species. We find little evidence for niche conservatism between mainland and island species of green anoles. The large population expansion after mainland colonization of the green anole (Campbell-Staton *et al.*, 2012; Tollis *et al.*, 2012) suggests that rapid ecological divergence and adaptation may have driven niche expansion of the continental lineage. This expansion has allowed mainland populations to occupy significantly colder and thermally variable environments than their Cuban sister species. Local adaptation to thermal environments has accompanied range expansion along with reduced gene flow between climatically differentiated populations. As a result, *A. carolinensis* now occupies higher latitudes than any other species of its genus.

Acknowledgments

Rachel Goodman provided tissue samples that were integral in the completion of this project. Erin O'Kelly and Kadeem Gilbert provided assistance in collecting thermal tolerance data. Thomas Sanger provided help in collection of lizards in southern Florida and incubation and care of hatchling lizards for the common garden experiment presented here. Allison Schultz, Tim Sackton, Phil Grayson, Simon Sin, Mary Stoddard, Christie Riehl, Maude Baldwin, Adam Freedman, Robert Reynolds, Ambika Kamath and Talia Moore provided helpful comments in the development of ideas and analytical approaches of this project. The Harvard Museum of Comparative Zoology Putnam Expedition

Grant, Miyata Award and Robert A. Chapman Memorial Scholarship funded field expeditions for this research. The National Science Foundation Doctoral Dissertation Improvement Grant (DDIG) funded our genome sequencing.

References

Alfoldi, J., Di Palma, F., Grabherr, M., Williams, C., Kong, L.S., Mauceli, E. *et al.* 2011. The genome of the green anole lizard and a comparative analysis with birds and mammals. *Nature* **477**: 587–591.

Berry, A. & Kreitman, M. 1993. Molecular analysis of an allozyme cline alcohol dehydrogenase in *Drosophila melanogaster* on the east coast of North America. *Genetics* **134**: 869–893.

Bishop, D.C. & Echternacht, A.C. 2004. Emergence behavior and movements of winter-aggregated green anoles (*Anolis carolinensis*) and the thermal characteristics of their crevices in Tennessee. *Herpetologica* **60**: 168–177.

Blair, L.M., Granka, J.M. & Feldman, M.W. 2014. On the stability of the Bayenv method in assessing human SNP-environment associations. *Hum. Genomics* **8**. doi: 10.1186/1479-7364-8-1.

Bogert, C.M. 1949. Thermoregulation in reptiles, a factor in evolution. *Evolution* **3**: 195–211.

Bohning-Gaese, K., Schuda, M.D. & Helbig, A.J. 2003. Weak phylogenetic effects on ecological niches of Sylvia warblers. *J. Evol. Biol.* **16**: 956–965.

Bolger, A.M., Lohse, M. & Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114–2120.

Campbell-Staton, S.C., Goodman, R.M., Backstrom, N., Edwards, S.V., Losos, J.B. & Kolbe, J.J. 2012. Out of Florida: mtDNA reveals patterns of migration and Pleistocene range expansion of the Green Anole lizard (*Anolis carolinensis*). *Ecol. Evol.* **2**: 2274–2284.

Catchen, J.M., Amores, A., Hohenlohe, P., Cresko, W. & Postlethwait, J.H. 2011. Stacks: building and genotyping loci de novo from short-read sequences. *G3 (Bethesda)* **1**: 171–182.

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A. & Cresko, W.A. 2013. Stacks: an analysis tool set for population genomics. *Mol. Ecol.* **22**: 3124–3140.

Chen, H.X., Weng, Q.Y. & Fisher, D.E. 2014. UV signaling pathways within the skin. *J. Investig. Dermatol.* **134**: 2080–2085.

Coop, G., Witonsky, D., Di Rienzo, A. & Pritchard, J.K. 2010. Using environmental correlations to identify loci underlying local adaptation. *Genetics* **185**: 1411–1423.

Cowles, R.B. & Bogert, C.M. 1944. A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* **83**: 263–296.

Dobzhansky, T. 1948. Genetics of natural populations. 16. Altitudinal and seasonal changes produced by natural selection in certain populations of *Drosophila pseudoobscura* and *Drosophila persimilis*. *Genetics* **33**: 158–176.

Doebeli, M. & Dieckmann, U. 2003. Speciation along environmental gradients. *Nature* **421**: 259–264.

Eales, J., Thorpe, R.S. & Malhotra, A. 2010. Colonization history and genetic diversity: adaptive potential in early stage invasions. *Mol. Ecol.* **19**: 2858–2869.

Edelaar, P., Alonso, D., Lagerveld, S., Senar, J.C. & Bjorklund, M. 2012. Population differentiation and restricted gene flow in Spanish crossbills: not isolation-by-distance but isolation-by-ecology. *J. Evol. Biol.* **25**: 417–430.

Edwards, S.V., Schultz, A.J. & Campbell-Staton, S.C. 2015. Next-generation sequencing and the expanding domain of phylogeography. *Folia Zool.* **64**: 187–206.

Endler, J.A. 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.

ESRI 2011. ArcGIS Desktop: Version 10.1. Environmental Systems Research Institute, Redlands, CA.

Flores-Moreno, H., Garcia-Trevino, E.S., Letten, A.D. & Moles, A.T. 2015. In the beginning: phenotypic change in three invasive species through their first two centuries since introduction. *Biol. Invasions* **17**: 1215–1225.

Freedman, A.H., Thomassen, H.A., Buermann, W. & Smith, T.B. 2010. Genomic signals of diversification along ecological gradients in a tropical lizard. *Mol. Ecol.* **19**: 3773–3788.

Funk, D.J., Nosil, P. & Etges, W.J. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proc. Natl. Acad. Sci. U.S.A.* **103**: 3209–3213.

Gao, Y., Nelson, D.W., Banh, T., Yen, M.I. & Yen, C.L.E. 2013. Intestine-specific expression of MOGAT2 partially restores metabolic efficiency in Mogat2-deficient mice. *J. Lipid Res.* **54**: 1644–1652.

Glor, R.E., Kolbe, J.J., Powell, R., Larson, A. & Losos, J.B. 2003. Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk ground anoles (*Anolis cybotes* group). *Evolution* **57**: 2383–2397.

Glor, R.E., Gifford, M.E., Larson, A., Losos, J.B., Schettino, L.R., Lara, A.R.C. *et al.* 2004. Partial island submergence and speciation in an adaptive radiation: a multilocus analysis of the Cuban green anoles. *Proc. R. Soc. B Biol. Sci.* **271**: 2257–2265.

Glor, R.E., Losos, J.B. & Larson, A. 2005. Out of Cuba: overwater dispersal and speciation among lizards in the *Anolis carolinensis* subgroup. *Mol. Ecol.* **14**: 2419–2432.

Graham, C.H., Ron, S.R., Santos, J.C., Schneider, C.J. & Moritz, C. 2004. Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution* **58**: 1781–1793.

Gunderson, A.R., Siegel, J. & Leal, M. 2011. Tests of the contribution of acclimation to geographic variation in water loss rates of the West Indian lizard Anolis cristatellus. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **181**: 965–972.

Gunther, T. & Coop, G. 2013. Robust identification of local adaptation from allele frequencies. *Genetics* **195**: 205–220.

Hadfield, J.D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* **33**: 1–22.

Haldane, J.B.S. 1948. The theory of a cline. *J. Genet.* **48**: 277–284.

Hendry, A.P. 2004. Selection against migrants contributes to the rapid evolution of ecologically dependent reproductive isolation. *Evol. Ecol. Res.* **6**: 1219–1236.

Hendry, A.P., Wenburg, J.K., Bentzen, P., Volk, E.C. & Quinn, T.P. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science* **290**: 516–518.

Hertz, P.E., Huey, R.B. & Nevo, E. 1983. Homage to Santa Anita – thermal sensitivity of sprint speed in agamid lizards. *Evolution* **37**: 1075–1084.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**: 1965–1978.

Hoekstra, H.E., Drumm, K.E. & Nachman, M.W. 2004. Ecological genetics of adaptive color polymorphism in pocket mice: geographic variation in selected and neutral genes. *Evolution* **58**: 1329–1341.

Hohenlohe, P.A., Bassham, S., Etter, P.D., Stiffler, N., Johnson, E.A. & Cresko, W.A. 2010. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *PLoS Genet.* **6**: 23.

Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D. & Serra, L. 2000. Rapid evolution of a geographic cline in size in an introduced fly. *Science* **287**: 308–309.

Keller, I. & Seehausen, O. 2012. Thermal adaptation and ecological speciation. *Mol. Ecol.* **21**: 782–799.

Kolbe, J.J., VanMiddlesworth, P.S., Losin, N., Dappen, N. & Losos, J.B. 2012. Climatic niche shift predicts thermal trait response in one but not both introductions of the Puerto Rican lizard *Anolis cristatellus* to Miami, Florida, USA. *Ecol. Evol.* **2**: 1503–1516.

Langmead, B. & Salzberg, S.L. 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**: 357–359.

Leal, M. & Fleishman, L.J. 2004. Differences in visual signal design and detectability between allopatric populations of Anolis lizards. *Am. Nat.* **163**: 26–39.

Leal, M. & Gunderson, A.R. 2012. Rapid change in the thermal tolerance of a tropical lizard. *Am. Nat.* **180**: 815–822.

Lee, C.E. 2002. Evolutionary genetics of invasive species. *Trends Ecol. Evol.* **17**: 386–391.

Legendre, P., Lapointe, F.J. & Casgrain, P. 1994. Modeling brain evolution from behavior: a permutational regression approach. *Evolution* **48**: 1487–1499.

Lenormand, T. 2002. Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **17**: 183–189.

Levins, R. 1968. *Evolution in Changing Environments*. Princeton University Press, Princeton, New Jersey.

Li, X., Liu, L., Yang, S.D., Song, N., Zhou, X., Gao, J. *et al.* 2014. Histone demethylase KDM5B is a key regulator of genome stability. *Proc. Natl. Acad. Sci. U.S.A.* **111**: 7096–7101.

Losos, J.B., Leal, M., Glor, R.E., de Queiroz, K., Hertz, P.E., Schettino, L.R. *et al.* 2003. Niche lability in the evolution of a Caribbean lizard community. *Nature* **424**: 542–545.

Malhotra, A. & Thorpe, R.S. 2000. The dynamics of natural selection and vicariance in the Dominican anole: patterns of within-island molecular and morphological divergence. *Evolution* **54**: 245–258.

Manly, B.F.J. 1986. Randomization and regression methods for testing for associations with geographical, environmental and biological distances between populations. *Res. Pop. Ecol.* **28**: 201–218.

McCalmon, S.A., Desjardins, D.M., Ahmad, S., Davidoff, K.S., Snyder, C.M., Sato, K. *et al.* 2010. Modulation of angiotensin II-mediated cardiac remodeling by the MEF2A target gene Xirp2″. *Circ. Res.* **106**: 952–960.

McCracken, K.G., Bulgarella, M., Johnson, K.P., Kuhner, M.K., Trucco, J., Valqui, T.H. *et al.* 2009. Gene flow in the face of countervailing selection: adaptation to high-altitude hypoxia in the beta a hemoglobin subunit of yellow-billed pintails in the andes. *Mol. Biol. Evol.* **26**: 815–827.

Morgenstern, E.K. 1996. *Geographic Variation in Forest Trees. Genetic Basis and Application of Knowledge in Silviculture*. UBC Press, Vancouver.

Munoz, M.M., Stimola, M.A., Algar, A.C., Conover, A., Rodriguez, A.J., Landestoy, M.A. *et al.* 2014. Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proc. R. Soc. B Biol. Sci.* **281**: 595–615.

Nagylaki, T. 1975. Conditions for existence of clines. *Genetics* **80**: 595–615.

Ng, J., Landeen, E.L., Logsdon, R.M. & Glor, R.E. 2013. Correlation between Anolis lizard dewlap phenotype and environmental variation indicates adaptive divergence of a signal important to sexual selection and species recognition. *Evolution* **67**: 573–582.

Noma, K., Oyama, N. & Liao, J.K. 2006. Physiological role of ROCKs in the cardiovascular system. *Am. J. Physiol. Cell Physiol.* **290**: C661–C668.

Nosil, P. 2004. Reproductive isolation caused by visual predation on migrants between divergent environments. *Proc. Biol. Sci.* **271**: 1521–1528.

Nosil, P. 2012. *Ecological Speciation*. Oxford University Press, Oxford.

Nosil, P., Vines, T.H. & Funk, D.J. 2005. Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* **59**: 705–719.

Obeidat, M., Li, L.J. & Ballermann, B.J. 2014. TIMAP promotes angiogenesis by suppressing PTEN-mediated Akt inhibition in human glomerular endothelial cells. *Am. J. Physiol. Renal Physiol.* **307**: F623–F633.

Olson, M.S., Levsen, N., Soolanayakanahally, R.Y., Guy, R.D., Schroeder, W.R., Keller, S.R. *et al.* 2013. The adaptive potential of *Populus balsamifera* L. to phenology requirements in a warmer global climate. *Mol. Ecol.* **22**: 1214–1230.

Payne, F., Colnaghi, R., Rocha, N., Seth, A., Harris, J., Carpenter, G. *et al.* 2014. Hypomorphism in human NSMCE2 linked to primordial dwarfism and insulin resistance. *J. Clin. Invest.* **124**: 4028–4038.

Peterson, A.T., Soberon, J. & Sanchez-Cordero, V. 1999. Conservatism of ecological niches in evolutionary time. *Science* **285**: 1265–1267.

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S. & Hoekstra, H.E. 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One* **7**: e37135.

Phillips, S.J., Dudík, M. & Schapire, R.E. 2004. A maximum entropy approach to species distribution modeling. *Proceedings of the Twenty-First International Conference on Machine Learning* pp. 655–662. ACM Press, Banff, Canada.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. 2006. Maximum entropy modeling of species geographic distributions. *Ecol. Model.* **190**: 231–259.

Poirier, C., Gorshkov, B.A., Zemskova, M.A., Bogatcheva, N.V. & Verin, A.D. 2011. TIMAP protects endothelial barrier from LPS-induced vascular leakage and is down-regulated by LPS. *Respir. Physiol. Neurobiol.* **179**: 334–337.

Prinzing, A., Durka, W., Klotz, S. & Brandl, R. 2001. The niche of higher plants: evidence for phylogenetic conservatism. *Proc. R. Soc. B Biol. Sci.* **268**: 2383–2389.

R Core Team. 2014. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Rank, N.E. & Dahlhoff, E.P. 2002. Allele frequency shifts in response to climate change and physiological consequences of allozyme variation in a montane insect. *Evolution* **56**: 2278–2289.

Rankinen, T., Church, T., Rice, T., Markward, N., Blair, S.N. & Bouchard, C. 2008. A major haplotype block at the rho-associated kinase 2 locus is associated with a lower risk of hypertension in a recessive manner: the HYPGENE study. *Hypertens. Res.* **31**: 1651–1657.

Ricklefs, R.E. & Latham, R.E. 1992. Intercontinental correlation of geographical ranges suggests stasis in ecological traits of relict genera of temperate perennial herbs. *Am. Nat.* **139**: 1305–1321.

Riento, K. & Ridley, A.J. 2003. Rocks: multifunctional kinases in cell behaviour. *Nat. Rev. Mol. Cell Biol.* **4**: 446–456.

Rundle, H.D. & Nosil, P. 2005. Ecological speciation. *Ecol. Lett.* **8**: 336–352.

Rupp, F., Payan, D.G., Magillsolc, C., Cowan, D.M. & Scheller, R.H. 1991. Structure and expression of a rat agrin. *Neuron* **6**: 811–823.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A. *et al.* 2001. The population biology of invasive species. *Annu. Rev. Ecol. Syst.* **32**: 305–332.

Salinas, S. & Munch, S.B. 2012. Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. *Ecol. Lett.* **15**: 159–163.

Savolainen, O., Pyhajarvi, T. & Knurr, T. 2007. Gene flow and local adaptation in trees. *Annu. Rev. Ecol. Evol. Syst.* **38**: 595–619.

Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science* **323**: 737–741.

Schluter, D. & Conte, G.L. 2009. Genetics and ecological speciation. *Proc. Natl. Acad. Sci. U.S.A.* **106**: 9955–9962.

Schmidt, P.S., Zhu, C.T., Das, J., Batavia, M., Yang, L. & Eanes, W.F. 2008. An amino acid polymorphism in the couch potato gene forms the basis for climatic adaptation in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* **105**: 16207–16211.

Seasholtz, T.M., Wessel, J., Rao, F.W., Rana, B.K., Khandrika, S., Kennedy, B.P. *et al.* 2006. Rho kinase polymorphism influences blood pressure and systemic vascular resistance in human twins – role of heredity. *Hypertension* **47**: 937–947.

Shafer, A.B.A. & Wolf, J.B.W. 2013. Widespread evidence for incipient ecological speciation: a meta-analysis of isolation-by-ecology. *Ecol. Lett.* **16**: 940–950.

Slatkin, M. 1973. Gene flow and selection in a cline. *Genetics* **75**: 733–756.

Smouse, P.E., Long, J.C. & Sokal, R.R. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Syst. Zool.* **35**: 627–632.

Stone, G.N., Nee, S. & Felsenstein, J. 2011. Controlling for non-independence in comparative analysis of patterns across populations within species. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **366**: 1410–1424.

Storz, J.F., Sabatino, S.J., Hoffmann, F.G., Gering, E.J., Moriyama, H., Ferrand, N. *et al.* 2007. The molecular basis of high-altitude adaptation in deer mice. *PLoS Genet.* **3**: 448–459.

Sultan, S.E., Horgan-Kobelski, T., Nichols, L.M., Riggs, C.E. & Waples, R.K. 2013. A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. *Evol. Appl.* **6**: 266–278.

Thibert-Plante, X. & Hendry, A.P. 2009. Five questions on ecological speciation addressed with individual-based simulations. *J. Evol. Biol.* **22**: 109–123.

Thorpe, R.S. & Stenson, A.G. 2003. Phylogeny, paraphyly and ecological adaptation of the colour and pattern in the Anolis roquet complex on Martinique. *Mol. Ecol.* **12**: 117–132.

Tollis, M., Ausubel, G., Ghimire, D. & Boissinot, S. 2012. Multi-locus phylogeographic and population genetic analysis of *Anolis carolinensis*: historical demography of a genomic model species. *PLoS One* **7**: e38474.

Vandamme, R., Bauwens, D. & Verheyen, R.F. 1990. Evolutionary rigidity of thermal physiology – the case of the cool temperate lizard *Lacerta vivipara*. *Oikos* **57**: 61–67.

Wang, I.J. 2013. Examining the full effects of landscape heterogeneity on spatial genetic variation: a multiple matrix regression approach for quantifying geographic and ecological isolation. *Evolution* **67**: 3403–3411.

Wang, I.J., Glor, R.E. & Losos, J.B. 2013. Quantifying the roles of ecology and geography in spatial genetic divergence. *Ecol. Lett.* **16**: 175–182.

Warren, D.L., Glor, R.E. & Turelli, M. 2010. ENMTools: a toolbox for comparative studies of environmental niche models. *Ecography* **33**: 607–611.

Weissing, F.J., Edelaar, P. & van Doorn, G.S. 2011. Adaptive speciation theory: a conceptual review. *Behav. Ecol. Sociobiol.* **65**: 461–480.

Wiens, J.J. & Graham, C.H. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu. Rev. Ecol. Evol. Syst.* **36**: 519–539.

Williams, E.E. 1969. Ecology of colonization as seen in zoogeography of anoline lizards on small islands. *Q. Rev. Biol.* **44**: 345–389.

Wilson, M.A. & Echternacht, A.C. 1987. Geographic variation in the critical thermal minimum of the green anole, *Anolis carolinensis* (Sauria, Iguanidae), along a latitudinal gradient. *Comp. Biochem. Physiol. A Physiol.* **87**: 757–760.

Wilson, M.A. & Echternacht, A.C. 1990. A comparison of heat and cold tolerance of 2 morphs of *Anolis carolinensis* (Iguanidae) from southern Florida. *J. Herpetol.* **24**: 330–333.

Yeh, H.M., Yu, C.Y., Yang, H.C., Ko, S.H., Liao, C.L. & Lin, Y.L. 2013. Ubiquitin-specific protease 13 regulates IFN signaling by stabilizing STAT1. *J. Immunol.* **191**: 3328–3336.

Zweier, C., Peippo, M.M., Hoyer, J., Sousa, S., Bottani, A., Clayton-Smith, J. *et al.* 2007. Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). *Am. J. Hum. Genet.* **80**: 994–1001.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Map of climatic niche models of the (a) eastern clade of *Anolis porcatus* (b) *A. allisoni* (c) western clade of *A. porcatus* and (d) *A. carolinensis*. Warm colours represent high probability of occurrence, whereas cooler colours represent lower probabilities of occurrence. All species models were trained on their native range (Cuba for *A. porcatus* and *A. allisoni* clades, south-eastern United States for *A. carolinensis*) and projected onto a common range including both Cuba and the south-eastern United States.

Figure S2 Graphical display of genomic outlier variants associated with (a) BIO10 (mean temperature of the

warmest quarter of the year), (b) BIO11 (mean temperature of the coldest quarter of the year), (c) BIO16 (precipitation of the wettest quarter of the year) and (d) BIO17 (precipitation of the driest quarter of the year). Within each graph, nonsignificant variants are signified by open grey dots. Significant outliers (those in the top 1% of both Byes Factor and Spearman's rank correlation coefficient distributions) are indicated by filled dots (BIO10: red, BIO11: blue, BIO16: green, BIO17: yellow).

Table S1 Summary of outlier analysis with bayenv2. Column A is the abiotic climate factor used in each association analysis. Columns B and C give the chromosomal/linkage group of each outlier SNP and position on that chromosome/linkage group, respectively.

Received 19 February 2016; revised 7 June 2016; accepted 24 June 2016