Dimorphism and divergence in island and mainland Anoles

HUGO H. SILICEO-CANTERO¹, ANDRES GARCÍA², R. GRAHAM REYNOLDS³, GUALBERTO PACHECO⁴ and BRADFORD C. LISTER⁵*

¹Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico
²Estación de Biología Chamela, Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico
³Department of Organismic and Evolutionary Biology & Museum of Comparative Zoology, Harvard University, Cambridge, MA, 02138, USA
⁴Posgrado en Ciencias Biológicas, Instituto de Ecología, Universidad Nacional Autónoma de México, Mexico City, Mexico
⁵Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY, 12180, USA

Received 21 August 2015; revised 23 December 2015; accepted for publication 23 December 2015

Relative to the West Indies, the ecology and evolution of anoles inhabiting islands off Central and South America have received little attention. The paucity of studies on continental islands has limited our ability to generalize and extend results based on the West Indian paradigm, as well as our understanding of the profound differences between the adaptive radiations of continental vs. Greater Antillean anoles. Here we compare the morphological, ecological, behavioural and genetic divergence between Anolis nebulosus populations inhabiting a small island in the Bay of Chamela, Mexico, and a nearby mainland forest. Notably, the two populations exhibit intra-sexual dimorphism with respect to head and limb sizes, the first such polymorphism documented for an Anolis species. We also compare the shape of island and mainland A. nebulosus with each other, the six West Indian ecomorphs and a hypothetical generalist species. Finally, we address the generalist convergence hypothesis for anoles on single species islands. We conclude that convergence on a generalist morphology is widespread among solitary anoles in the West Indies. We present data on a limited sample of solitary anoles with mainland ancestors that suggest a parallel convergence on a similar generalist morphology, probably due to similar adaptive landscapes shaped by selective forces common to small island environments. © 2016 The Linnean Society of London, Biological Journal of the Linnean Society, 2016, 118, 852–872.


INTRODUCTION

Since Darwin landed in the Galapagos and Wallace explored the Malay Archipelago, islands have played a major role in elucidating the mechanisms of evolutionary change. In keeping with this tradition, Anolis lizards inhabiting islands in the West Indies have emerged as model organisms for studies of evolution at both the micro- and the macro-evolutionary scale. The diversity of the genus (nearly 400 species in the West Indies, Central and South America), combined with the sheer number of islands harbouring at least one resident anole, provides a rich array of natural experiments that biologists have mined to address a broad range of questions in ecology and evolution (Losos, 2009).

Relative to the West Indies, anoles inhabiting islands off the coasts of Central and South America have received little attention. Besides the research reported here, and related work by Senczuk et al.
expansion on single species islands did not entail diversifying selection for specialized phenotypes and a concomitant increase in adaptive variation, as predicted by the niche variation hypothesis (Van Valen, 1965; Bolnick et al., 2007). Rather, solitary species of West Indian anoles appeared to converge on a generalist phenotype capable of exploiting a variety of perch sites, thermally diverse habitats and a broad range of prey sizes. If widely replicable, this pattern would represent a reversal of evolutionary direction and an example of deterministic, microevolutionary convergence at the species level (Losos, Irschick & Schoener, 1994; Wollenberg et al., 2013).

METHODS

STUDY AREAS AND FOCAL SPECIES

Our mainland site was located within the Chamela-Cuixmala Biosphere Reserve (Ch-CBR) in the state of Jalisco on the west coast of Mexico (19°22′03″–19°35′11″N and 104°56′13″–105°03′25″W). The Ch-CBR encompasses 13 142 ha of tropical dry forest. The study area was approximately 1 km from the National Autonomous University of Mexico’s Estacion de Biologia Chamela.

Our second study site was the small island of San Agustín located 0.47 km from the coast and 5.5 km from the mainland study area. The island has a length of 280 m, a width of 230 m and an area of 3.3 ha. All the islands in the Bay of Chamela are land bridge islands and were separated from the mainland during a rapid rise in sea level that took place between 7000 and 8000 years BP (Ramírez-Herrera, Kostoglodovb & Urrutia-Fucugauchi, 2004). Compared with nearby islands, San Agustín’s vegetation is more open, less scrubby and consists of tropical dry forest which is much more similar in structure to the mainland forest than to that of other islands in the bay (see Fig. 6). The focal species of this research, Anolis nebulosus, is a small tree-dwelling anole, endemic to western Mexico and widely distributed from eastern Sonora to southern Guerrero. Mainland males average approximately 42 mm in snout–vent length (SVL), with a maximum SVL of 49 mm and a mean body weight of 1.6 g (3.0 g maximum). Females are smaller, with a mean SVL of 36 mm (maximum 45 mm), and mean body weight of 1.0 g (maximum 1.6 g; Supporting Information, Table S1).

All permissions for fieldwork and specimen collections associated with this study were carried out under a licence to A. Garcia from the National Autonomous University of Mexico.
MORPHOLOGY

From February to October 2011, and in January 2014, 183 A. nebulosus individuals were captured, 78 in the mainland study area and 105 on San Agustín. The sex of each individual was identified based on dewlap size. Body weight was measured using a Pesola 5-g scale, and several other body measurements were taken using Vernier calipers. We also recorded the perch height and perch diameter used by all individuals. To avoid resampling the same individuals, captured lizards were marked by toe clipping and released at the point of capture. While no studies to date have demonstrated reduced survival due to this technique (Losos, 2009), we minimized any potential effects on clinging ability by clipping just the tip of the toe, an area free of lamellae.

We measured ten morphological variables for 46 insular females, 66 insular males, 35 mainland females and 43 mainland males: SVL from the tip of the snout to the anterior end of the cloaca; tail length (TaL) from the anterior of the cloaca to the tip of the tail; snout length (SL) from the tip of the snout to the skull base; mandibular width (MW), the widest part of the jaw; upper arm length (UAL) from the point at which the forelimb enters the body to the apex of the elbow; forearm length (FAL) from the apex of the elbow to the centre of the wrist; hand length (HL) from the centre of the wrist to the tip of the hand; and lower limb length (LLL) as FeL + FaL, and upper arm length (UAL) and lower limb length (LLL) as FeL + TiL. ULL in anoles is often measured from the distal end of the longest toe to the point of insertion of the limb in the body wall. Here we use the correlation between our measure of ULL and ULL + HL averaged 0.98 for male morphs and 0.97 for female morphs. Our results are the same regardless of which measure is employed to represent upper limb length.

BEHAVIOURAL OBSERVATIONS, INSECT SAMPLES, POPULATION DENSITIES AND PREDATION EXPERIMENTS

Methods employed for behavioural observations and insect sampling were the same as those described by Lister & Garcia (1992). Density estimates and behavioural observations were conducted every 6 months from July 1990 to July 1991. On the island, population densities of anoles were estimated in 15-m by 15-m quadrats. Captured lizards were weighed to the nearest 0.1 mg with a Pesola spring balance, and marked on the dorsal surface with fast drying Testors acrylic model airplane paint. The SVL of captured lizards was also measured to the nearest 0.5 mm. Quadrats were censused twice a day until six consecutive censuses yielded no unmarked lizards. At this point, we considered the anoles within the quadrat to be close to completely marked out. On the mainland, we censused A. nebulosus along existing trails through the forest. We marked the locations of every lizard encountered on a map, and then estimated the area searched on either side of the trails to determine the approximate number of individuals per 225 m².

To estimate relative rates of predation, we tethered at a height of 2.0 m ten adult A. nebulosus to trees in the mainland and island forests over 12 days during the summer wet season and 10 days during the winter dry season. The tethers were 0.5-m lengths of black 6-pound test fishing line tied around the waist of the lizards directly above the rear legs and secured to the tree trunks with a small nail. Lizards were checked each day during the morning and afternoon, and any missing individuals were recorded. Every 3 days, remaining lizards were replaced with newly caught individuals. The data were analysed using the survival analysis routines in IBM SPSS 21.

VEGETATION STRUCTURE

Variation in local vegetation structure can confound comparisons of perch site utilization and microhabitat niche breadth between different habitats (Johnson et al., 2006). The greater the similarity in habitat structure, the greater the probability that differences in microhabitat use between populations are due to differences in selectivity and behaviour. To compare the vegetation of the island and mainland forests, we measured the heights and diameters of all woody plants within 1 m to the left and right of two 50-m transects through both habitats.

GENETIC DATA AND ANALYSES

We obtained 23 tail tips from individuals on San Agustín and 27 from the mainland study area, and preserved the samples in 95% EtOH. We extracted whole genomic DNA using the Promega Wizard SV DNA purification system according to the manufacturer’s protocol, and stored the extracts at −20 °C. We used PCRs to amplify a portion of the mitochondrial locus ND2 (NADH subunit II, conditions as specified by Revell et al., 2007), and purified and sequenced products in both directions on an automated sequencer (ABI 3730XL) at Massachusetts General Hospital DNA Core Facility, Cambridge, MA. We assembled contigs and manually verified
ambiguous base calls using GENEIOUS 7.1.2 (Biomatters). We then aligned sequences using the CLUSTALW 2.1 (Larkin et al., 2007) algorithm implemented in GENEIOUS with reference sequences and default parameters. We deposited representative sequences from all loci in GenBank (accession numbers KU757434-KU757453). We estimated genetic variation within populations as nucleotide (π) and haplotype (h) diversity, as well as ΦST between populations. We conducted the above analyses, as well as inferred haplotypes and connection distances between haplotypes, in ARLEQUIN 3.5.1.3 (Excoffier & Lischer, 2010). We determined the significance of ΦST values via the maximum number of permutations in ARLEQUIN, and visualized haplotype connections using HapStar (Teacher & Griffiths, 2010).

We additionally selected a subset of ten individuals (five mainland, five island) for RAD (restriction site associated DNA) sequencing followed by single nucleotide polymorphism (SNP) genotyping. We used the nextRAD (Nextera – tagmented reductively amplified DNA) genotyping-by-sequencing (GBS) approach as implemented by SNPaurus (Institute of Molecular Biology, Eugene, OR, USA). Briefly, nextRAD uses selective primers to amplify fragments across the genome, as opposed to using restriction enzymes followed by size selection as in similar RADseq approaches (Etter & Johnson, 2012). These fragments are initially generated using Illumina Nextera tagmentation, followed by selective PCR amplification and ligation of sequencing adapters and barcoded indices, as in Russello et al. (2015). We quantitated each of our genomic DNA (gDNA) samples using a Qubit 2.0 system prior to library preparation. Genomic DNA was fragmented using the Nextera reagent (Illumina), which also ligates short adapter sequences to the ends of each fragment. This reaction calls for 15 ng of gDNA per sample, although 22.5 ng of gDNA was included to compensate for degraded DNA in our samples. These fragments were then amplified using a selective primer complementary to the adapter sequences, which also includes a 9-bp selective sequence (GTGTAGAGC). Amplification was conducted using PCR at 73 °C for 26 cycles, during which only fragments of gDNA that were able to hybridize with the selective sequence were amplified.

Sequencing was performed on an Illumina Next-Seq 500 at the Institute of Molecular Biology, Eugene, OR, using a high-output run to generate ~400 million single-end sequencing reads 75 bp in length. Reads were quality-filtered resulting in a mean of 3656 509 reads per individual and SNPs were called using the SNPaurus nextRAD pipeline. This pipeline consists of custom scripts (SNPaurus, LLC) that create a de novo reference from abundant reads. All sequencing reads are then mapped to the reference with an alignment identity threshold of 93% (BBMap, http://sourceforge.net/projects/bbmap/). Genotypes were called using Samtools and bcftools using the following flags: (samtools mpileup –gu –Q 10 –t DP,DPR –f ref.fasta –b anolis.align_samples | bcftools call –cv – > anolis.vcf). We converted the resulting variant-call format (.vcf) genotype file to subsequent input files using PDGSViper (Lischer & Excoffier, 2012) after excluding loci with only missing data and non-polymorphic loci, as well as SNPs with Phred scores less than 10. This resulted in 34 543 polymorphic SNPs among 18 018 loci. We further trimmed this dataset to include only the first SNP from each locus in order to retain only presumably independent loci for clustering analyses (Pritchard, Stephen & Donnelly, 2000).

We assessed potential genetic structure between populations with the Bayesian clustering algorithm STRUCTURE (Pritchard et al., 2000), which uses a Markov chain Monte Carlo approach to cluster K groups based on individual genotype profiles. We used the admixture model and five replications of K = 1–4, with each replication having a run length of 100 000 generations and a burn-in of 20%. We selected values of K using the AK method of Evanno, Regnaut & Goudet (2005) in STRUCTURE HARVESTER (Earl & von Holdt, 2012). We also used a discriminant analysis of principal components (DAPC; Jombart, Devillard & Balloux, 2010) implemented in the R 3.1.2 (R Institute for Statistical Computing) adegenet package (Jombart, 2008) to identify genotypic clusters. This method attempts to maximize genetic differentiation between groups, and minimize variation within groups, by clustering individual genotypes with a principal components transformation of the genetic data prior to discriminant analysis. We used a Bayesian information criterion approach to obtain the predicted number of clusters between K = 1 and 20 after retaining all PCs. To perform the DAPC, we selected the optimal number of PCs using optim.a.score() in adegenet with 1000 replications, resulting in retention of the first two PCs and the first eigenvalue in the analysis. We calculated summary statistics using ARLEQUIN for each of the groupings delimited in the above analyses, and we estimated FST between groups in ARLEQUIN via the maximum number of permutations.

**Statistical analyses**

Prior to statistical analysis, measurements of all morphological variables were transformed by taking natural logarithms. To comply with the linearity assumptions of the statistical procedures employed, we used the cube root of individual body weight...
(CMASS) rather than raw body weight measurements (Butler & Losos, 2002). In the initial multivariate morphological space comparisons, we were interested in the actual size differences within and between the island and mainland populations, and used the log-transformed morphological data without size adjustment. For comparisons of shape divergence using multivariate analysis of variance (MANOVA), principal components analysis (PCA) and discriminant analysis (DA), we employed Mosimann’s (1970) geometric mean method to adjust the log-transformed data for size differences (Butler & Losos, 2002). Unless otherwise noted, statistical analyses were carried out in IBM SPSS 21.

RESULTS

To assess morphological divergence between the island and mainland populations, we first analysed differences in intra- and intersexual dimorphism, overall size and the multivariate morphological space occupied by the two populations. We then analysed divergence in shape and compared the morphology of male *A. nebulosus* with the six canonical West Indian ecomorphs and a hypothetical generalist phe- notype. Finally, we documented ecological, beha- vioural and genetic differences between island and mainland *A. nebulosus*.

INTRASEXUAL SIZE DIMORPHISM

Our exploratory analysis of the morphological data for male and female *A. nebulosus* included his- tograms of all the measured variables, as well as bivariate plots of all variables against SVL. These graphs revealed that both males and females from the mainland and island populations were dimorphic for snout length, mandibular width, upper arm length and forearm length. Each sex in each location has a large-headed, short-limbed morph, and a small-headed, long-limbed morph (Supporting Information, Table S1; Fig. 1). On the island, the SVLs of the large-headed, small-limbed male and female morphs were significantly higher than the SVLs of the corresponding small headed, long-limbed morphs (Mann–Whitney *U* test, *P* < 0.01). The scatterplots in Figure 1 illustrate the dimorphism by plotting snout length against forearm length for island and mainland males. The bimodal histograms for snout lengths, mandibular widths and forearm lengths are given in Supporting Information (Fig. S1). As Supporting Information (Table S1) shows, the large-headed, short-limbed males and females were the most common morphs, comprising 75 and 77% of the island and mainland male samples, and 87 and 91% of the island and mainland female samples, respectively.

Numerous studies have shown that *Anolis* limb dimensions are correlated with the structural features of the microhabitat that a given species characteristically occupies (Losos et al., 1994; Losos, Warheit & Schoener, 1997). In particular, relative hind limb length increases as perch diameter increases, both within and between species (Losos, 2009). In mainland species, forelimb length is nega- tively correlated with perch height, while no such relationship is found in Greater Antillean anoles (Irschick et al., 1997). Given these patterns, we analysed the relationship between the means of the size-adjusted lower and upper limb lengths and the average logarithms of the perch heights and perch diameters utilized by the island and mainland morphs. There was no significant correlation between height and limb length. However, as Figure 2 illustrates, there was a positive association

![Figure 1](image.png)

**Figure 1.** Snout length vs. forearm length for adult male *A. nebulosus* from the island (A) and mainland (B) populations. Circles: large-headed, short-limbed morph; triangles: small-headed, long-limbed morph.
between size-adjusted forelimb and hind limb length and perch diameter. The Spearman correlation between the size-adjusted upper limb lengths and mean perch diameters was significant ($\rho = 0.833$, $P = 0.010$), while the correlation between the size-adjusted lower limb lengths and mean perch diameters was marginally insignificant ($\rho = 0.667$, $P = 0.071$). We also conducted $t$-tests for the differences between the logarithms of the perch diameters occupied by the male and female morphs. In all four comparisons the small-headed, long-limbed morph utilized perches with larger diameters. However, none of the $t$-tests was significant ($P > 0.35$).

### SIZE AND SEXUAL SIZE DIMORPHISM

On the island, body sizes (LnSVL) of both male morphs were significantly larger than the corresponding female morphs (Supporting Information, Table S1; ANOVA, $P < 0.0001$, followed by Tamahane post-hoc tests, $P < 0.01$). On the mainland, the body size of the large-headed, short-limbed male morph was also significantly larger than the large-headed, short-limbed female morph (ANOVA, $P < 0.00001$, Tamahane post-hoc test, $P < 0.00001$). Sample size of the mainland small-headed, long-limbed female morph was insufficient to test for differences in body size.

Overall, island morphs were larger than the corresponding mainland morphs (Supporting Information, Table S1). Taking ratios of mean SVLs, island male morphs were 1.26 and 1.17 times larger than the corresponding mainland morphs, and the large-headed, short-limbed female morph was 1.29 times larger than the corresponding mainland female morph. For both island males and females, body sizes (LnSVL) of the large-headed, short-limbed morphs were significantly larger than their mainland counterparts (ANOVA, $P < 0.000001$, Tamahane post-hoc test, $P < 0.00001$). SVLs of the small-headed, long-limbed island males were also significantly larger than those of the corresponding mainland morph (ANOVA, $P < 0.001$; Tamahane post-hoc test, $P = 0.014$). Sample sizes were insufficient to test for differences in body size between the island and mainland small-headed, long-limbed female morphs.

The average sexual size dimorphisms (SSDs) of male morphs to female morphs were similar on island and mainland for both the ratio of mean SVLs (1.16 vs. 1.10) and the ratio of maximum SVLs (1.12 vs. 1.17; Supporting Information, Table S2). To add perspective to these differences, the histograms on the left of Figure 3 show the distribution of SSDs (mean male SVL/mean female SVL) for 102 Greater Antillean species compared with 28 solitary species inhabiting smaller islands in the West Indies. The histograms on the right show the SSDs for 60 mainland anoles vs. four solitary species from islands off Central and South America that are derived from mainland ancestors, including *A. nebulosus* on San Agustin. The median SSDs for the Greater Antillean species (1.16) and the West Indian solitary anoles (1.32) were significantly different (Mann–Whitney U test, $P < 0.000001$). The median SSD for the mainland species was 1.03 compared with 1.29 for the solitary island species with mainland ancestors, and these medians were also significantly different (Mann–Whitney U test, $P = 0.00013$).

### MORPHOLOGICAL SPACE COMPARISONS

Intraspecific divergence in size and shape between sexes is common in anoles, and can significantly increase both morphological and ecological diversity.
within Anolis communities (Schoener, 1967; Butler, Sawyer & Losos, 2007). Here we analyse the joint impact of dimorphism within and between sexes on the area of multivariate morphological space occupied by the island and mainland populations.

Figure 4 illustrates the results of a PCA based on the natural logarithms of seven of the measured morphological characters. The first two PCs accounted for 86 and 78% of the variation in the data for the island and mainland populations, respectively. The characters were not size adjusted, and hence the multivariate space represents differences in absolute size rather than shape.

We defined the area of the PCA morphospace occupied by each of the island and mainland morphs by connecting the points in Figure 4 that delimited the outer boundaries of the PC scores. We then calculated the areas of the resulting polygons. The total and unique areas for each morph, as well as the total morphospace occupied by all four morphs in each environment, are given in Supporting Information (Table S3). Unique area is defined as that part of a given morph’s PC space that does not overlap with another morph. On island and mainland, males and females each contributed an average of 50% to the total morphological space occupied by a given morph. Hence, within morphs, sexual dimorphism approximately doubles the total morphological space. On the island, the combined area of the male and female large-headed, short-armed morphs was 2.09 vs. 1.93 PC units² for the small-headed, long-armed male and female morphs, respectively, giving a total morphospace area of 4.02 PC units². On the mainland, the combined area occupied by the male and female large-headed, short-armed morphs was 1.90 vs. 1.89 PC units² for the small-headed, long-armed male and female morphs, respectively, giving a total morphospace area of 3.79 units². Hence, in each environment, the existence of a second morph essentially doubles the total morphological breadth of the respective A. nebulosus population.

In continuously growing species such as anoles, variance and coefficients of variation of SVLs are highly correlated with average SVL (Lister &
McMurtrie, 1975). Given the larger SVLs of island A. nebulosus, we would expect the CV of their SVLs to be greater than their mainland counterparts. The means and standard deviations of SVLs for all morphs and sexes combined were 42.89 (6.84) and 34.1 (3.98) mm for the island and mainland, respectively. The CV of the SVLs for all the island morphs and sexes combined was 0.159 vs. 0.116 for all the mainland morphs and sexes. The difference was significant ($P = 0.0037$) via a likelihood ratio test (Miller, 1991). We also calculated Shannon diversity indices ($H$) for the total SVL distributions of the island and mainland populations. The $H$ values for the mainland and island were 2.57 and 2.12, respectively. Hutcheson’s $t$-test for Shannon diversity indices (Hutcheson, 1970) rejected the null hypothesis ($P < 0.0002$), and we conclude that the size diversity of the island population was significantly greater than that of the mainland population.

**DISCRIMINATION AND CLASSIFICATION OF THE MORPHOLOGICAL GROUPS**

To determine if differences in shape as well as size could be used to define and classify the various morphs, we conducted a discriminant function analysis (DFA) using eight size-adjusted variables (Supporting Information, Table S4). In the preliminary one-way MANOVA, with morpho-group as the main factor and the size-adjusted morphological measurements as the dependent variables, all tests for overall differences in the size-adjusted means were significant ($P < 0.00001$; Supporting Information, Table S5). All tests of between-morph effects for the size-adjusted variables were also significant ($P < 0.0001$).

The first two discriminant axes accounted for 98.6% of the variation in the discriminant scores, and both functions were significant (Wilks’ $\lambda$ = 0.011 and 0.374, $P < 0.00001$). The loadings of the size-adjusted variables on each discriminant axis are given in Supporting Information (Table S4). The discriminant analysis classified 63% of all individuals correctly with respect to morph group. The discriminant scores for all morphs are plotted in two-dimensional discriminant space in Supporting Information (Fig. S3).

**ISLAND–MAINLAND DIVERGENCE IN SHAPE**

To further analyse island and mainland populations with respect to shape, we conducted a PCA utilizing the same eight size-adjusted variables employed in the discriminant analyses. Figure 5 shows the relative positions of the male and female morphs in the morphological space defined by the first two PC axes. Supporting Information (Fig. S4) illustrates the loadings of the size-adjusted values on these axes. The size-adjusted values for size (CMASS), snout length, mandibular width and upper limb length were all highly correlated with the first PC axis, while tail length, hand length and rear foot length were most highly correlated with the second axis.

Following a significant one-way MANOVA (Supporting Information, Table S5), a Tukey’s post-hoc test was conducted to assess the significance of the
differences between equivalent mainland and island morphs with respect to the eight size-adjusted variables. Both mainland male morphs had proportionally longer snouts and wider mandibles than their island counterparts. The mainland female morphs also had consistently longer size-adjusted snout lengths and wider mandibles than the corresponding island morphs. However, only the difference in relative mandibular width between the island and mainland small headed, long-armed female morphs was significant (Tukey’s post-hoc test, $P = 0.0004$; Supporting Information, Table S6). Differences in size-adjusted upper limb lengths between the island and mainland morphs were marginally insignificant ($P = 0.09$). As a measure of the morphological divergence between the island and mainland populations, the Euclidean distances between the centroids shown in Figure 5 were calculated (Supporting Information, Table S7), and ranged from 0.75 to 1.16. Comparative interpretation of these distances is hampered by the use of different morphological variables in different studies.

**Comparison with West Indian ecomorphs and the hypothetical generalist anole**

In an investigation of the generalist convergence hypothesis for solitary anoles, Losos & de Queiroz (1997) compared the morphology of male anoles belonging to the six canonical Greater Antillean ecomorphs with anoles from one- and two-species islands in the Lesser Antilles and Caribbean. Utilizing the same data set, we conducted a PCA that included corresponding morphological data for *A. nebulosus* large-headed, short-limbed males from the island and mainland habitats. Our aim was to determine if males from the island and mainland populations could be classified as one of the West Indian ecomorphs, and whether island males were more similar to the generalist phenotype than mainland males.

Figure 6 compares the positions, in the same PC space utilized by Losos & de Queiroz (1997), of island and mainland males with each other and with the six canonical *Anolis* ecomorphs. Morphologically, both *A. nebulosus* populations are clearly separated from the West Indian ecomorphs, as well as from the putative generalist morphology, and are more similar to each other than to any of the West Indian species. The Euclidean distances from the centroids of the island and mainland males to the ecomorph centroids (Supporting Information, Table S8) indicate that the centroid for the trunk ground ecomorph was closest to the centroids for both mainland (4.12 PC units) and island (3.98 PC units) males. In comparison, the average distance to the nearest ecomorph centroid for the 33 West Indian species in the Losos & de Queiroz (1997) study was 1.36 PC units. The distance to the postulated centroid of the generalist anole was also much lower for the West Indian spe-
lizard-eating mammals including the white nosed coati (*Nasua narica*), the Tayra (*Eira barbara*), the pygmy spotted skunk (*Spilogale pygmaea*) and the raccoon (*Procyon lotor*). Over the past 25 years we have not encountered any snakes on San Agustín, or any mammals. Given the paucity of predators, the survivorship of resident *A. nebulosus* on San Agustín was expected to be significantly greater than on the mainland. Both observational and experimental data support this hypothesis.

On the mainland, the average survival time of adult males and females was 2.4 (± 0.7) months, and only 16% of the lizards alive at the beginning of the year were still alive after 6 months (A. Ramirez-Bautista, pers. comm.). Our census data indicate that on San Agustín, average survival time of adult males and females was 7.1 (± 1.1) months, and 60% of the individuals alive in January were still alive after 6 months. Overall, survivorship was significantly lower on the mainland (Mantel-Cox log rank test, \( P = 0.00001 \)). As our survivorship data utilized cohorts of marked adult lizards, life expectancy at birth could not be calculated. In general, the life span of mainland anoles rarely exceeds a year (Andrews & Nichols, 1990), while island anoles consistently have longer life expectancies (Lister, 1981; Schoener & Schoener, 1982). Our tethering experiments also suggest that predation rates are considerably greater on the mainland. During both wet and dry seasons, survivorship of the tethered lizards was significantly higher on San Agustín (Supporting Information, Fig. S2; Wilcoxon test, \( P = 0.0001 \) dry season; \( P = 0.003 \) wet season).

To gain insight into relative levels of intraspecific competition, we also estimated the densities of the island and mainland *A. nebulosus* populations, and measured resource levels in the wet and dry seasons using sticky traps (Fig. 7). Combined densities of males and females in the 15-m by 15-m census quadrat on San Agustín totalled 128 individuals. This is over 60 times higher than the mainland density of about two individuals per 225 m\(^2\), which in turn is somewhat lower than the density of ten individuals per equivalent area found in a mainland forest in Nayarit, Mexico, 360 km north of the Ch-CBR forest (Jenssen, 1970). The density of *A. nebulosus* on San Agustín is equivalent to *Anolis* densities on islands in the West Indies. For example, estimated *Anolis* density per 225 m\(^2\) is 140 individuals in the Luquillo rainforest (Lister, 1981; B. C. Lister & A. Garcia, unpubl. data), 150 on Bimini, 119 on St. Croix and 80 on Dominica (Stamps, Losos & Andrews, 1997).

The biomass of arthropods caught in sticky traps was consistently higher on the mainland, ranging from 4.3- to 8.8-fold greater depending on the season (Fig. 7). The mainland results are similar to those

**Figure 6.** Position of the centroids for the island (grey diamond) and mainland (black diamond) male *A. nebulosus* compared with the six canonical West Indian ecomorphs within the morphological space defined by PC axes 1 and 4 (A), and 1 and 2 (B). The X indicates the position of the hypothetical generalist anole, intermediate in morphology with respect to the six canonical ecomorphs. The polygons outline the centroids of species belonging to the same ecomorph. TG, trunk-ground; TC, trunk-crown; TR, trunk; CG, crown-giant; GB, grass-bush; TW, twig; NI, *A. nebulosus* island males; NM, *A. nebulosus* mainland males. Data for West Indian anoles provided by Jonathan Losos.

**ECOLOGICAL AND BEHAVIOURAL DIVERGENCE**

**Predation and competition**

Predators are known to have a major impact on the survival of island and mainland anoles (Schoener, Losos & Spiller, 2005). Like other tropical mainland habitats (Losos, 2009), the Ch-CBR forest abounds with predators. Of the 108 resident bird species, at least 15 are documented lizard predators, 16 of the 24 most common snake species are known to include lizards in their diet, and six of the 15 resident lizard species prey upon other lizards, including *A. nebulosus*. The mainland also harbours several

of vegetation height and diameter as the dependent diameter, a MANOVA was conducted with the logs obtained by Lister & Garcia (1992) in the same Ch-CBR study area, and, as these authors point out, represent one of the highest seasonal fluctuations in arthropod resources reported to date within a tropical ecosystem. Such large reductions in food resources suggest that interspecific competition on the mainland and intraspecific competition on the island may be particularly pronounced during prolonged, El Nino-driven dry seasons.

Siliceo-Cantero & Garcia (2014) recently published a comparison of the San Agustin and mainland populations of A. nebulosus with respect to growth rate, body condition, habitat use and food availability. While habitat use matched the data presented here, results for arthropod abundance are not comparable due to differences in methodology. In 2011 and 2015, two major hurricanes, Jova and Patricia, made landfall near the Ch-CBR forest and the Bay of Chamela and impacted habitat structure as well as the population density and survivorship of anoles on San Agustin (H. H. Siliceo-Cantero and A. Garcia, unpubl. results). These data are being prepared for publication.

**Niche shifts**

Analysis of the vegetation sampling data revealed considerable overlap in the structure of the Ch-CBR and San Agustin forests (Supporting Information, Fig. S5A). The average height of woody plants within the island transects was 351 cm vs. 394 cm for the mainland transects. Woody plant diameter averaged 3.9 and 2.9 cm, respectively, on the island and mainland. To test for differences in vegetation height and diameter, a MANOVA was conducted with the logs of vegetation height and diameter as the dependent variables, and location as the independent variable. The MANOVA was significant (Wilk’s $\lambda = 0.958$, $P = 0.001$). There were also significant univariate main effects for perch diameter differences between the island and mainland vegetation, ($P = 0.007$), but not for perch height ($P = 0.247$).

Supporting Information (Fig. S5B) shows the distribution of perch heights and perch diameters occupied by the island and mainland populations (males plus females). A MANOVA indicated significant differences in the mean logarithms of perch sites between the island and mainland populations (Wilks $\lambda = 0.892$, $P = 0.000039$). There were significant univariate main effects for perch height ($P = 0.00007$), but not for perch diameter ($P = 0.93$). The mean perch height of island males was 111 cm vs. 78 cm for mainland males, and the mean perch heights of island and mainland females were 98 and 71 cm, respectively. An ANOVA with a post-hoc Tamahane test was employed to test the null hypothesis of no difference amongst the island and mainland male and female morphs with respect to perch height. The ANOVA was significant ($P = 0.00008$), and the Tamahane post-hoc test indicated that island males (both morphs combined) perched significantly higher than the combined mainland male morphs ($P = 0.005$), and the combined mainland female morphs ($P = 0.003$), but not significantly higher than island females. There was also no significant difference in perch height between mainland males and females ($P > 0.90$). The similarity of the vegetation on island and mainland suggests that the observed shifts are not artefacts of differences in available perch sites.

**Niche expansion**

As a measure of niche width, the Simpson diversity index was calculated for the utilization of height, perch diameter, and perch height and diameter combined by the male and female island and mainland populations. There were no notable changes in the diversity of perch diameters utilized by island compared with mainland A. nebulosus. However, perch height diversity of island males was almost twice that of the mainland males (9.8 vs. 5.2, Supplementary Information, Table S9). The Simpson niche width of 9.8 for heights utilized by island males is greater than the Simpson indices for height utilization found amongst male anoles on several single-anole islands in the West Indies. For example, the perch height diversities for A. sagrei on Swan Island, Cayman Brac and Duck Key are 7.2, 7.8 and 5.9, and 7.8 for A. monensis on Mona Island (Lister, 1976a).

The diversity of the combined utilization of perch heights and diameters by all morphs was also higher.
on the island (3.46 vs. 2.78). Excluding the small male and female morphs, the diversity of the combined perch heights and diameters utilized by the large male and female morphs was 2.84. Hence, the small male and female morphs increased microhabitat diversity by a substantial 18%. On the mainland the small male and female morphs contributed 10% to the overall perch height–perch diameter diversity of 2.78. Employing the methods of Roughgarden (1974), perch site niche width was partitioned into within-morph (WMC) and between-morph (BMC) components. BMC measures the proportion of total niche width that is due to niche differences between the morphs, while WMC gives the proportion of total niche width due to differences in resource utilization among individuals in the population. WMC on San Agustín was 75.6% and BMC was 24.4%. WMC for the mainland was 82.8% and BMC was 17.2%. The higher BMC on the island suggests that overlap in microhabitat is less amongst the island morphs, which would be expected if intraspecific competition is higher on the island.

**Behaviour**

Between 1989 and 1993 we observed adult males of *A. nebulosus* on San Agustín for a total of 84 h and adult females for 78 h. We also accumulated 73 h of observation on adult males within the Ch-CBR forest over the same period, which, when combined with the observations of Lister & García (1992), yielded a total observation time of 231 h for mainland males and 89 h for mainland females. From these detailed observations, we calculated the per cent of time spent in social behaviour and in resting/hiding by insular and mainland males. During the wet (breeding) season, island males spent much more time in social behaviour, mainly courting females, than mainland males (95 vs. 49%), and far less time resting and hiding (1 vs. 44%). There was no difference in the number of moves per hour between island and mainland males during the dry season (2.5 vs. 2.6 h⁻¹). Over two wet seasons, the average number of movements per hour for island males (37.2) was 3.8-fold higher than for mainland males over five wet seasons (9.7), and feeding rates were 5–10 times higher than those of mainland males (Supporting Information, Figs S7, S8). The maximum dewlap rate during the breeding season was also close to five times higher for island males (72 h⁻¹) than for mainland males (15 h⁻¹). Only *A. carolinensis* males at 75–78 displays h⁻¹ (Johnson & Wade, 2010) match the display rate of male *A. nebulosus* on San Agustín. Like mainland females, island females are considerably more sedentary than island males, especially during the wet season (4.9 vs. 37.2 moves h⁻¹).

**Genetic divergence**

We obtained a maximum of 758 bp of mitochondrial ND2 sequence data [partial coding sequence (cds) plus some tRNAs] from 23 island and 27 mainland individuals of *A. nebulosus*, resulting in a total of 20 haplotypes. Genetic diversity was low on San Agustín (*h* = 0.50; π = 0.0007) relative to the mainland (*h* = 0.95; π = 0.0006), and the populations were fairly divergent (*F*<sub>ST</sub> = 0.34; Fig. 8A). We selected five mainland and five island individuals for RADseq, including island individuals sharing mtDNA haplotypes with the mainland population. Our reduced dataset for clustering analyses consisted of 16 382 SNPs, resulting from the first SNP from each locus. Our mainland dataset consisted of an average of 92% genotypic coverage per locus (SD = 12%) and 8.3% missing data (missing alleles across loci). For San Agustín, we obtained 91% coverage per locus.

(SD = 12%) and 10.5% missing allelic data. Island and mainland individuals grouped cleanly (K = 2, from our ΔK analysis) in both STRUCTURE and DAPC analyses (Fig. 8B), with individuals showing 100% posterior support in STRUCTURE for membership in their respective populations (Island, Mainland). We further found evidence for divergence between these two populations \( (F_{ST} = 0.18) \), as well as lower genetic diversity on the mainland \( (h = 0.38) \) relative to San Agustín \( (h = 0.32) \); Supplementary Information, Table S10).

**DISCUSSION**

Given the above results, we return to our initial questions.

1. To what extent has the island population of *A. nebulosus* diverged morphologically and genetically from the ancestral mainland population?

**Retention of intrasexual dimorphism**

The island population has retained the same dimorphisms in relative head and limb size that evolved in the mainland population. Such intrasexual polymorphisms involving morphology, coloration, behaviour and life history have been reported for a variety of vertebrate taxa including birds, reptiles, fish and amphibians (Knudsen et al., 2006; Huber et al., 2007; Martin & Pfennig, 2009; Higham et al., 2015). In anoles, research to date has focused on polymorphisms in female dorsal pattern (Paemelaere, Guyer & Dobson, 2011). To our knowledge, the only documented example of non-sexual, morphological dimorphism in an anole other than *A. nebulosus* is for *Anolis agassizi* on Malpelo Island, an oceanic island off the west coast of Colombia (López-Victoria, Herrn & Botello, 2011). Here male *A. agassizi* have two morphs which differ in maximum SVL (84 vs. 110 mm) and coloration.

The evolution of intrasexual dimorphism in a mainland anole is somewhat surprising, given that increases in ecomorphological diversity often occur on islands and other areas where reduced competition can lead to niche expansion and character release (Lister, 1976a, b; Butler et al., 2007). For example, the degree of sexual size dimorphism in Caribbean anoles is inversely related to the number of co-occurring congeners (Schoener, 1977). In theory, as the number of sympatric species increases, size differences between males and females contract to lessen the impact of interspecific competition for food and other resources. However, given that no other *Anolis* species inhabits the Ch-CBR forest, or most other parts of *A. nebulosus*’ range in western Mexico (Garcia & Ceballos, 1994), *A. nebulosus* is an unusual example of a solitary mainland anole. This absence of congeners may have resulted in vacant niches and the evolution of different morphs to fill them.

Several studies suggest that the densities and behaviour of mainland anoles are primarily shaped by predation (Andrews, 1979; Lister & Garcia, 1992). In theory, such top-down control should alleviate intense inter- and intraspecific competition, and consequently reduce the effects of density-dependent, disruptive selection. Under these conditions, competition for enemy-free space rather than food could be the driving force behind the evolution of mainland polymorphism (Jeffries & Lawton, 1984). Like resource competition, competition for enemy-free space could promote shifts in behaviour and divergence in microhabitat use. While not as common as those arising from competitive interactions, polymorphisms resulting from predator-driven disruptive selection are well known and include the green and brown colour morphs of the Pacific tree frog (Morey, 1990), the dorsal pattern polymorphism of female *A. sagrei* (Calsbeek & Cox, 2011) and the behaviour–coloration polymorphism in garter snakes (Brodie, 1992).

While specification of the selective forces underlying intrasexual dimorphism in *A. nebulosus* awaits further research, previous studies suggest that multiple selection pressures may shape the direction and degree of such polymorphisms. Intersexual differences in microhabitat use are common in anoles and often associated with shape dimorphisms similar to those found amongst the morphs of *A. nebulosus*. Many male anoles, for example, perch higher than females, and often have relatively longer legs and larger heads (Butler et al., 2007). Manipulative field experiments have also demonstrated that intraspecific differences in limb length in *A. sagrei* result from density-dependent, disruptive selection for separate performance optima on narrow and broad surfaces (Calsbeek & Smith, 2008).

Our current understanding of adaptive radiation in anoles is based on a paradigm of ecological and allopatric speciation due to evolutionary change in continuously varying traits driven by interspecific competition and niche divergence. The existence of intraspecific dimorphisms in *A. nebulosus* and *A. agassizi* raises the possibility that sympatric speciation, driven by intraspecific competition, predation or sexual selection, may have played a role in the origin of new *Anolis* species, as is apparently the case in a variety of other taxa (Knudsen et al., 2006; Huber et al., 2007; Takahashi, Takahashi & Parris,
conclude that intersexual size and shape dimorphism have played an important role in the adaptive radiation of anoles. We would expect the same conclusion to hold for intrasexual dimorphism, assuming that its existence in *A. nebulosus* and *A. agassizi* is not unique in the evolutionary history of anoles.

Island–mainland divergence in shape

The characters that separate phenotypes on island and mainland are known to be ecologically relevant and adaptively important in anoles. Species with longer limbs, such as trunk-ground anoles, run faster on broad surfaces than short-limbed species, while species with shorter legs are more agile on thinner perches (Losos *et al.*, 1998). These differences in performance are functionally significant in escaping from predators, foraging and reproducing (Calsbeek & Irschick, 2007). The positive relationship between size-adjusted limb length and perch diameter documented here among *A. nebulosus* morphs is consistent with a general pattern that holds across the Greater Antillean ecomorphs, as well as within species such as *A. sagrei* and *A. carolinensis* (Losos, 2009).

The ecology and evolution of head size have been extensively studied in anoles and other lizards. Head size and prey size covary in anoles, and even differences as small a 1 mm in head size are associated with significant differences in prey size distributions (Schoener & Gorman, 1968). Within and between species, larger head size is also highly correlated with greater bite force, and individuals with greater bite force consume larger, harder prey items (Herrel *et al.*, 2006; Runemark, Sagonas & Svensson, 2015; Van Kleeck, Chiaverano & Holland, 2015). Hence, divergence in head shape between morphs may promote intrasexual resource partitioning that parallels intersexual differences in diet in sexually dimorphic anoles and other lizard species (Schoener, 1967; Herrel *et al.*, 2006). Larger heads and stronger bites are also excellent predictors of the outcome of aggressive disputes between male lizards (Lailvaux & Irschick, 2007), and appear to enhance defence against predators as well (Leal, 1999; Runemark *et al.*, 2015). Given these results, it appears likely that the relatively larger head size of mainland *A. nebulosus* is associated with utilization of larger arthropods that require greater bite force to capture and consume, and possibly with predator defence, but further data are needed to test these hypotheses.

Based on the results of our genomic analyses, it is doubtful that gene flow from the nearby mainland, or loss of genetic diversity through genetic drift, have constrained the shape divergence of the island population relative to the mainland source population, and hence contributed to stasis (Calsbeek & Smith, 2003). While we do not know when San Agustín was colonized, evolution of shape in response to local conditions can occur rapidly in insular anoles (Stuart *et al.*, 2014), and limited divergence in shape due to limited adaptation time is unlikely. Translocation experiments within and between islands have also shown that the magnitude of divergence of experimental populations from the source population is a function of the degree of difference in vegetation (Malhotra & Thorpe, 1991; Losos *et al.*, 1997). As our Supporting Information (Fig. S5) indicates, the structures of the island and mainland habitats are quite similar, and this may have contributed to the minimal shape divergence between the two populations.

Divergence in size

The major morphological difference between island and mainland *A. nebulosus* is the larger size of the island morphs. Anoles and other island-dwelling lizards often show a size-diversity effect in which size increases as the number of other lizard species inhabiting the same island decreases (Schoener, 1970; Meiri, 2008). The traditional explanation of this trend is that interspecific competition declines in direct proportion to the number of co-occurring species, which in turn leads to ecological release and niche expansion. Phenotypes, including larger bodies, jaws and beaks, that facilitate utilization of a broader set of resources are then selected for (Owens *et al.*, 2006). Available evidence suggests strongly that on San Agustín competition with other saurian and avian insectivores should be considerably reduced compared with the adjacent mainland. In the mainland forest, *A. nebulosus* coexists with 14 other lizard species (Garcia, 2008; Garcia, Valterria & Lister, 2010), and up to 30 species of insectivorous birds that can reach densities of 60–70 individuals per hectare during the winter dry season when insect abundances are comparatively low (Hutto, 1980). While small flocks of mainland birds occasionally forage on San Agustín, the only resident breeding bird species is the largely granivorous mourning dove *Zenaida macroura*.

Our comparisons of survivorship and predation between island and mainland are in keeping with the prediction of life history theory that lower extrinsic mortality on islands should result in the evolution of larger body sizes (Palkovacs, 2003). A more complete understanding of the relative impacts of increased density, competition and relaxed predation on size awaits more extensive comparisons such as those conducted by Li *et al.* (2011). These authors analysed an extensive data set on the body sizes, resources, predators and life history parameters of rice frogs (*Rana limnocharis*) on 20 islands and two mainland sites, and demonstrated convincingly that

© 2016 The Linnean Society of London, *Biological Journal of the Linnean Society, 2016, 118, 852–872*
reduced predation was the major force driving body gigantism of the island frogs.

**Divergence in behaviour**

Increases in population density and intraspecific competition on islands can promote greater competition amongst males for mates as well as food. In theory, increased sexual competition can also lead to selection for larger body (Stamps et al., 1997). In anoles and other lizards, larger size confers a number of advantages. Larger males are socially dominant, mate with more females and are more likely to win skirmishes over territories and females than smaller males (Trivers, 1976; Losos, 2009). Our comparisons of *A. nebulosus* behaviour on San Agustin and in the Ch-CBR forest revealed striking differences which suggest that intrasexual competition among island males may be an important factor in the evolution of larger body size. In particular, the extraordinary amount of time and effort that island males expend in courtship and display is a likely indicator of intense rivalry for access to females.

Mainland *A. nebulosus* are amongst the most sedentary lizards known, and Lister & Garcia (1992) interpreted their lack of movement and prolonged periods of hiding as adaptations that reduced exposure to predation. Release from mainland predators probably enables the island population to adopt a much more active life style including greater number of movements per hour, greater distances moved per hour and prey capture rates that are 4- to 5-fold greater per hour (Supporting Information, Figs S7, S8). Similar behavioural shifts occur in populations of *A. wattsii* inhabiting small islands near Antigua where predatory *Ameiva* are missing (Kolbe, Colbert & Smith, 2008). Other island–mainland comparisons have also documented marked differences in activity, with island anoles moving on average 4–5 times more frequently per hour than mainland anoles (Losos, 2009).

Given intense male–male competition and the likelihood of increased intersexual selection, we hypothesize that island males should have evolved relatively larger testes (Byrne, Roberts & Simmons, 2002), bigger dewlaps (Harrison & Poe, 2012; Harrison, 2014) and greater signalling complexity (Ord, Blumstein & Evans, 2001) than their mainland counterparts. In contrast, island females that spend the majority of their day avoiding male advances might be especially recalcitrant owing to their capacity for sperm storage and long-term retention of fertilized eggs in the highly variable climate of Mexico’s west coast (Lister & Garcia, 1992).

**Genetic divergence**

Genetic analyses showed some evidence for mtDNA haplotype sharing between island and mainland populations, although private haplotypes were observed in each group. The island population is less diverse than the mainland population in mtDNA (*h* = 0.50 vs. 0.95), but not nucDNA (*h* = 0.38 vs. 0.32). Interestingly, our broad sampling of the nuclear genome resulted in a clear distinction between the island and mainland populations, indicating a relative lack of gene flow. Taken together, our results are likely to be capturing some evidence of incomplete lineage sorting in the mtDNA, and generally show evidence of a relaxation population genetic model, whereby the island population is evolving under the relative influences of drift and lack of migration. Senczuk et al. (2014) also genetically analysed San Agustin and mainland Ch-CBR *A. nebulosus* and found that the island population likewise exhibited reduced genetic variation relative to the mainland. That study, which utilized only mtDNA from a smaller sample of individuals, also failed to detect haplotype sharing between island and mainland populations. Those authors interpreted this result as a single colonization of the island from the mainland, followed by minimal or non-existent gene flow (relative isolation). Although not stated, the reduced genetic diversity of the island population is, as in our study, probably due to either a historical founder effect and/or a relaxation of allelic diversity (drift). In both studies, genetic data suggest reduced or non-existent gene flow from the mainland to the island populations and that island populations are evolving independently of mainland populations.

2. DOES THE MORPHOLOGY OF THE ISLAND AND MAINLAND POPULATIONS OF *A. NEBULOSUS* CORRESPOND TO ANY OF THE SIX WEST INDIAN ECROMORPHS?

While the behaviour and ecology of mainland *A. nebulosus* most closely resemble West Indian trunk-ground anoles (Lister & Garcia, 1992), the same predictable relationships between morphology and habitat use found in the West Indies do not apply to continental anoles, and the great majority of species that utilize habitats similar to West Indian anoles have not converged on similar morphologies (Irschick et al., 1997; Pinto et al., 2008; however, see Moreno-Arias & Calderon-Espinosa, 2015) for recent evidence indicating greater convergence between mainland and Caribbean morphotypes than previously realized). From data presented by Schaad & Poe (2010), the probability that a mainland species has converged on any of the West Indian ecomorphs is 0.07–0.12 depending on the confidence level of the assignment. The conditional probability of convergence, given that the mainland species occupies a trunk-ground microhabitat, is 0.0–0.016. Hence, our
initial expectation that the morphology of *A. nebulosus* would not correspond to any of the West Indian ecomorphs was confirmed. Like *A. nebulosus*, the shapes of the solitary, mainland-derived species *A. concolor* and *A. pinchoti* do not fully match any of the West Indian ecomorphs (Calderon-Espinosa & Barragan-Forero, 2011). To date, a comparative analysis of shape in *A. agassizi* has not been carried out.

3. IN THE ABSENCE OF MOST MAINLAND COMPETITORS, HAS THE ISLAND POPULATION OF *A. NEBULOSUS* UNEARTHED NICHE EXPANSION AND EVOLVED TOWARDS A GENERALIST PHENOTYPE?

Losos & de Queiroz (1997) tested the prediction that anoles on one- and two-species islands in the Lesser Antilles and northern Caribbean should have evolved convergent, generalist morphologies. They defined the generalist morphology as a hypothetical phenotype intermediate between the shapes of the six major Great Antillean ecomorphs. Only four West Indian species met this criterion. While the San Agustín population of *A. nebulosus* has expanded the range of perch sites utilized relative to the mainland population, it has largely retained its ancestral phenotype and has not evolved greater similarity to the putative generalist morphology compared with the mainland population (Fig. 6).

While these results suggest limited support for the generalist convergence hypothesis, we suggest that this conclusion is unwarranted for three reasons. First, the definition of the generalist phenotype assumes that anoles on small, biologically depauperate islands would somehow experience the net selection pressure resulting from the exploitation of the same set of microhabitats utilized by all six ecomorphs. While this is theoretically possible, it is extremely doubtful that the diversity of vegetation on most small islands would encompass the required range of microhabitats. Even if such islands existed, the niches of resident anoles would have to expand sufficiently to encompass the entire range of vegetation occupied by all of the more specialized ecomorphs. Second, the effects of predation, intraspecific competition and changes in life history are all assumed to remain constant on small islands. The third problem arises from defining the generalist phenotype with respect to shape in the first place. Below we offer an alternative definition that is consistent with known patterns in the morphology of solitary anoles, and suggest that convergent evolution on a generalist phenotype should be a common phenomenon on continental as well as West Indian islands.

As Schoener (1970) originally observed, anoles on one- and two-species islands are largely convergent with respect to size. Among 40 monospecific islands in the West Indies, 34 of 40 resident male anoles have SVLs between 55 and 75 mm, and, on 37 of the 40 islands, female SVLs are between 40 and 60 mm (Schoener, 1970). The maximum sizes of the large-headed adult male and female morphs on San Agustín lie within these ranges, as well as the males and females of the solitary species *A. concolor* on Colombia’s San Andrés Island (Calderon-Espinosa & Barragan-Forero, 2011). While the SVLs of female *A. pinchoti*, the other solitary species investigated by Calderon-Espinosa & Barragan-Forero (2011), are within the West Indian size range, the mean and maximum SVLs of males are somewhat less than 55 mm (Corn & Dalby, 1973). Like *A. ferreus* in the Lesser Antilles, *A. agassizi* is an outlier with respect to the sizes of most solitary anoles. The maximum SVLs of the male morphs are 84 and 110 mm, while females have a maximum SVL of 83 mm (López-Victoria et al., 2011). Comparisons of *A. nebulosus* and other island and mainland anoles with *A. agassizi* should be especially revealing given the latter species’ large size, polymorphism, unique habitat and foraging behaviour, lack of territoriality and origins in mainland Dactyloa rather than the Norops clade (Rand, Gorman & Rand, 1975; Losos, 2009).

The evolution of increased body size of anoles on San Agustín and other islands has important implications for niche expansion and intraspecific competition. As maximum size increases, the range and variance of prey sizes taken by a given population will increase as well (Lister, 1976a). Moreover, as Bolnick et al. (2007) have shown, as the range of prey size expands, the degree of prey size specialization among individuals increases significantly, as predicted by the niche variation hypothesis (Van Valen, 1965). Greater between-individual partitioning of prey size results in reduced intraspecific competition and higher population densities. Mathematical analysis of growth in continuously growing species indicates that as mean size increases, variance in size automatically increases as well (Lister & McMurtrie, 1975). This effect is evident in the increased coefficient of variation of SVLs on San Agustín (Supporting Information, Fig. S6). Such adaptive increases in size variation, and concomitant increases in niche width and individual specialization, are, in essence, emergent properties of the evolution of larger size.

As Schoener (1977) also discovered, solitary anoles in the West Indies are convergent in their degree of sexual dimorphism as well as size (Fig. 3). Like increases in overall size, greater sexual dimorphism increases both morphological and ecological diversity within *Anolis* populations, as Figure 4 and the Supporting Information (Fig. S6) illustrate, and hence are important components in the evolution of niche width (Butler & Losos, 2002; Butler et al., 2007). While
SSDs are available for only four solitary species with mainland origins (Fig. 3), these initial data suggest a trend similar to West Indian counterparts. We predict that additional samples from continental island populations should cluster around a modal SSD of approximately 1.3. Finally, it follows that the behavioural release from predation exhibited on San Agustín should also occur on most small islands lacking mainland predators. We suggest that solitary anoles should converge on a repertoire of behaviours similar to insular *A. nebulosus*, behaviours that drive selection for larger male body size, and possibly proportional increases in testes and dewlap size as well as similar endocrine functioning and display complexity.

Multiple variables, including climatic variation, distance to source of colonists, prey size distribution and vegetation, will undoubtedly impact the evolution of shape, size, SSD and behaviour on any given island. However, the primary forces affecting size in solitary anoles, reduced predation, increased intraspecific competition and sexual selection, should create similar adaptive landscapes on many small islands, and are probably the major drivers of convergence in size, sexual dimorphism and behaviour. Together, size and sexual size dimorphism are the building blocks underlying the evolution of a generalist phenotype capable of utilizing an expanded set of resources, reducing intraspecific competition, and enhancing male fitness under conditions of high density and intense competition for mates. As Poe, Goheen & Hulebak (2007) point out, exaptation in size may also be an important driver of size convergence in solitary anoles. The data in Figure 3 further suggest that both ecomorph type and SSD might also serve as exaptations that pre-adapt anoles for life on single-species islands. We conclude that solitary anoles represent an example of evolutionary convergence at the species level. Divergence in shape between island and source environments, while undoubtedly important in increasing fitness and more efficient exploitation of resources, largely involves the fine tuning of colonizing morphologies to local conditions.

ACKNOWLEDGEMENTS

We thank Sarahi Torobio, Eduardo Avila, Helda Hernandez and Katherine Lister for their help with field work. Hector Alvarez’s assistance and knowledge of the islands in the Bay of Chamela were invaluable in collecting data on San Agustín, and in surveys of several other islands. Jonathan Losos graciously shared his data on the morphology of anoles belonging to the six ecomorphs, and also provided many helpful comments during the formulation and writing of this article. We thank Eric Johnson and Paul Etter for assistance with genotyping. The suggestions and comments of three anonymous referees were greatly appreciated and helped to improve the article in many ways. We also acknowledge the support of the graduate programme in the biological sciences at the Instituto de Biología (IB) Universidad Nacional Autonoma de Mexico (UNAM) and the Estacion de Biologia Chamera from IB-UNAM. Brad Lister was funded by National Science Foundation grant 1038013. The authors declare no conflict of interests through any commercial or other relationships.

REFERENCES


**SUPPORTING INFORMATION**

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

**Figure S1.** Histograms of adult male *A. nebulosus* snout and forearm lengths for the island (A, B) and mainland (C, D) populations. For both locations, the light grey histobars correspond to the long-armed, small-headed morph, and the dark grey histobars to the short-armed, large-headed morph.

**Figure S2.** Cumulative survival and hazard functions for *A. nebulosus* individuals tethered at 2 m height to tree trunks on San Agustin (blue) and the Ch-CBR forest (red) during the wet and dry seasons. The overall mean survival time on the island was 7.4 days vs. 2.8 days on the mainland. During both seasons, survival rates were significantly higher on the island (Mantel-Cox log rank test, *P* < 0.0001).

**Figure S3.** Morphological groupings of the four mainland and four island morphs in two-dimensional discriminant space. Polygons encompassing the outermost scores for each morph are shown. Sixty-seven per cent of individual cases were correctly classified with respect to the eight morph-groups. The large-headed, short-limbed and longer SVL morphs are on the right of the diagram, and the small-headed, long-limbed and shorter SVL morphs are grouped on the left.

**Figure S4.** Vectors of the loadings of the labelled variables on the two PC axes shown in Figure 5. A, male morphs. For males, the two PC axes accounted for 65% of the total variation in the size-adjusted variables. B, female morphs. For females, the two PC axes accounted for 55% of the total variation in the size-adjusted variables. Note the negative loadings along PC1, the axis associated with relative size differences, for snout length and mandibular width, and the positive loadings for forelimb and hind limb length.

**Figure S5.** A, plot of ln height vs. ln diameter of woody plants measured along transects through the island (triangles) and mainland (circles) study areas. Island filled triangle: $\bar{x}$ ln height = 5.68 cm; $\bar{x}$ ln diameter = 1.30 cm. Mainland filled circle: $\bar{x}$ ln height = 5.74 cm; $\bar{x}$ ln diameter = 1.10 cm. B, plot of ln perch height vs. ln perch diameter for adult male and female *A. nebulosus* morphs combined from the island (triangles) and mainland (circles) study areas. Island filled triangle: $\bar{x}$ ln perch height = 4.5 cm; $\bar{x}$ ln perch diameter = 1.63 cm. Mainland filled circle: $\bar{x}$ ln perch height = 3.9 cm; $\bar{x}$ ln perch diameter = 1.63 cm.
**Figure S6.** Distribution of snout–vent length measurements for all morphs combined from the island (A) and mainland (B) populations. Both the coefficient of variation (CV) and the Shannon diversity measure ($H$) were significantly higher for the island population.

**Figure S7.** Number of moves per hour (A) and total distance moved per hour (B) for male and female *A. nebulosus* observed in the mainland and island study areas during the wet and dry seasons. Mainland males dark red; mainland females light red; island males dark blue; island females light blue.

**Figure S8.** Number of prey captures per hour for adult male *A. nebulosus* observed in the mainland (red) and island (blue) study areas as a function of the number of arthropods captured in sticky traps during the time period of observation. Mainland males red; island males blue. Best fit linear regression lines have been drawn through the island and mainland data.

**Table S1.** Descriptive statistics for male and female *A. nebulosus* morphs.

**Table S2.** Sexual size dimorphism (SSD) indices calculated by taking the ratios of mean and maximum SVLs of male morphs to the mean and maximum SVLs of the corresponding female morphs.

**Table S3.** The morphospace areas calculated from Figure 4 for the island and mainland male and female morphs.

**Table S4.** Loadings of the size-adjusted predictor variables on the first two discriminant axes in the Supporting Information (Fig. S3).

**Table S5.** Results of the one-way MANOVA with Morph Group as the independent variable and the eight size-adjusted morphological variables as the dependent variables.

**Table S6.** Results of Tukey’s post-hoc test for differences between size-adjusted snout lengths (SL) and mandible widths (MW) of equivalent mainland and island morphs.

**Table S7.** Euclidean distances between the centroids in Figure 5 for the island and mainland male (IslM and MLM) and island and mainland female (IslF and MLF) morphs.

**Table S8.** Euclidean distances between the centroids of insular and mainland *A. nebulosus* males, the centroids of the West Indian male ecomorphs, and the putative generalist morphology, in Figure 6.

**Table S9.** Mean trunk heights and diameters in the island and mainland study areas, mean perch diameters and heights utilized by adult males and females on the island and mainland, and niche widths (Simpson measure) of perch site utilization.

**Table S10.** Mitochondrial DNA summary statistics for island and mainland populations of *A. nebulosus*. 