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Left–right Dewlap Asymmetry and Phylogeography of *Anolis lineatus* on Aruba and Curaçao

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Abstract

Anolis lizards exhibit a remarkable degree of diversity in the shape, colour, pattern and size of their dewlaps. Asymmetry, where one side of the dewlap differs in pattern or colour from the other, has only been reported in one species, *Anolis lineatus*, and then on only one of the two islands from which it occurs. Given the importance of the dewlap in intra- and interspecific signalling, we expanded on previous work by (1) investigating whether the reported asymmetry actually occurs and, if so, whether it occurs on animals from both Aruba and Curaçao; (2) examining whether populations differ in other aspects of their morphology or ecology; and (3) resolving the evolutionary relationships and the history of the two populations. We confirmed the presence of the asymmetrical dewlap on Curaçao and found that the asymmetry extends to populations on Aruba as well. Animals on Curaçao were smaller overall than populations from Aruba with relatively shorter metatarsals, radii, and tibiae but relatively deeper heads, longer jaws, and wider and more numerous toepads on fore and hind feet. Habitat use did not differ significantly between the islands. We found populations on Aruba and Curaçao to be reciprocally monophyletic with an early Pleistocene divergence of populations on the two islands. Neutrality tests indicate that neither population has seen any recent reduction in population size, making it unlikely that the asymmetry is a result of founder effects or is some other consequence of reduced genetic variation. A variety of factors likely account for the remarkable and unique dewlap morphology exhibited by this species, although more detailed field studies are required to test these hypotheses.

Keywords

geographical variation, Lesser Antilles, lined anole

Introduction

'The dewlap is large in the male; extended it has a wide border with bright orange skin around a black central spot ... The border on one side is closely set with yellow or whitish scales, on the other side the scales are rudimentary and colored like skin. About half the males have the scales well developed on the right side of the dewlap, about half on the left. From several feet away the asymmetry is still apparent, one side of the dewlap appears to have a bright orange border, the other side a yellow orange border.'

The almost 400 described species of *Anolis* exhibit a remarkable diversity of dewlap shapes, colours, patterns, and size. Moreover, species vary in the extent of sexual dimorphism in size, some species are sexually dichromatic, and several species have no dewlap at all. Despite this enormous panoply of dewlap variation, only one species, *Anolis lineatus* (DAUDIN 1802) has been reported to possess an asymmetric dewlap, with the pattern on one side not matching that on the other (Fig. 1). Perhaps even more unexpected, given the extraordinary amount of research that has been conducted on anoles, including on their dewlaps (Losos, 2009), no study has ever followed up on the report by Rand and Rand of dewlap asymmetry in *A. lineatus*.



Figure 1. Left (A) and right (B) views of the asymmetrical dewlap of an adult male *Anolis lineatus* from Curaçao. The left side (A) is the more orange side referred to by Rand and Rand (1967), as well as throughout the present study, whereas the right side (B) is the lighter or more yellow side of the dewlap.

Anolis lineatus, the lined anole of Curaçao and Aruba, is a very little-known species. The only publication on any aspect of its natural history is that of a brief report of 3 days of observations on the species in Curaçao by Rand & Rand (1967). Moreover, as far as we are aware, no study has ever compared populations of the species on the two islands. Curaçao and Aruba are separated by deep water and thus have never been physically connected. Consequently, the species must have evolved on one of the islands and then colonized the other. This realization, in turn, suggests that the unusual asymmetry of *A. lineatus* on Curaçao could be the consequence of loss of genetic variation during a founder event (Leary & Allendorf, 1989; Leamy & Klingenberg, 2005). More generally, absolutely nothing is known about the biology of Aruban *A. lineatus*, much less how populations on the two islands differ.

For these reasons, in the present study, we set out to determine: (1) does asymmetry in the dewlap of *A. lineatus* exist and, if so, is it characteristic of the entire species, or just of populations on Curaçao; (2) do populations on the two islands differ in morphology and ecology; and (3) what are the evolutionary relationships of the populations and is there any evidence for a loss of genetic variation that might be related to the evolution of dewlap asymmetry?

Material and Methods

We visited Curaçao and Aruba on 11–19 January 2012 to collect specimens and habitat use data.

Morphological comparisons

We examined all complete *A. lineatus* specimens from the Harvard Museum of Comparative Zoology (MCZ) Herpetology collection. Lizard localities were split between Aruba ($N = 32$) and Curaçao ($N = 63$). The sex of each lizard was determined by the presence or absence of two enlarged post-anal scales. Dewlap asymmetry was examined from photographs taken of both sides of the dewlap from living, field-collected animals. Lizards from Curaçao were examined in the field; those from Aruba were studied in the laboratory at the MCZ. The animals photographed did not come from multiple site and likely comprised a single population.

We measured 18 morphological traits (13 continuous and five meristic; Table 1) known to vary with habitat use and environment in *Anolis* lizards using previously published methods (Losos, 2009; Mahler et al., 2010). All continuous measures were made with a Mitutoyo digital caliper (ABSOLUTE Coolant Proof Caliper Series 500 IP67, <http://www.mitutoyo.com>). In some instances (e.g. lamella width), photographs were taken with a tripod

mounted digital camera (Nikon d300s) and measurements were taken from photographs using ImageJ software (<http://imagej.nih.gov/ig/>). Meristic traits were counted from photographs or by eye under a dissecting microscope. Measurements were taken from the left side of the animals only and all measurements were taken at least twice to assess repeatability using the ICCest package (Wolak, Fairbairn & Paulsen, 2012). We used the log₁₀ transformed mean of our two measurements in all subsequent statistical analyses. Data did not violate assumptions of normality.

Table 1. List of traits used in comparison of animals from Aruba and Curaçao

	Trait	N (M/F)	(ICC)*	Mean (mm)		CV %	
				Male	Female	Male	Female
1	SVL	49/42	0.997	69.14	58.07	10.84	10.09
2	Femur	49/42	0.990	16.78	13.55	12.14	9.53
3	Tibia	49/42	0.990	18.51	14.84	12.12	9.33
4	Metatarsal IV	49/42	0.980	10.44	8.75	10.44	8.29
5	Toe IV	48/41	0.978	12.81	10.54	10.02	7.90
6	Humerus	49/42	0.980	14.09	11.20	11.38	9.44
7	Radius	48/41	0.980	11.46	9.23	13.55	11.95
8	Finger IV	49/41	0.898	7.01	5.69	12.30	10.04
9	Head height	49/42	0.985	8.86	7.15	11.69	10.02
10	Head length	49/41	0.989	14.12	12.03	10.39	9.34
11	Jaw length	49/42	0.995	19.81	16.56	10.56	8.58
12	Lamella toe IV width	46/36	0.988	1.74	1.37	14.67	14.64
13	Lamella finger IV width	45/39	0.991	1.49	1.15	16.66	14.73
14	Supralabial scales (count)	48/42	0.975	8.59	8.83	9.94	7.87
15	Sublabial scales (count)	49/42	0.974	7.59	7.50	7.68	8.96
16	Ventral scales (count)	47/39	0.738	63.94	61.41	6.70	4.76
17	Lamellae toe IV (count)	47/40	0.804	32.26	31.19	5.05	5.37
18	Lamellae finger IV (count)	47/42	0.800	20.17	18.76	6.73	6.48

*Repeatability measured as intraclass correlation coefficient (ICC; see Material and methods).

All $P > > > 0.01$.

Traits 1–13 are continuous and 14–18 are meristic (counts). Columns are traits measured, sample sizes for males and females, repeatability (as measured using the intraclass correlation coefficient; see Material and methods). Mean values by sex and coefficients of variation are expressed as a percentage for each sex.

CV, coefficient of variation; SVL, snout–vent length.

We examined how variation in our traits was distributed among all animals with a principal components analysis using residual log₁₀-transformed values [from univariate regressions with snout–vent length (SVL)] for continuous traits and log₁₀-transformed values for meristic traits (not size corrected). Differences in body size (measured as SVL) between islands and sexes were assessed using a two-way analysis of variance (ANOVA). Variation in all other variables as a result of Island and Sex were examined using analysis of covariance (ANCOVA) with SVL as a covariate. For variables in which SVL did not covary with a trait, two-way ANOVAs were used. We ran full models (main effects of Sex and Island plus Sex × Island interaction) for each of our measured traits. If the interaction term was nonsignificant, we removed it from the model and examined Island and Sex effects from ANCOVAs with parallel slopes. We did not correct for multiple comparisons because of the low statistical power of such tests when most results are significant.

Habitat use

Habitat use of *A. lineatus* was conducted using standard methods (Rand & Rand, 1967; Schoener & Gorman, 1968; Losos, 2009) at a variety of sites on both islands. Data were collected by slowly walking through the study site and noting for every lizard observed, the substrate type upon which it was perched, as well as its height and

diameter. Data were not included for lizards that were moving when first detected and thus may have been disturbed by the observer. Island and Sex effects on habitat use data (perch height and perch diameter) were assessed using two-way ANOVAs, whereas Island and Sex differences in perch type were detected using chi-squared tests.

Molecular methods

We extracted genomic DNA from tail clips using the DNeasy Blood and Tissue kit (Qiagen). A polymerase chain reaction (PCR) was used to amplify an approximately 1070-bp fragment of the mitochondrial protein-coding gene *ND2* (NADH dehydrogenase subunit 2) and adjacent tRNAs using primers LVT_Metf.6_AnCr (AAGCTATTGGGCCCATACC) and LVT_5617_AnCr (AAAGTYTTGAGTTGCATTCA [Rodríguez-Robles, Jezkova & Garcia, 2007]) and fragments of three nuclear protein-coding genes from a subset of four individuals from each island: an 820-bp fragment of *MKL1* (megakaryoblastic leukaemia 1) using primers MKL1-f1 (GTGGCAGAGCTGAAGCARGARCTGAA) and MKL1-r2 (GCRCTCKRTTGGTCACRGTGAGG) (Townsend *et al.*, 2008); a 550-bp fragment of *NGFB* (nerve growth factor) using primers NGFB-f2 (GATTATAGCGTTTCTGATYGGC) and NGFB-r2 (CAAAGGTGTGTGTWGTGGTGC) (Townsend *et al.*, 2008); and a 960-bp fragment of *RAG1* (recombination activating gene 1) using primers RAG1-AnF1 (GAAATTCAAGCTCTTCAAAGTGAGAT) and RAG1-AnR1 [TGTCAAKGAAAGTAAGTGTGCTTG (present study)]. We used the following PCR profile: an initial 5-min denaturation at 95 °C followed by 32 cycles of denaturation (30 s at 95 °C), annealing (45 s at 52–56 °C) and extension (1 min at 72 °C), followed by a final extension of 5 min at 72 °C. PCR clean-up and Sanger sequencing in both directions was performed by Beckman Coulter Genomics (Danvers, MA, USA). Sequences were edited and aligned using SEQUENCHER, version 4.8 (Gene Codes). Protein-coding sequences were translated into amino acids using MESQUITE, version 2.75 (Maddison & Maddison, 2011) to confirm alignment and ensure there were no premature stop codons. Gametic phase of nuclear haplotypes was resolved manually because there was only one heterozygous site in the nuclear dataset (see results).

We sequenced mitochondrial DNA from 24 individuals (Table 2). An additional *A. lineatus* *ND2* sequence from Curaçao (Jackman *et al.*, 2002) was downloaded from GenBank ([AF294287](https://www.ncbi.nlm.nih.gov/nuclseq/AF294287)) creating a mitochondrial DNA dataset of 25 individuals (14 from Aruba and 11 from Curaçao) with an aligned length (with outgroup taxa) of 1088 bp. We obtained nuclear gene sequences from eight individuals: four from Aruba (MCZ25773, MCZ25719, MCZ29306, MCZ25716) and four from Curaçao (C1, C3, C16, C8). All new sequences have been deposited in GenBank (KC952902-KC952949).

Table 2. Specimens used for the molecular phylogenetic analyses

Species	Specimen ID	Locality	ND2 Haplotype #	GenBank ID
<i>Anolis lineatus</i>	MCZ25772	Downtown Oranjestad, Aruba	1	KC952902
<i>Anolis lineatus</i>	MCZ25719	Downtown Oranjestad, Aruba	2	KC952903
<i>Anolis lineatus</i>	MCZ25712	Downtown Oranjestad, Aruba	2	KC952915
<i>Anolis lineatus</i>	MCZ25767	Downtown Oranjestad, Aruba	3	KC952904
<i>Anolis lineatus</i>	MCZ29308	Downtown Oranjestad, Aruba	3	KC952906
<i>Anolis lineatus</i>	MCZ29305	Downtown Oranjestad, Aruba	3	KC952913
<i>Anolis lineatus</i>	MCZ25715	Downtown Oranjestad, Aruba	4	KC952905
<i>Anolis lineatus</i>	MCZ25709	Downtown Oranjestad, Aruba	5	KC952907
<i>Anolis lineatus</i>	MCZ29307	Downtown Oranjestad, Aruba	6	KC952908
<i>Anolis lineatus</i>	MCZ29306	Downtown Oranjestad, Aruba	7	KC952909
<i>Anolis lineatus</i>	MCZ25716	Downtown Oranjestad, Aruba	8	KC952910
<i>Anolis lineatus</i>	MCZ25773	Downtown Oranjestad, Aruba	9	KC952911
<i>Anolis lineatus</i>	MCZ25717	Downtown Oranjestad, Aruba	10	KC952912

<i>Anolis lineatus</i>	MCZ25711	Downtown Oranjestad, Aruba	11	KC952914
<i>Anolis lineatus</i>	C3	Lodge Kura Hulanda, near Westpunt, Curaçao	12	KC952917
<i>Anolis lineatus</i>	C1	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952918
<i>Anolis lineatus</i>	C13	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952919
<i>Anolis lineatus</i>	C12	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952920
<i>Anolis lineatus</i>	C15	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952924
<i>Anolis lineatus</i>	No ID	Lodge Kura Hulanda, near Westpunt, Curaçao	13	KC952916
<i>Anolis lineatus</i>	C8	Lodge Kura Hulanda, near Westpunt, Curaçao	14	KC952921
<i>Anolis lineatus</i>	C9	Lodge Kura Hulanda, near Westpunt, Curaçao	14	KC952922
<i>Anolis lineatus</i>	C16	Lodge Kura Hulanda, near Westpunt, Curaçao	14	KC952923
<i>Anolis lineatus</i>	C10	Lodge Kura Hulanda, near Westpunt, Curaçao	14	KC952925
<i>Anolis lineatus</i>	LSUMZ-H5450	Curaçao	15	AF294287
<i>Anolis chrysolepis</i>	MPEG26568	Faro, Pará, Brazil	NA	JN191530
<i>Anolis chrysolepis</i>	BPN780	Ralleighvallen, Suriname	NA	JN191534
<i>Anolis chrysolepis</i>	BPN1587	Saul, French Guiana	NA	JN191536
<i>Anolis tandai</i>	LSUMZ-H14098	Rio Ituxi, Amazonas, Brazil	NA	JN191542
<i>Anolis tandai</i>	MPEG25029	Juruti, Pará, Brazil	NA	JN191546
<i>Anolis tandai</i>	LSUMZ-H13599	Rio Juruá, Acre, Brazil	NA	JN191548
<i>Anolis planiceps</i>	BPN96	Kartabo, Guyana	NA	JN191552
<i>Anolis planiceps</i>	LSUMZ-H12300	Rio Ajarani, Roraima, Brazil	NA	JN227867
<i>Anolis brasiliensis</i>	CHUNB43282	Brasília, Distrito Federal, Brazil	NA	JN191555
<i>Anolis brasiliensis</i>	CHUNB45075	Minapu, Goiás, Brazil	NA	JN191559
<i>Anolis brasiliensis</i>	CHUNB37527	Paraná, Tocantins, Brazil	NA	JN191562
<i>Anolis brasiliensis</i>	CHUNB37528	São Domingos, Goiás, Brazil	NA	JN191563
<i>Anolis scypheus</i>	LSUMZ-H12543	Reserva Faunística Cuyabeno, Sucumbios	NA	AF337802
		Province, Ecuador		
<i>Anolis scypheus</i>	KU222147	Loreto, Peru	NA	JN191569
<i>Anolis bombiceps</i>	KU222145	Loreto, Peru	NA	JN191570
<i>Anolis meridionalis</i>	LF166692	Canindeyu, Paraguay	NA	AY909760
<i>Anolis auratus</i>	LSUMZH13928	Alter do Chão, Pará, Brasil	NA	JN191571
<i>Anolis auratus</i>	CIEZAH1163	Estado Falcón, Venezuela	NA	AY909740
<i>Anolis auratus</i>	LACM148978	Gamboa, Panama	NA	DQ377353
<i>Anolis onca</i>	CIEZAH1156	Estado Falcón, Venezuela	NA	DQ377357
<i>Anolis annectens</i>	CIEZAH1160	Estado Falcón, Venezuela	NA	DQ37734
<i>Anolis garmani</i>	REG3211	Portland, Jamaica	NA	EU107944

NA, not available.

Molecular diversity and population genetic structure

We calculated standard indices of population genetic diversity for each locus using DNASP, version 5.10.01 (Librado & Rozas, 2009) including the number of haplotypes, number of segregating sites (*S*), haplotype diversity (*h*) and nucleotide diversity (π). We estimated genetic differentiation between Aruba and Curaçao populations by calculating the number of fixed differences between populations, nucleotide divergence (*Dxy*; Nei, 1987), and the nearest-neighbour statistic (*Snn*; Hudson, 2000). Net between-group distance (Tamura, Nei & Kumar, 2004) between Aruba and Curaçao populations for the mitochondrial data was calculated using MEGA, version 5.05 (Tamura et al., 2011) with standard error estimated using 500 bootstrap replicates.

We examined demographic parameters of Aruba and Curaçao populations by estimating *Fu*'s F_s (*Fu*, 1997) and the R_2 statistic (Ramos-Onsins & Rozas, 2002) for each population separately using mitochondrial ND₂ data. Both statistics test for demographic expansion or contraction by determining whether sequences depart from neutral expectations and both tests are generally robust to small sample sizes (Ramos-Onsins & Rozas, 2002).

Test statistics and statistical significance (estimated using 1000 coalescent simulations) were calculated in DNASP (Librado & Rozas, 2009).

Phylogenetic analysis and divergence dating

We estimated phylogenetic relationships among *A. lineatus* haplotypes and related *Anolis* taxa from mitochondrial sequence data using maximum likelihood implemented in RAXML, version 7.3.2 (Stamatakis, 2006; Stamatakis, Hoover & Rougemont, 2008) via the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010). Data were divided into four partitions: one partition for each codon and another partition for tRNAs. We used 1000 bootstrap replicates to assess nodal support. Additional *ND₂* sequences were downloaded from GenBank with related taxa chosen from published anole phylogenies (Poe, 2004; Nicholson *et al.*, 2005; Nicholson, Mijares-Urrutia & Larson, 2006; D'Angiolella *et al.*, 2011) and *Anolis garmani* was chosen as an outgroup. *ND₂* haplotypes were collapsed using FABOX (Villesen, xref ref-type="bibr" rid="bij12131-bib-0001">2002).

Divergence dates between *A. lineatus* populations on Aruba and Curaçao were estimated using a Bayesian relaxed molecular clock in BEAST, version 1.7.3 (Drummond *et al.*, 2012). We utilized *ND₂* sequences from every individual and used a log-normal clock model, constant size coalescent tree prior, and a unweighted pair group method with arithmetic mean starting tree. The preferred model of sequence evolution (HKY+G+I) was determined using the Bayesian information criterion in JMODELTEST, version 0.1 (Posada, 2008). The absence of appropriate *Anolis* fossils to use as an external calibration prompted us to use a per lineage molecular clock rate of 0.65% Mya⁻¹ derived from *ND₂* using several other lizard groups (Macey *et al.*, 1998, 1999). We ran two analyses for 10 million generations each, sampling every 1000 generations. Trees were combined in LOGCOMBINER, version 1.7.3 (Drummond *et al.*, 2012) and summarized in TREEANNOTATOR, version 1.7.3 (Drummond *et al.*, 2012) with a 10% burn-in. Convergence in BEAST analyses was assessed by visual inspection of split frequencies using AWTY (Nylander *et al.*, 2008) and inspection of likelihood values and the effective sample size of output statistics using TRACER, version 1.5 (Drummond *et al.*, 2012).

Results

Morphological comparisons

We confirm that asymmetry exists in the dewlaps of *A. lineatus* in both males and females and in populations on both Aruba and Curaçao: all of the individuals we examined were notably asymmetric in dewlap patterning, although, for one female from Curaçao, the differences were relatively slight. The asymmetry occurs primarily as a series of lateral scale rows of increasing lightness on the lower portion of one side of the dewlap, which borders a large dark spot (Fig. 2). The other side lacks these light coloured scale rows. This asymmetry gives the overall impression of one side of the dewlap being lighter, or more yellow, than the other, more orange, side. *Sensu* Rand & Rand (1967), we refer to the lighter side with more obvious transverse scale rows as the yellow side and the darker side without the rows less visible as the orange side. We caution, however, that these descriptions are subjective, and may be variable between investigators. We emphasize that future work should entail more analytical and quantitative analyses of dewlap colour in accordance with published methods (Fleishman, Leal & Persons, 2009). Photographs from 15 living specimens from Aruba (Fig. 2A, B; 15 males and six females) and 13 from Curaçao (Fig. 2C, D; 10 males, three females) show the asymmetry to be evenly distributed in males between the right and left sides (Aruba: $N = 5$ right, 4 left; Curaçao, six right, four left). In females, the yellow side was found only to occur on the left side of the dewlap in Aruba, whereas, in Curaçao, two females were clearly more scaly on the left side, and one animal was very slightly more yellow on the right based upon our subjective assessment. Moreover, the dark spot on animals from Curaçao appears to be much darker than those from Aruba. However, because animals from the two islands were photographed under

different conditions, we cannot say whether this is a viable difference or simply an artefact of varying lighting conditions (e.g. colour temperature, angle) when photographing lizards.

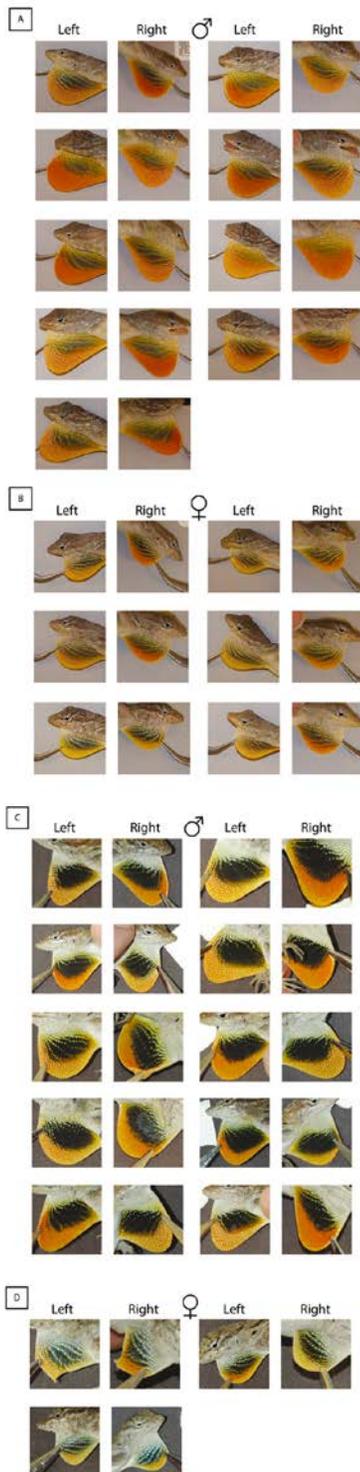


Figure 2. Photographic pairs of the left and right sides of nine male (A) and six female (B) *Anolis lineatus* from Aruba and ten male (C) and three female (D) *A. lineatus* from Curaçao. In the first pair of images, the yellow side is the left side of the dewlap and the orange side is on the right.

Repeatability for all traits was highly significant ($P \ll 0.01$; Table 1). A principal components analysis on data from all lizards describes four primary axes of variation accounting for almost 60% of the morphological variation measured (no other axis had Eigenvalues > 1.4 or loadings greater than 0.6): PC1 loads most strongly for residual limb and jaw-lengths, PC2 for residual lamellae widths and numbers of lamellae, PC3 for labial scale counts, and PC4 for residual head length (Table 3). The results from a two-way ANOVA of body size (SVL) with Sex and Island (interaction term nonsignificant) indicate that males were larger than females ($\beta_{\text{sex}} = 0.073$, d.f. = 2, 88, $P \ll 0.001$) and animals from Curaçao were smaller than those from Aruba ($\beta_{\text{island}} = -0.576$, d.f. = 2, 88, $P \ll 0.001$). All continuously measured variables increased significantly with body size (SVL; $P \ll 0.01$; Table 4), whereas meristic traits (supralabial, sub-labial, and ventral scales, and lamella numbers) did not. Males had significantly larger size-corrected limb dimensions for all limb traits measured ('Sex'; Table 4) but generally did not differ from females in size-corrected head dimensions. Size-corrected differences in head dimensions (head height and jaw length) were found between lizards from the two islands, as were differences in several limb dimensions ('Sex+Island'; Table 4). Animals on Curaçao were smaller, and had relatively shorter metatarsals, radii, and tibiae but relatively deeper heads (head height), longer jaws, longer toes, and wider and more numerous toepads on both hind and forefeet ('Island'; Table 4). Relative head lengths and labial and ventral scale counts showed no significant effects of either Island or Sex. Only one trait, tibia length, showed a significant Sex \times Island Interaction (Fig. 3). Males have relatively longer tibiae than females and no difference exists in the mean values between the islands, although males from Aruba have disproportionately longer tibiae than males from Curaçao or females from either island, thus driving the interaction.

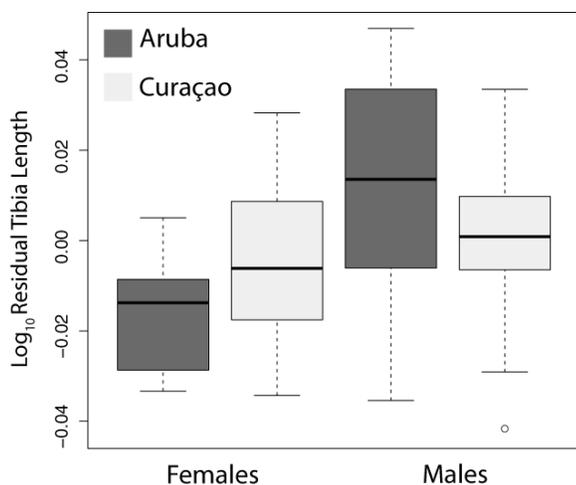


Figure 3. Boxplot of residual \log_{10} tibia length showing Sex \times Island interaction.

Table 3. Loadings, eigenvalues, and variance explained on size-corrected variables used in analysis of *Anolis lineatus* from Aruba and Curaçao

	Trait	PC1	PC2	PC3	PC4
Continuous	Femur	0.605	-0.395	0.035	-0.221
	Tibia	0.591	-0.416	-0.151	-0.300
	Metatarsal IV	0.573	-0.501	-0.189	-0.049
	Toe IV	0.734	0.156	0.253	0.060
	Humerus	0.618	-0.175	0.117	-0.158
	Radius	0.367	-0.456	-0.324	-0.155
	Finger IV	0.564	-0.221	0.292	-0.011
	Head height	0.491	0.391	-0.232	0.073
	Head length	0.370	0.157	-0.366	0.698
	Jaw length	0.751	0.024	-0.209	0.484
	Lamellae width (toe IV)	0.201	0.635	-0.040	-0.134

	Lamellae width (finger IV)	0.366	0.600	-0.059	0.136
Meristic	Supralabials	0.012	-0.264	0.569	0.455
	Sublabials	0.110	-0.211	0.750	0.250
	Ventral scales	0.285	0.479	0.224	-0.436
	Lamellae (toe IV)	0.384	0.568	0.209	-0.066
	Lamellae (finger IV)	0.411	0.278	0.184	-0.266
	Eigenvalue	3.945	2.577	1.578	1.493
	Percentage of variance	23.21	15.16	9.28	8.78
	Cumulative	23.2	38.4	47.7	56.4

PC, principal component.

Table 4. Effects of body size, island and sex on 18 continuous and meristic traits in *Anolis lineatus*

Major effect	Trait	=	Intercept	+	Log ₁₀ SVL	+	Sex	+	Island	R ²	SEE
Sex	Femur	=	-0.532	+	0.944	+	0.020	+	-0.001	0.89	0.022
	Tibia*	=	-0.422	+	0.903	+	0.041	+	0.001	0.94	0.017
	Humerus	=	-0.538	+	0.901	+	0.031	+	-0.003	0.92	0.020
	Finger IV	=	-0.906	+	0.937	+	0.020	+	0.012	0.82	0.029
Sex + Island	Metatarsal IV	=	-0.404	+	0.767	+	0.018	+	-0.011	0.93	0.015
	Toe IV	=	-0.603	+	0.911	+	0.017	+	0.029	0.90	0.019
	Radius	=	-0.926	+	1.076	+	0.012	+	-0.011	0.94	0.019
	Ventral scales (Count)	=	1.770	+		+	0.018	+	0.026	0.30	0.023
	Lamellae toe IV (Count)	=	1.481	+		+	0.015	+	0.020	0.26	0.021
	Lamellae finger IV (Count)	=	1.000	+		+	0.021	+	0.019	0.28	0.029
Island	Head height	=	-1.075	+	1.083	+	0.011	+	0.030	0.88	0.024
	Jaw length	=	-0.451	+	0.944	+	0.006	+	0.008	0.97	0.010
	Lamellae width toe IV	=	-2.465	+	1.454	+	-0.003	+	0.046	0.80	0.038
	Lamellae width finger IV	=	-2.718	+	1.557	+	-0.004	+	0.044	0.86	0.034
None	Head length	=	-0.555	+	0.926	+	-0.001	+	0.004	0.96	0.012
	Supralabials (count)	=	0.941	+		+	-0.012	+	0.006	0.03	0.040
	Sublabials (count)	=	0.871	+		+	0.006	+	0.004	0.01	0.036

*Showed significant Sex × Island interaction (Fig. 3).

Traits are ordered according to major effect in multiple regressions [i.e. whether Sex, Island, or both, are significant (significant predictors are highlighted)]. All continuous traits covaried significantly with snout–vent length (SVL), whereas no meristic traits did and thus SVL was omitted from those regression models. Three traits showed no effect of Sex or Island, whereas one trait (Tibia) showed a significant interaction between Sex and Island (see Discussion). SEE, standard error of the estimate.

Habitat use

The type of perch or substrate on which an animal was found ('perch type') did not differ between islands ($\chi^2 = 8.42$, d.f. = 4, $P = 0.074$; Fig. 4A), nor among the sexes ($\chi^2 = 1.91$, d.f. = 4, $P = 0.75$; Fig. 4B). Males were found on significantly thicker perches than females (perch diameter; $\beta = 4.0016$, d.f. = 2, 133, $P = 0.035$; Fig. 5A) and had a slight (but marginally nonsignificant) tendency to perch higher than females ($\beta = 0.19$, d.f. = 2, 164, $P = 0.062$; Fig. 5B). There were no significant Sex × Island interactions.

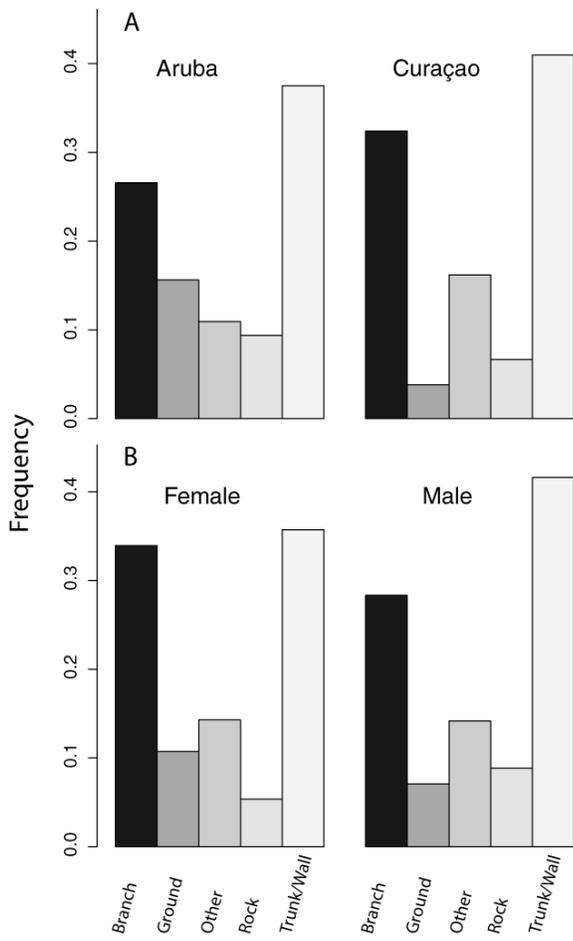


Figure 4. Perch types used by *Anolis lineatus* across (A) islands and (B) sexes. There were no significant differences in perch type used among animals from different islands or between sexes, although substantially fewer animals were found on the ground on Curaçao.

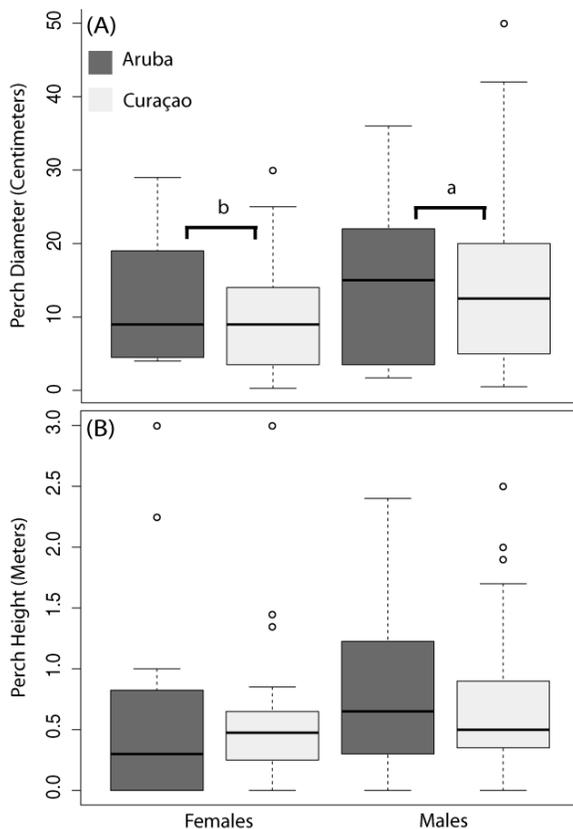


Figure 5. Variation in perch diameter (A) and perch height (B). Males on the two islands did not differ in the diameter of the perches used but did significantly differ from females on both islands (as indicated by ‘a’ and ‘b’, respectively). Males on both islands also had a tendency to perch slightly higher than females (although this was not significant). There were otherwise no differences in perch height or diameter between islands and no Sex \times Island interactions.

Molecular diversity and population genetic structure

We identified 15 unique mitochondrial haplotypes among our samples (Table 5). Both haplotype (h) and nucleotide diversity (π) were higher in Aruba samples than in Curaçao samples. No shared mitochondrial haplotypes were found between *A. lineatus* on Aruba and Curaçao and 18 fixed nucleotide differences occurred between the two populations. Nucleotide divergence (D_{xy}), which estimates the mean number of nucleotide substitutions per site between populations, was 0.02534. The nearest-neighbour statistic (S_{nn}) indicated that populations on the two islands are highly differentiated ($S_{nn} = 1.00$, $P = 0.000$). Net between group distance (p -distance) between Aruba and Curaçao populations was 2.1% (SE = 0.4%).

Table 5. Haplotype and nucleotide diversity among *Anolis lineatus* samples for sampled loci

	<i>N</i> samples	<i>N</i> haplotypes	Segregating sites (<i>S</i>)	Haplotype diversity (h) (SD)	Nucleotide diversity (π) (SD)
Aruba					
<i>ND2</i>	14	11	21	0.956 (0.045)	0.00621 (0.045)
<i>RAG1</i>	4	1	0	0	0
<i>MKL1</i>	4	1	0	0	0
<i>NGFB</i>	4	1	0	0	0
Curaçao					
<i>ND2</i>	11	4	9	0.709 (0.099)	0.00221 (0.00069)
<i>RAG1</i>	4	2	1	0.49 (0.169)	0.00045 (0.00018)
<i>MKL1</i>	4	1	0	0	0

NGFB	4	1	0	0	0
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Both MKL₁ and NGFB datasets consisted of a single haplotype and were invariant between populations. RAG1 was more diverse with three haplotypes. One unique haplotype was shared among all Aruba samples, whereas Curaçao samples had two haplotypes (Table 5); one fixed nucleotide difference was found between the two populations. Two of the Curaçao individuals were heterozygous for RAG₁. RAG₁ nucleotide divergence (D_{xy}) was 0.00130 and the nearest-neighbour statistic (S_{nn}) indicated that populations on the two islands are highly differentiated ($S_{nn} = 1.00$, $P = 0.001$).

Neutrality tests for *A. lineatus* in both Aruba and Curaçao indicate stable population sizes for both populations. Both Fu's F_s and the R_2 statistic had nonsignificant P-values and do not differ significantly from neutral expectations (Aruba: Fu's $F_s = -2.749$, $P = 0.094$; $R_2 = 0.1389$, $P = 0.473$; Curaçao: Fu's $F_s = 1.165$, $P = 0.757$; $R_2 = 0.1767$, $P = 0.541$).

Phylogenetic analysis and divergence dating

Phylogenetic relationships among sampled *Anolis* species (Fig. 6) were largely concordant with previously published phylogenies (Nicholson et al., 2005, 2006; D'Angiolella et al., 2011). We recovered *A. lineatus* as the sister taxon to the *A. chrysolepis* species group sensu D'Angiolella et al. (2011). Aruba and Curaçao haplotypes were reciprocally monophyletic with haplotypes from Aruba further divided into two well-supported clades.

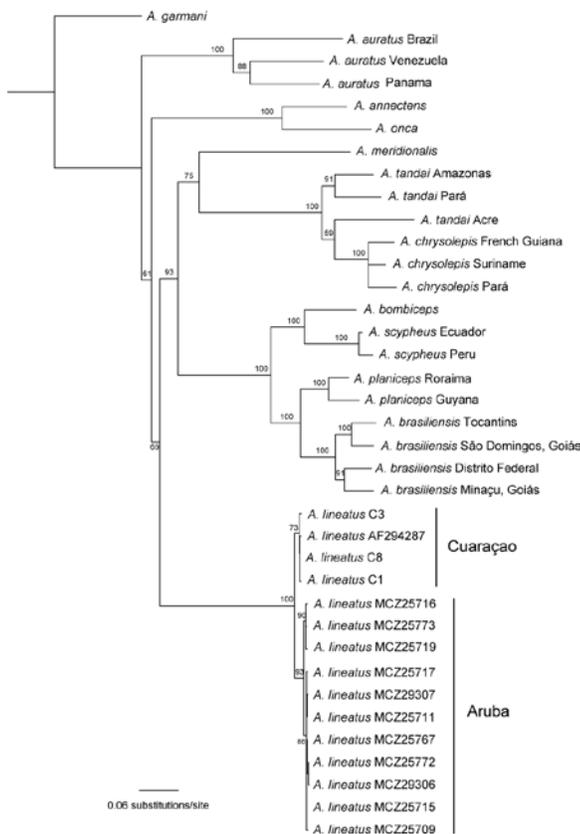


Figure 6. Phylogenetic relationships among unique *Anolis lineatus* haplotypes and related *Anolis* species produced by a maximum likelihood analysis of mitochondrial *ND2* data. Numbers at nodes are maximum likelihood bootstrap values; only values > 50% are shown.

Both Bayesian relaxed clock analyses converged and data from both runs were combined for final analyses (Fig. 7). Divergence between sampled Aruba and Curaçao haplotypes occurred between 1.3–3.8 Mya (mean = 2.5 Mya). The crown age of sampled Aruba haplotypes was 0.4–1.5 Mya (mean = 0.9 Mya), whereas the crown age of sampled Curaçao haplotypes was 0.2–0.8 Mya (mean = 0.4 Mya). Our estimates of population divergence times should be interpreted cautiously because they rely on the application of a single rate of molecular evolution, which is only an approximation because time-dependency and lineage-specific rate variation are widespread (Bromham, 2002; Bromham, 2009; Ho *et al.*, 2011). In addition, gene tree divergence times occur before the divergence of the sampled populations and the mismatch between the two dates can increase as effective population size (N_e) increases (Edwards & Beerli, 2000; Arbogast *et al.*, 2002).

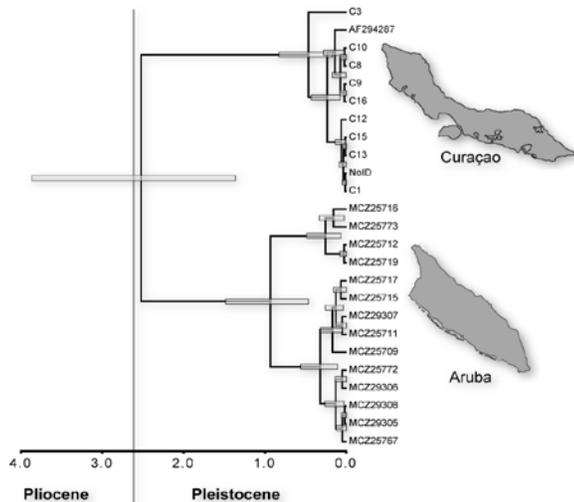


Figure 7. Bayesian time-calibrated phylogeny of sampled *Anolis lineatus*. Individuals are designated by sample ID numbers. Shaded horizontal bars indicate 95% highest posterior density (HPD) interval. Samples from Aruba and Curaçao are indicated. Time is shown as millions of years before present.

Discussion

Anolis lineatus was reported 46 years ago to be asymmetric in dewlap colour (Rand & Rand, 1967), with one side of the dewlap being darker, or more orange, along its border than the other, a phenomenon that is unique among the 400 species of anoles and all other dewlap-bearing lizards of which we are aware. The study by Rand & Rand (1967) has barely been commented upon in the literature (11 citations according to Google Scholar) and the putative asymmetry has never been investigated, other than a report posted on the Internet in 2012 (<http://www.anoleannals.org/2012/01/03/asymmetrical-dewlap-color-in-anolis-lineatus-on-curacao/>). In the present study, we confirm the existence of this asymmetry and demonstrate that it characterizes populations of the species on Aruba, as well as on Curaçao.

We also confirm, as stated by Rand & Rand (1967), that the asymmetry is nondirectional in males, with approximately half of the individuals being more yellow on the left side and the other half on the right. Curiously, there was a suggestion of a directional bias in asymmetry among females; eight individuals were strongly asymmetric with the left side scaller, whereas the sole individual skewed in the other direction was the only animal in our sample in which the differences between the two sides of the dewlap were relatively minor. Whether this is a real trend or a statistical aberration resulting from our small sample of females remains to be determined.

The existence of this asymmetry leads to inevitable questions about its explanation, particularly in light of the fact that asymmetry in secondary sexual characteristics is sometimes considered to be associated with reduced

reproductive success (Palmer & Strobeck, 1986; Trokovic *et al.*, 2012). Our finding that the asymmetry occurs on both islands would appear to rule out the possibility that it is the result of a founder effect associated with the colonization of one of the islands. Nonetheless, to provide greater insight on the evolutionary history of the species, we conducted a phylogenetic analysis, for the first time examining relationships among the populations of *A. lineatus*.

Molecular analysis

Anolis lineatus populations on Aruba and Curaçao are reciprocally monophyletic using mitochondrial DNA (*ND2*), with divergence between the populations occurring in the early Pleistocene. Shared haplotypes between the two islands in two of the three nuclear loci examined are likely the result of incomplete lineage sorting. Mitochondrial loci are expected to achieve reciprocal monophyly much more rapidly than nuclear genes as a result of the smaller effective population sizes (N_e) of the haploid mitochondrial genome, which is one-quarter of the size of the diploid nuclear genome (Hudson & Coyne, 2002). The early Pleistocene divergence of *A. lineatus* populations on Aruba and Curaçao coupled with the relatively stable demography on both islands indicates that neither population is the result of human-mediated transport from one island to the other and the species is a long-term resident on both islands.

The observed sequence divergence between the Aruba and Curaçao *A. lineatus* populations (2.1%) is comparable to *ND2* sequence divergences (*p*-distances) among populations in other *Anolis* species, which vary from 0.6–9.0% (Glor, Vitt & Larson, 2001; Glor, Losos & Larson, 2005; Kolbe *et al.*, 2008; Rodríguez-Robles, Jezkova & Leal, 2010; Tollis *et al.*, 2012). Sequence divergences between closely-related *Anolis* species, on the other hand, are typically greater and range from 5.0–22.5% (Creer *et al.*, 2001; Glor *et al.*, 2001, 2005; D'Angiolella *et al.*, 2011). This does not mean that we advocate species delimitation solely on some predetermined amount of sequence divergence, which would be difficult, if not impossible, to implement as a result of variation in coalescent times related to differences in effective population sizes and differences in lineage-specific substitution rates (Bromham, 2002; Moritz & Cicero, 2004; Hickerson, Meyer & Moritz, 2006; Pons *et al.*, 2006). That said, the amount of sequence divergence among populations may help identify taxa deserving additional study. Given the low levels of mitochondrial sequence divergence, coupled with the conserved morphological and ecological traits described below, we do not think that *A. lineatus* requires any taxonomic changes at this time, although, previously, these allopatric populations would have been ideal candidates for the description of subspecies. We note in passing that, as far as we are aware, there are no names in the literature that have been proposed for the separate island populations of this species. Neutrality tests indicate that neither population has suffered from any recent reduction in population size, driving the final nail into the founder effect hypothesis.

Morphology and habitat use

Concordant with the genetic differentiation, we found significant morphological differences between the islands. Males on Curaçao were significantly smaller than lizards on Aruba. However, animals on Curaçao showed larger relative jaw-lengths, head-heights, toe lengths, and wider and more numerous toe-pads on the hind and forefeet. Anole species commonly exhibit sexual dimorphism in both overall body size and in the relative size of body parts (Butler & Losos, 2002; reviewed in Losos, 2009). *Anolis lineatus* exhibits patterns common among Caribbean anoles; males are substantially larger than females, have more toepads on both fore- and hindfeet, and are longer limbed. However, *A. lineatus* males do not differ from females in head dimensions (length, height, and width), lamella width on the fore- and hind-feet or in labial scale counts. Although differences in limb, toepad, and head dimensions are associated with differences in habitat use and diet among species and populations of other anoles (Elstrott & Irschick, 2004; Herrel & O'reilly, 2006; Herrel, McBrayer & Larson, 2007; reviewed in Losos, 2009), the explanations for such differences in *A. lineatus* are unclear.

We did not detect any differences in habitat use between populations on the two islands and the climate, floral, and faunal composition of both Aruba and Curaçao are very similar: dry, desert-like climates (van Buurt, 2005; Peel, Finlayson & McMahon, 2007) with distinct wet and dry seasons and suppression of summer rains (during what is otherwise the 'rainy season' at the latitudes of Aruba and Curaçao). Hence, the interpopulational differences in morphology cannot be explained at this point as a result of different natural histories. As is found in many anoles, males use broader surfaces than females and have longer hindlimbs. Biomechanical studies on anoles reveal that, on narrow surfaces, lizards with shorter legs are more able to move without difficulty, and hence the intersexual differences in limb length might reflect these differences in habitat use (Butler & Losos, 2002).

Anoles in the multispecies communities of the Greater Antilles specialize to use particular microhabitats that differ in perch height and perch diameter. At the extremes, 'trunk-ground ecomorphs' use broad surfaces near the ground, whereas 'twig ecomorphs' generally are found on narrow surfaces, often high in trees. By contrast, species occurring on smaller islands by themselves (*A. lineatus* is the sole member of the genus on both islands), often have broader, less-specialized habitat use, especially with regard to perch height (Lister, 1976; Losos, 2009). Rand & Rand (1967) reported that *A. lineatus* generally uses broad surfaces, such as tree trunks, rocks, and building walls, a finding that we have confirmed. They also reported that this species uses a broader range of perch heights than is typical for Greater Antillean ecomorph species, being more similar in this respect to one-island species. Our findings for both Curaçao and Aruba are not in agreement. Whereas Rand and Rand found that only one-third of their animals perched at a height of ≤ 1 m, we found that 81% of our animals (137 of 169) perched at such heights, including 9% (16 animals) on the ground. By contrast to Rand and Rand's conclusions, we observed *A. lineatus* to be similar in habitat use to trunk-ground anoles of the Greater Antilles. Of course, the observations of both Rand and Rand and ourselves were conducted over a short period of time at few sites; more intensive sampling is required to fully understand the ecology of this species, how it varies both within and between islands, and how, if at all, it is related to inter-island and intersexual differences in morphology.

The mystery of the asymmetric dewlap

Anole dewlaps are used as signals in male–male and male–female encounters and even in interactions with predators (Losos, 2009). Of what adaptive use might be the uniquely asymmetric dewlap of *A. lineatus*? One hypothesis is that each side of the dewlap in *A. lineatus* serves as a separate signal. If one accepts that dewlap colour or pattern is an honest signal of some aspect of fitness, for which there is some suggestion in anoles (Vanhooydonck *et al.*, 2005; Lailvaux & Irschick, 2007; Lailvaux, Gilbert & Edwards, 2012), then having two honest signals of different aspects of fitness might even be better. We are unaware of any comparable example in other animals. Alternatively, in recent years, considerable attention has been paid toward the importance that signals be detectable in the environment in which they occur (Leal & Fleishman, 2004; Fleishman *et al.*, 2009). With regard to anole dewlap colour and pattern, recent research has shown that different dewlap colours are most detectable in different light environments (Fleishman *et al.*, 2009). Hence, an alternative hypothesis is that the dewlap asymmetry provides the bearer with the opportunity to maximize signal detection by using whichever side of the dewlap is more detectable in the particular light environment and background in which it finds itself. Mitigating against this possibility is the fact that the difference in colour between the two sides of the dewlap does not appear to be that large in magnitude, although more sophisticated measurements (Fleishman *et al.*, 2009) are necessary to quantify these differences. Tests of these hypotheses will require detailed data on the display behaviour of lizards and quantification of the light environment in which displays occur. In particular, research should examine whether lizards use different sides of their dewlaps in different social contexts or light environments, as well as whether the different sides of the dewlap differ in detectability in their natural environment (Leal & Fleishman, 2004).

Of course, it is also possible that the asymmetry has resulted as a result of non-adaptive mechanisms. We rule out the possibility that it has resulted from a founder effect on Curaçao, although ruling out a role for random genetic drift is always difficult. Nonetheless, we find this possibility unlikely given the critical role the dewlap plays in both intra- and interspecific communication in *Anolis*.

Directional asymmetry (in which bilateral sides of a structure not only differ, but also one side is consistently larger than the other) are rare in the animal world. The adaptive significance of having a more yellow left side of the dewlap in females is not clear; one possibility might be that it is related to the brain lateralization and differential use of different eyes in different behavioural contexts, which has been reported in anoles and other lizards (Deckel, 1998; Hews & Worthington, 2001; Hews, Castellano & Hara, 2004). Obviously, more data are needed on how females use their dewlaps and, more generally, more females need to be examined to insure that this finding is not a statistical accident.

The asymmetric dewlap of *A. lineatus* is unique among anoles and perhaps among lizards, and we know of few parallel examples anywhere in the animal world. Some have argued that asymmetry in secondary sexual characteristics is associated with reduced reproductive success (Palmer & Strobeck, 1986; Kodric-Brown, 1997; Leamy & Klingenberg, 2005; Trokovic *et al.*, 2012), yet all *A. lineatus* have this asymmetry, particularly males. Perhaps the asymmetry was selected for and driven to fixation because (counter to conventional wisdom) females preferred asymmetrical over symmetrical males. There are certainly many intriguing untested evolutionary hypotheses regarding the origins of dewlap asymmetry in *Anolis lineatus*; how and why this asymmetry has evolved in this species is currently unknown and would make an excellent subject for detailed behavioural and evolutionary investigation.

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