


Climate-related environmental variation in a visual signalling device: the male and female dewlap in *Anolis sagrei* lizards

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Abstract

Animals communicate using a variety of signals that differ dramatically among and within species. The astonishing dewlap diversity in anoles has attracted considerable attention in this respect. Yet, the evolutionary processes behind it remain elusive and have mostly been explored for males only. Here, we considered *Anolis sagrei* males and females to study signal divergence among populations. First, we assessed the degree of variation in dewlap design (size, pattern and colour) and displays by comparing 17 populations distributed across the Caribbean. Second, we assessed whether the observed dewlap diversity is associated with variation in climate-related environmental conditions. Results showed that populations differed in all dewlap characteristics, with the exception of display rate in females. We further found that males and females occurring in ‘xeric’ environments had a higher proportion of solid dewlaps with higher UV reflectance. In addition, lizards inhabiting ‘mesic’ environments had primarily marginal dewlaps showing high reflectance in red. For dewlap display, a correlation with environment was only observed in males. Our study provides evidence for a strong relationship between signal design and prevailing environmental conditions, which may result from differential selection on signal efficacy. Moreover, our study highlights the importance of including females when studying dewlaps in an evolutionary context.

Introduction

Animals communicate to one another using a wide variety of signals that can differ dramatically among and even within species. A thorough understanding of the evolutionary processes giving rise to this signal diversity seems crucial, as it provides insights into how communication can contribute to population differentiation and speciation events (Boughman, 2002; Smith & Harper, 2003; Rundle & Nosil, 2005). In the absence of long-term time series, within-species geographical variation provides one of the best tools in studying factors that shape the evolution of communication signals (Wilczynski & Ryan, 1999). Differences in signalling systems among

populations can arise through random genetic drift (Campbell *et al.*, 2010), as a by-product of selection on other characteristics (e.g. Nevo & Capranica, 1985; O’Neill & Beard, 2011), or by direct differential selection. Adaptive changes in signalling systems may occur in direct response to competitors (e.g. Hobel & Gerhardt, 2003), predators (e.g. Brandley *et al.*, 2013) or conspecifics (Fisher, 1958; Endler, 1993a; Wilczynski & Ryan, 1999), but may also arise to accommodate variation in climatic conditions (e.g. Möller, 2011; Snell-Rood, 2012; Martín & López, 2013) and physical habitat characteristics (e.g. Boughman, 2002; Seehausen *et al.*, 2008; Baeckens *et al.*, 2015). Here, we specifically test the idea that the physical environment may select for different signal characteristics in an anole lizard.

With nearly 400 species, *Anolis* constitutes an extremely diverse lizard genus that is distributed throughout the Neotropics (i.e. Caribbean and mainland Americas) and often used as model system for studying ecology and evolutionary biology (Losos, 2009). For

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communicating, anoles typically use an extendable flap of skin attached to the throat, called a 'dewlap' (Jensen, 1977; Carpenter, 1978). Although the exact functions are not fully comprehended, this visual signalling device is primarily used in a context of mating and territorial defence (Greenberg & Noble, 1944; Jenssen, 1970; Crews, 1975; Sigmund, 1983; Jenssen *et al.*, 2000), in species recognition (Rand & Williams, 1970; Losos, 1985) and even, in predator deterrence (Leal & Rodríguez-Robles, 1995, 1997). Anole species can differ dramatically in dewlap design and display behaviour, and therefore, these elements have frequently been used to delineate species boundaries (Schwartz & Henderson, 1991 and references therein; Nicholson *et al.*, 2007). In addition, dewlap characteristics can also show considerable variation among and even within populations (Vanhooydonck *et al.*, 2009). In attempt to disentangle the astonishing dewlap diversity and its role in speciation, anole researchers have proposed several nonmutually exclusive mechanisms including sexual selection (e.g. Greenberg & Noble, 1944; Crews, 1975; Sigmund, 1983; Harrison & Poe, 2012), species recognition (e.g. Rand & Williams, 1970; Webster & Burns, 1973; Losos, 1985; Macedonia & Stamps, 1994; Nicholson *et al.*, 2007), predation pressure (Leal & Rodríguez-Robles, 1997; Vanhooydonck *et al.*, 2009) and sensory drive (e.g. Leal & Fleishman, 2002, 2004; Ord *et al.*, 2010, 2011). Many of these authors have suggested that dewlap colour and display repertoire, in particular, may have played a critical role in anole speciation.

For several reasons, we are particularly interested in how interpopulational variation in dewlap design and display relates to climate and associated environmental conditions, in both sexes of *Anolis sagrei*. First, interpopulational differences provide an excellent framework for investigating incipient stages of speciation (Foster *et al.*, 1998). Examining them may thus help identify the mechanisms leading to signal divergence and possibly speciation, even for entire radiations. Second, *A. sagrei* seems a highly appropriate species to examine dewlap diversity. In contrast to most anole species, which exhibit little intraspecific variation, *A. sagrei* is reported to show considerable interpopulational variation in several aspects of dewlap design (Vanhooydonck *et al.*, 2009). Dewlap colour can, for example, range from dull or bright ultraviolet (UV) yellow to deep red, and the dewlap can additionally exhibit a variety of colour patterns. Both sexes of *A. sagrei* possess a dewlap, but the structure is more elaborate in males, that is larger and more conspicuously coloured dewlaps as well as higher display rates in comparison with females (Jenssen *et al.*, 2000; Steffen & McGraw, 2009; Vanhooydonck *et al.*, 2009; Partan *et al.*, 2011; Driessens *et al.*, 2014, 2015). Evolutionary processes driving divergence in dewlap design and display might therefore be sex specific. In a previous study on *A. sagrei*, Vanhooydonck *et al.* (2009) have indeed shown that

sexual selection acts differently on dewlap size in both sexes, whereas predation pressure and species recognition drive dewlap size and pattern, respectively, in the same direction. Unfortunately, research on anoline dewlap diversity has almost exclusively been conducted in males (e.g. Echelle *et al.*, 1978; Losos & Chu, 1998; Nicholson *et al.*, 2007; but see for example Johnson & Wade, 2010; Harrison & Poe, 2012). Incorporating data on female dewlap design and display behaviour seems thus highly necessary, also because we already know that *A. sagrei* females use their dewlap (Driessens *et al.*, 2014). Third, to our knowledge, the contribution of climate-related environmental conditions in explaining dewlap divergence has never been investigated within *A. sagrei*. The species is distributed across a large geographical area, characterized by a variety of environments, which provides an excellent framework for testing. Moreover, two anole studies addressing interpopulational signal divergence in relation to environmental heterogeneity revealed partially discordant results (Leal & Fleishman, 2004; Ng *et al.*, 2013a). Leal & Fleishman (2004) reported that *Anolis cristatellus* populations occupying dark signalling environments (i.e. mesic forest) exhibited brighter and more UV-reflecting dewlaps than populations inhabiting brighter signalling environments (i.e. xeric forests), which were shown to have relatively dull dewlaps with low UV reflectance and red appearance. The same authors additionally proved that the *A. cristatellus* populations were more detectable in their respective natural habitat. Instead, Ng *et al.* (2013a) found that *Anolis distichus* populations occurring in mesic habitats exhibited less bright and more orange dewlaps in comparison with populations occupying drier environments, which were characterized by brighter and more yellow dewlaps. They also noted that dewlaps were more orange in cooler environments with more tree cover. Testing associations between signal design and prevailing environmental conditions within additional anole species can improve our understanding of the directions in which dewlap colours may adaptively diverge.

In this study, we have two main objectives. First, we aim to document the occurrence and degree of variation in dewlap characteristics among *A. sagrei* populations distributed across islands in the Caribbean. Second, we assess whether the observed interpopulational variation in dewlap design and display behaviour correlates with variation in prevailing environmental conditions, studied on a macro-ecological scale. To do this, we linked dewlap data (i.e. size, pattern, colour and display) to climatic parameters (i.e. temperature, precipitation, incoming radiation and vegetation cover) from 17 *A. sagrei* island populations, while accounting for phylogenetic relationships. If physical aspects of the environment shape the evolution of the signalling system in *A. sagrei*, we expect to see significant correlations. In complement to previous studies, we did not



Fig. 1 Brown anole (*Anolis sagrei*) from Cuba. (a) Displaying male and (b) male and female (left and right, respectively) *Anolis* basking in the sun. Photographs by Steven De Decker (April 2012). Photograph (a) already published in Baeckens *et al.* (2016).

exclusively test males, but considered females too. We hypothesize differences in prevailing environmental conditions to be important in shaping dewlap diversity among populations for *A. sagrei* males, and potentially also for *A. sagrei* females.

Materials and methods

Animals

For this study, we used data of 17 *A. sagrei* populations distributed across the Caribbean (Figs 1 and 3b). We incorporated data from seven populations in the Bahamas (Acklins, Andros, Chub Cay, Crooked Island, Grand Bahama, Pigeon Cay and Staniel Cay; April–May 2003) published by Vanhooydonck *et al.* (2009). An additional ten populations were sampled on Jamaica (March 2012), Cuba (Santa Clara, Soroa 1, Soroa 2; April–May 2012), San Salvador (January 2013), Cayman Islands (Cayman Brac, Grand Cayman, Little Cayman, March 2013), South Abaco and South Bimini (March 2015). All data were collected during the breeding season (March–September; Lee *et al.*, 1989), with exception of one population (i.e. San Salvador). A total of 425 male and 362 female individuals were caught by noose and kept individually in plastic bags for maximum 48 h before releasing back at the location of capture (see Table 1 for sample sizes). We measured snout-vent length (SVL) with callipers (Mitutoyo CD-15DC, accuracy 0.01 mm) and quantified dewlap design (i.e. size, pattern and colour) for each captured lizard. Of the latter ten populations, another 235 male and 189 female *A. sagrei* individuals were filmed to quantify dewlap use.

Dewlap

Size

We positioned each lizard on its left side against a 1 cm² grid and gently pulled the base of the ceratobranchial forward with a pair of forceps until the

dewlap was fully extended parallel to the grid (Bels, 1990). We then photographed the dewlap using a Nikon D70 camera mounted on a tripod. We used Adobe Photoshop CS3 extended software (AP CS3, version 10.0) to trace the outer edge of the dewlap on the digital images and to calculate its area. A similar method for measuring dewlap dimensions has produced highly repeatable results in a previous study (Vanhooydonck *et al.*, 2005). We corrected for body size by regressing the log₁₀-transformed SVL against log₁₀-transformed dewlap size for males and females separately. The obtained residuals were subsequently used as estimates of relative dewlap size.

Pattern

One or two distinct colours, that is red and yellow, create a degree of patterning in the dewlap of *A. sagrei* that can vary among individuals. According to well-defined descriptions of dewlap pattern categories (Nicholson *et al.*, 2007), we assigned dewlaps to one of the following pattern categories: solid (uniformly coloured, Fig. 2a), marginal (uniformly reddish-coloured centre with yellowish margin, Fig. 2b) or spotted (yellowish spots across the reddish centre, regardless of the presence of a margin, Fig. 2c). The same observer who had prior experience with classifying *A. sagrei* dewlap patterns (Driessens *et al.*, 2015) assigned a total of 425 male and 362 female dewlaps (17 study populations) to one of the given pattern categories based on high-quality digital photographs. The proportion of individuals classified under each of the three pattern categories was then calculated for every population.

Colour

We measured dewlap reflectance using an Avantes spectrometer (AvaSpec-2048 USB2-UA-50, range 250–1000 nm) and deuterium–halogen light source (AvaLight-DHS) equipped with a fibre-optic probe. The probe was mounted within a metal holder to ensure readings at a fixed distance from the surface and was

Table 1 Descriptive statistics for dewlap measurements in (a) males and (b) females. Population means \pm standard deviation or proportions (prop.) are displayed for each population. Sample size (*N*) can vary according to dewlap measurement, and therefore, ranges are provided for some populations. Proportion of variation among groups is also shown (=100 * the coefficient of intraclass correlation).

Population	<i>N</i>	Dewlap size			Dewlap pattern			Dewlap colour			Dewlap display		
		Size (cm ²)	Prop. solid	Prop. marginal	Prop. spotted	Brightness	Hue	RF 365 nm	RF 655 nm	DE rate (counts/min)	Prop. DE		
(a) Males													
Acklins	11	2.58 \pm 0.68	0.91	0.09	0.00	–	–	–	–	–	–	–	
Andros	24	1.21 \pm 0.33	0.00	0.13	0.88	–	–	–	–	–	–	–	
Cayman Brac	23–28	1.53 \pm 0.39	0.43	0.54	0.04	11353 \pm 1317	567.3 \pm 4.9	0.0015 \pm 0.0002	0.0048 \pm 0.0003	0.16 \pm 0.46	0.22	–	
Chub Cay	23	1.67 \pm 0.49	0.00	0.04	0.96	–	–	–	–	–	–	–	
Crooked Island	24	1.81 \pm 0.61	0.50	0.46	0.04	–	–	–	–	–	–	–	
Grand Bahama	28	1.59 \pm 0.41	0.25	0.68	0.07	–	–	–	–	–	–	–	
Grand Cayman	24–27	1.64 \pm 0.41	0.00	0.93	0.07	8746 \pm 1213	550.8 \pm 3.8	0.0011 \pm 0.0002	0.0053 \pm 0.0005	0.94 \pm 1.57	0.50	–	
Jamaica	22–35	1.17 \pm 0.27	0.60	0.11	0.29	10439 \pm 1791	554.7 \pm 12.2	0.0017 \pm 0.0003	0.0043 \pm 0.0004	0.35 \pm 0.65	0.32	–	
Little Cayman	23–29	2.00 \pm 0.56	0.45	0.52	0.03	8637 \pm 1449	555.6 \pm 8.7	0.0011 \pm 0.0001	0.0053 \pm 0.0003	0.04 \pm 0.11	0.13	–	
Pigeon Cay	17	1.56 \pm 0.39	0.82	0.06	0.12	–	–	–	–	–	–	–	
San Salvador	24–27	1.96 \pm 0.75	0.59	0.41	0.00	7164 \pm 1138	558.6 \pm 7.7	0.0018 \pm 0.0002	0.0040 \pm 0.0004	0.08 \pm 0.14	0.33	–	
Santa Clara	24–27	2.06 \pm 0.36	0.00	0.41	0.59	–	–	–	–	2.28 \pm 1.79	0.96	–	
Soraa 1	23–24	1.91 \pm 0.45	0.00	0.75	0.25	10659 \pm 1418	550.3 \pm 5.0	0.0011 \pm 0.0001	0.0052 \pm 0.0003	2.15 \pm 2.24	0.67	–	
Soraa 2	22–30	2.27 \pm 0.46	0.00	0.73	0.27	10006 \pm 1520	550.1 \pm 1.7	0.0011 \pm 0.0002	0.0052 \pm 0.0003	3.57 \pm 2.62	0.97	–	
South Abaco	21–28	1.35 \pm 0.48	0.00	0.64	0.36	8374 \pm 1038	550.5 \pm 5.9	0.0015 \pm 0.0002	0.0042 \pm 0.0003	0.35 \pm 0.61	0.43	–	
South Bimini	20–27	1.62 \pm 0.45	0.00	0.00	1.00	9081 \pm 1332	551.8 \pm 10.2	0.0016 \pm 0.0002	0.0042 \pm 0.0003	0.51 \pm 0.74	0.55	–	
Staniel Cay	27	1.91 \pm 0.69	0.48	0.41	0.11	–	–	–	–	–	–	–	
Among-population variation		27.60%				46.65%	33.65%	70.00%	90.00%				
(b) Females													
Acklins	12	0.33 \pm 0.06	1.00	0.00	0.00	–	–	–	–	–	–	–	
Andros	19	0.25 \pm 0.05	0.21	0.68	0.11	–	–	–	–	–	–	–	
Cayman Brac	25–29	0.28 \pm 0.03	0.38	0.62	0.00	13143 \pm 1339	563.3 \pm 4.9	0.0019 \pm 0.0002	0.0039 \pm 0.0003	0.01 \pm 0.04	0.08	–	
Chub Cay	20	0.26 \pm 0.06	0.45	0.25	0.30	–	–	–	–	–	–	–	
Crooked Island	18–19	0.30 \pm 0.05	0.68	0.21	0.11	–	–	–	–	–	–	–	
Grand Bahama	23	0.21 \pm 0.04	0.22	0.78	0.00	–	–	–	–	–	–	–	
Grand Cayman	22–29	0.30 \pm 0.05	0.00	0.86	0.14	10543 \pm 1277	547.2 \pm 3.0	0.0014 \pm 0.0002	0.0042 \pm 0.0005	0.03 \pm 0.13	0.05	–	
Jamaica	14–15	0.27 \pm 0.04	0.64	0.36	0.00	12215 \pm 1690	557.3 \pm 18.2	0.0022 \pm 0.0003	0.0034 \pm 0.0004	0.00	0.00	–	
Little Cayman	22–30	0.30 \pm 0.04	0.00	1.00	0.00	9821 \pm 1565	552.1 \pm 5.4	0.0014 \pm 0.0002	0.0043 \pm 0.0003	0.00	0.00	–	
Pigeon Cay	8	0.32 \pm 0.06	1.00	0.00	0.00	–	–	–	–	–	–	–	
San Salvador	22–24	0.34 \pm 0.06	0.13	0.87	0.00	8929 \pm 1296	561.3 \pm 7.2	0.0016 \pm 0.0001	0.0044 \pm 0.0002	0.02 \pm 0.09	0.08	–	
Santa Clara	15–24	0.30 \pm 0.05	0.13	0.71	0.17	–	–	–	–	0.02 \pm 0.04	0.13	–	
Soraa 1	8–21	0.40 \pm 0.05	0.00	1.00	0.00	10386 \pm 1007	549.4 \pm 2.3	0.0013 \pm 0.0002	0.0047 \pm 0.0003	0.03 \pm 0.07	0.14	–	
Soraa 2	17–24	0.39 \pm 0.06	0.04	0.92	0.04	10207 \pm 1294	549.6 \pm 1.9	0.0013 \pm 0.0001	0.0048 \pm 0.0003	0.1 \pm 0.24	0.18	–	
South Abaco	20–25	0.28 \pm 0.05	0.00	0.40	0.60	11549 \pm 1887	548.2 \pm 8.8	0.0017 \pm 0.0002	0.0037 \pm 0.0003	0.08 \pm 0.15	0.25	–	

Table 1 (Continued)

Population	Dewlap size		Dewlap pattern		Dewlap colour			Dewlap display			
	N	Size (cm ²)	Prop. solid	Prop. marginal	Prop. spotted	Brightness	Hue	RF 365 nm	RF 655 nm	DE rate (counts/min)	Prop. DE
South Bimini	22–24	0.37 ± 0.05	0.46	0.21	0.33	10856 ± 1267	547.0 ± 8.6	0.0017 ± 0.0002	0.0039 ± 0.0003	0.00	0.00
Staniel Cay	20	0.26 ± 0.06	0.81	0.19	0.00	–	–	–	–	–	–
Among-population variation		50.38%				44.05%	42.39%	69.63%	56.19%	–	–

DE, dewlap extension, '–', missing data; more details about the variables can be found in the 'Materials and methods' section.

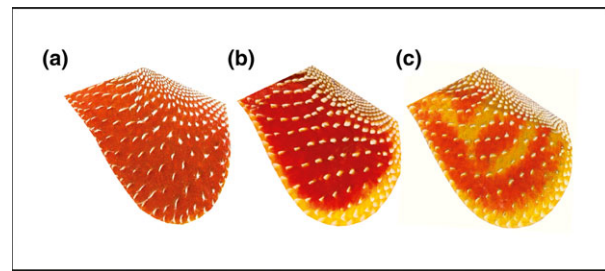


Fig. 2 Dewlap pattern categories used in this study, for both sexes of *Anolis sagrei*. (a) Solid, uniformly coloured (b) marginal, uniformly coloured centre with yellow margin and (c) spotted, yellow spots across a red centre regardless of the presence of a margin.

held perpendicular to the surface of the maximally extended dewlap (measuring point is ± 1 mm in radius). All measurements were expressed in relation to a white reference tile (WS2, Avantes). For both males and females, spectral reflectance was measured at the dewlap centre region (more details are provided in Driessens *et al.*, 2015). Reflectance data were collected for wavelengths from 300 to 700 nm, including the lower range of photon absorption by UV-sensitive photoreceptor cones published for anoles (Fleishman *et al.*, 1993). To investigate dewlap colour variation, we interpolated each spectrum to 1-nm wavelength intervals and extracted four variables following Ng *et al.* (2013a): brightness, hue, reflectance in the UV (RF 365 nm) and in red (RF 655 nm) (Montgomerie, 2006). We calculated brightness as the total area under the uncorrected spectral curve (300–700 nm) (Andersson *et al.*, 1998; Smiseth *et al.*, 2001). For the remaining three colour variables (i.e. hue, RF365 nm and RF 655 nm), we corrected each spectrum for brightness by making the area under the curve equal to 1 to allow the identification of differences in spectral shape independent of brightness (Endler, 1990). Hue was defined as the cut-on wavelength, that is the midpoint between baseline and maximum reflectance (Andersson *et al.*, 1998; Keyser & Hill, 2000; Saks *et al.*, 2003; Cummings, 2007). We decided to extract reflectance specifically in UV (365 nm) and red (655 nm), as the *A. sagrei* dewlap spectrum shows maxima and a high level of intraspecific variation at both wavelengths (Steffen & McGraw, 2007; Driessens *et al.*, 2015). The spectral measurements were made for 242 males and 217 females in total, distributed across nine populations. We do not have spectral data for the seven populations sampled by Vanhooydonck *et al.* (2009), or for the population from Central Cuba (Santa Clara) due to technical problems with the spectrometer. All analyses of spectral data were run in R using the 'pavo' package (Maia *et al.*, 2013).

Display

We observed each lizard ($N = 20$ –30 males and $N = 8$ –25 females per population; 10 populations in total) for

10 min, using a high-definition camera (Sony, HDR-CX260VE). We first located lizards by walking quietly through their natural habitat until an apparently undisturbed individual was spotted. We then videotaped the lizard from a distant location using the camera zoom function (30× optical zoom), to minimize any disturbance caused by our presence. Video recordings were only made during sunny or partly cloudy weather and between 9:00 and 16:30 h, to avoid possible confounding effects of weather and time on the lizard's activity level (Huey, 1982; Hertz *et al.*, 1993). All behavioural recordings were scored offline, using JWatcher 1.0 event recorder software (Blumstein *et al.*, 2000). To quantify dewlap display, we calculated the dewlap extension (DE) rate, expressed in counts per minute; one DE was defined as the complete extension and retraction of the throat fan. At the population level, we defined 'prop. DE' as the proportion of individuals that extended their dewlap at least once while they were filmed.

Climate-related environmental conditions

For each population, a set of environmental parameters was obtained from both climate (WorldClim, <http://www.worldclim.org>; Hijmans *et al.*, 2005; Meteorological Archival and Retrieval System, <https://software.ecmwf.int>) and Moderate Resolution Imaging Spectroradiometer (NASA-MODIS/Terra data set, <http://modis.gsfc.nasa.gov>) databases. Consistent with Ng *et al.* (2013a), we extracted annual precipitation (BIO12) and land surface temperature (LST) (MODIS MOD11A2 product). We additionally obtained annual global incoming shortwave radiation data (MARS) and the normalized difference vegetation index (NDVI) (MODIS MOD13Q1 product), which is the most frequently used remotely sensed indicator of vegetation and land-cover changes (Rouse *et al.*, 1974). Using the 'Global Subsetting Tool' (available online at http://daacmodis.ornl.gov/cgi-bin/MODIS/GLBVIZ_1_Glb/modis_subset_order_global_col5.pl), MOD13Q1 provided 16-day NDVI time series at a 250-m spatial resolution and we extracted from this data set a four-by-four pixel area centred at the sampling locality. The described environmental parameters (with exception of LST) can alter both the ambient light environment and background vegetation in a lizard's habitat; two factors that are known to strongly influence signal visibility (Endler, 1993a). Besides, the brown anole lizard is a territorial species, often moving and spending time in a variety of structural microhabitats within its home range (Schoener & Schoener, 1982; McMann, 1998). Based on the above arguments, we believe that the set of environmental parameters extracted for each population (spatial resolution of ca. 1 km²) is reliable for estimating the prevailing environmental conditions in which *A. sagrei* populations occur (consistent to Ng *et al.*, 2013a, using *A. distichus*).

Phylogeny

To account for the nonindependency of our data points (population means), we conducted our analyses in an explicit phylogenetic context (Felsenstein, 1985; Harvey & Pagel, 1991). We constructed a hypothesis on the phylogenetic relationships among our study populations, based on mitochondrial DNA genes (ND2 and tRNA's) available in GenBank (<http://www.ncbi.nlm.nih.gov>). In particular, we used mtDNA haplotypes obtained by Kolbe *et al.* (2004), as they were previously proven effective in assessing interpopulational relationships in *A. sagrei*. The islands, Andros, Crooked, Grand Bahama, Grand Cayman, Little Cayman, San Salvador, Cuba, South Abaco, South Bimini and Staniel Cay, have been sampled by Kolbe *et al.* (2004), and we assumed that our study populations occurring on these islands had the same genetic identity. No specific information was listed for Acklins, Chub Cay and Pigeon Cay, and we therefore assumed that lizards of these study populations are closest related to specimens collected from the nearest islands, that is Crooked Island, Berry Islands and Staniel Cay, respectively (R.G. Reynolds, personal communication). Notably, for Acklins, we incorporated the genetic sequence of a specimen from Crooked Island and this specimen was different from the one we used to represent our Crooked Island study population; the same working strategy was adopted for Chub Cay and Pigeon Cay (see Supporting information, Table S1 for all details of the used *A. sagrei* specimens with corresponding GenBank accession numbers). On Jamaica, Kolbe *et al.* (2004) described the co-occurrence of two haplotypes and possible hybridization. We performed separate analyses with either of the haplotypes, which resulted in the same phylogenetic tree. Consequently, we selected randomly one haplotype, as a representative for our Jamaican study population.

Prior to analysis, sequences were aligned using MAFFT v7 (Katoh & Standley, 2013), and refined with Gblocks v0.91b (Castresana, 2000; Talavera & Castresana, 2007). Phylogenetic trees were built through Bayesian inference as implemented in MrBayes v3.2 (Ronquist *et al.*, 2012), and the GTR+I+ Γ model was selected as the optimal model (jModelTest 2; Guindon & Gascuel, 2003; Durraba *et al.*, 2012). We performed 20×10^6 generation runs (Markov chain Monte Carlo), with trees sampled every 1000 generations, and a burn-in period of 4.0×10^6 generations. A 50% majority rule tree was constructed with the burn-in excluded using the 'sumt' command in MrBayes, and nodes with ≥ 0.95 posterior probability were considered supported. Overall, we found strong support for all nodes (i.e. posterior probability ≥ 0.95). A representation of the obtained Bayesian tree together with our sampling localities is provided in Fig. 3a.

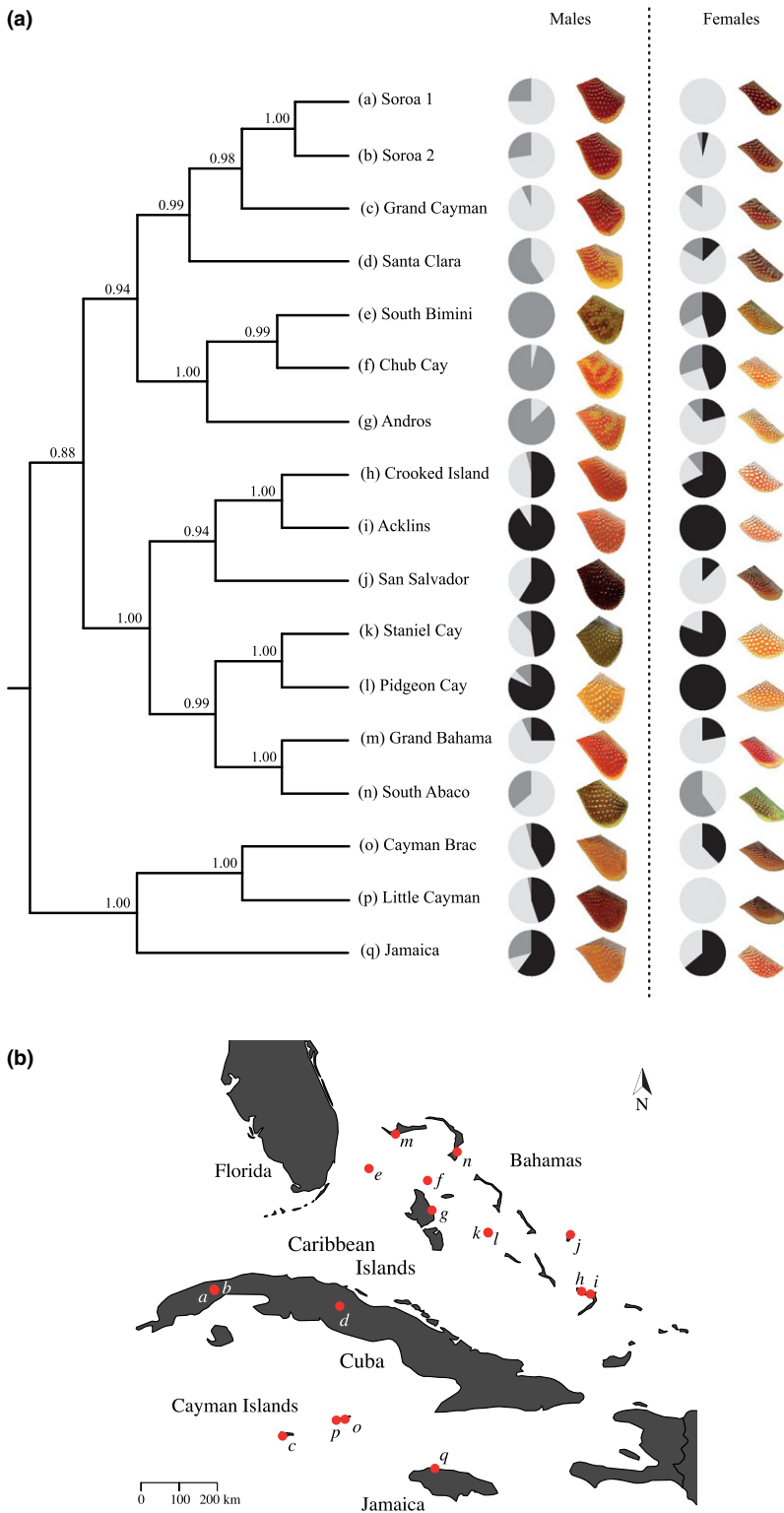


Fig. 3 Phylogenetic relationships among our seventeen *Anolis sagrei* study populations presented with corresponding sampling sites distributed across the Caribbean. (a) Phylogenetic relationships were estimated using a Bayesian analysis using mtDNA haplotypes from Kolbe *et al.* (2004); details on the specimens incorporated and the methods used to construct this phylogenetic tree are provided in the Materials and methods section (Phylogeny). Posterior probabilities > 0.95 are considered supported (shown at the nodes). Pie charts illustrate dewlap pattern proportions for each population per sex (black = solid; light grey = marginal; dark grey = spotted). Photographs represent male and female dewlaps of typical individuals from every population. The shown dewlap pattern corresponds with the patterning occurring in the largest proportion of individuals for a given population. Photographs were scaled differently (especially female dewlaps) and therefore provide no information about dewlap size. (b) Map of the Caribbean indicating sampling sites. Letters on the map and in the phylogeny refer to the respective study populations. Exact coordinates of the sampling localities can be found in the Supporting information (Table S1).

Statistics

All statistical analyses were run separately for males and females, due to a high degree of sexual dimorphism detected in most dewlap measurements.

To test for interpopulational differences in dewlap design, we ran separate one-way analyses of variance (ANOVAS), including population as factor; when the assumption of homogeneity of variances was not met, the Welch test was used instead. For dewlap pattern and display, Kruskal–Wallis tests were applied to deal with ordinal and not normally distributed variables, respectively.

For all further statistical analyses, we only considered interpopulational variation and therefore used population means and proportions of individuals per population as data points. We applied an arcsine square root transformation to the proportion data (dewlap pattern) to meet normality assumptions (Sokal & Rohlf, 1995). To reduce dimensionality and get rid of redundancy problems, we applied separate principal component analyses (PCAs) for dewlap display and the environmental parameters. We first assessed the validity of PCAs on our data sets by performing Bartlett tests of sphericity and the Kaiser–Meyer–Olkin Measure of Sampling Adequacy (MSA) (Bartlett, 1950, 1954; Kaiser, 1970; Dziuban & Shirkey, 1974). Only PC axes with eigenvalues greater than one were interpreted and used in subsequent regression analyses (i.e. PC dewlap display and PC environment).

To test whether variation in climate-related environmental conditions (i.e. PC environment) might explain interpopulational differences in dewlap design and display, we ran a series of univariate regression analyses incorporating population means and phylogenetic relationships. Specifically, we applied the phylogenetic generalized least squares (PGLS) method described in Revell (2010) and implemented by the `pgls()` function in R (caper package, Orme *et al.*, 2013). This method uses maximum likelihood to simultaneously estimate the regression model and phylogenetic signal (i.e. Pagel's λ) of the residual error (Garland & Ives, 2000; Revell, 2010). It has been shown to do better than *a priori* tests of phylogenetic signal to estimate the appropriateness of phylogenetically corrected tests, especially when sample sizes are smaller than 20 (Blomberg *et al.*, 2003; Revell, 2010; Kamilar & Cooper, 2013). Because data from one population (San Salvador) could only be obtained outside the breeding season, we performed an additional set of the same regression analyses excluding these particular data. *P*-values resulting from the phylogenetic regression analyses were corrected for multiple testing using the Benjamini–Hochberg procedure, which is described as a powerful method based on adjustment of the false discovery rate (Benjamini & Hochberg, 1995).

Statistical analyses were conducted in *SPSS* version 20 (SPSS, Chicago, IL, USA) and R version 3.2.1 (R Core Team 2015).

Results

Interpopulational variation in dewlap design and display

For males, we found substantial differences among populations for each dewlap characteristic we measured (ANOVAS or Welch tests: relative dewlap size $F_{16,140.29} = 18.55$, $N = 425$; brightness $F_{8,233} = 24.46$, $N = 242$; hue $F_{8,94.53} = 40.10$, $N = 242$; RF 365 nm $F_{8,95.14} = 92.22$, $N = 242$; RF 655 nm $F_{8,95.18} = 71.80$, $N = 242$, all $P < 0.001$; Kruskal–Wallis test: dewlap pattern $\chi^2(16) = 227.92$, $N = 428$; DE rate $\chi^2(9) = 108.89$, $N = 235$, all $P < 0.001$). Similar results were obtained for females with the exception of DE rate that did not differ among populations (ANOVAS or Welch tests: relative dewlap size $F_{16,112.17} = 20.41$, $N = 361$; brightness $F_{8,208} = 19.90$, $N = 217$; hue $F_{8,79.40} = 36.18$, $N = 217$; RF 365 nm $F_{8,208} = 47.69$, $N = 217$; RF 655 nm $F_{8,81.15} = 47.70$, $N = 217$, all $P < 0.000$; Kruskal–Wallis test: dewlap pattern $\chi^2(16) = 140.35$, $N = 364$, $P < 0.000$; DE rate $\chi^2(9) = 15.73$, $N = 189$, $P = 0.073$). Population means and standard deviations for all tested dewlap variables are provided in Table 1a, b.

PCAs and interpretations

Bartlett tests of sphericity and MSA yielded appropriate values, allowing us to perform valid PCAs on the population means. For dewlap display, PCAs were run separately for males and females incorporating mean DE rate and proportions of individuals showing DE (prop. DE). We obtained one significant axis per sex (PC dewlap display, males: eigenvalue = 1.92; females: eigenvalue = 1.83) explaining 95.90% of the variation in males and 91.71% in females. Higher values of PC dewlap display correspond in both sexes to more individuals showing DEs and a higher DE rate, which can be generally considered as more dewlap use (Table 2). For the PCA incorporating mean annual precipitation, radiation, NDVI and LST, also one PC axis was obtained (PC environment, eigenvalue = 2.85), explaining 64.57% of the variation. High values of PC environment correspond to relatively dark, dense green habitats with more rainfall (hereafter referred to as 'mesic' environments), whereas low values are consistent with more open, less green sites characterized by a higher incoming radiation ('xeric' environments) (Table 2).

Dewlap design and display linked to climate-related environmental conditions

Excluding San Salvador from the `pgls` regressions did not alter our obtained results for males, and the presented results will therefore always include this particular population (Table 3). For females, however, results from both regression sets are reported because data of

Table 2 Loadings of principal component analyses (PCAs) on dewlap display and climate-related environmental parameters with eigenvalues and variance (%).

Variables	PC (males)	PC (females)
Dewlap display (N = 10)		
Prop. DE	0.979	0.958
DE rate	0.979	0.958
Eigenvalue	1.92	1.83
Percentage variation	95.89	91.71
Environment (N = 17)		
Annual precipitation		0.957
Annual radiation	−0.902	
Annual NDVI		0.730
Annual LST		0.565
Eigenvalue		2.58
Percentage variation		64.57

Mean population values were always included in the PCAs with exception of the proportion of individuals showing a dewlap extension (Prop. DE). PCAs were run separately per sex for dewlap display. For environment, one PCA was performed and the same PC values were used in both sexes. Abbreviations: DE, dewlap extension; NDVI, normalized difference vegetation index; LST, land surface temperature.

San Salvador influenced the results for some variables substantially (Table 3). No associations were found between relative dewlap size and PC environment in males, nor in females ($P > 0.4$, Table 3). For dewlap pattern, we found PC environment to be a major predictor for the proportion of individuals with a solid or marginal, but not with a spotted dewlap (Table 3). Our results for dewlap pattern variation were similar for both sexes and showed that for populations with a low PC environment value (i.e. xeric environments), a higher proportion of solid dewlaps were present. In contrast, populations characterized by high PC environment values (i.e. mesic environments) contained a higher proportion of individuals with marginal dewlap patterns (Table 3). We are aware of a possible constant sum issue with respect to the proportional dewlap pattern variables and its implications for interpreting our results. We therefore report that no negative correlation between the proportion of individuals with solid and marginal dewlap pattern was obtained for the male sex (Spearman's correlation: $\rho = -0.34$, $P = 0.186$, $N = 17$). In females, however, a highly significant negative correlation was seen (Spearman's correlation: $\rho = -0.88$, $P = 0.000$, $N = 17$), presumably because of the low abundance of the spotted dewlap pattern in most of the study populations (Table 1b).

Dewlap colour was highly correlated with prevailing environmental conditions in males. In females, similar results were obtained as for males, only after excluding data of San Salvador (Table 3). Colour results specifically revealed a negative link between PC environment and the relative reflectance at UV (RF 365 nm)

(Fig. 4a, b). In addition, dewlaps from mesic environments (high values for PC environment) reflected more in red (RF 655 nm) (Fig. 4c, d). It is, however, important to note that RF 365 nm and RF 655 nm were strongly negatively correlated, in each sex (Pearson correlation: males, $r = -0.96$, $P < 0.001$, $N = 9$ and females, $r = -0.91$, $P < 0.001$, $N = 9$). Surprisingly, no significant relationships were found between environmental conditions and the other components of dewlap coloration, including brightness and hue (Table 3).

Lastly, we found that male lizards from populations with higher PC environment values use their dewlaps more frequently (Table 3). No such correlation between dewlap display and environment was seen in females.

Running standard (traditional) regression analyses without the incorporation of phylogenetic relationships revealed similar results.

Discussion

In the first step, we focused on documenting the inter-population variation in dewlap design and display of *A. sagrei* males and females, distributed across the Caribbean. In accordance with previous findings (Vanhooydonck *et al.*, 2009), we found that dewlap size and pattern differed significantly among populations of *A. sagrei*. Also dewlap colour, which was defined by brightness, hue and reflectance in UV and red, showed a high degree of inter-population variation. These results were obtained for both sexes separately, which highlight the importance of also incorporating data on female dewlap design in studies addressing dewlap diversity. For dewlap display, however, we only found inter-population differences in *A. sagrei* males. The low number of female individuals that actually exhibited dewlap extensions (DE) together with the low DE rates that were obtained may largely explain this result. The observation that females use dewlap displays far less compared to males is a commonly seen pattern that has already been reported for several anole species including *A. sagrei* (Partan *et al.*, 2011; Driessens *et al.*, 2014). Besides significant variation in dewlap phenotypes among populations, our data also reveal extensive variation within populations (see Table 1a, b for relative contributions), even within sexes.

In a second step, we assessed whether variation in climate-related environmental conditions could explain this remarkable dewlap diversity observed among populations. Our results showed that *A. sagrei* lizards occurring in xeric environments had a higher proportion of solid dewlaps with relatively high reflectance at UV. In addition, we found that lizards inhabiting mesic environments had primarily marginal dewlaps showing relatively high reflectance in red. Excluding data sampled outside the breeding season gave the same results in males. For females, however, dewlap colour (i.e. RF 365 nm and RF 655 nm) correlated significantly with

Table 3 Univariate phylogenetic linear regression analyses (pgls) of dewlap design and display vs. prevailing environmental conditions.

Dewlap variables	N	PC environment		P-value	r ²
		B	SE		
Males					
Relative dewlap size	17	0.017	0.018	0.408	0.055
Dewlap pattern – solid	17	-0.320	0.087	0.011	0.471
Dewlap pattern – marginal	17	0.287	0.090	0.014	0.402
Dewlap pattern – spotted	17	0.012	0.116	0.921	0.001
Dewlap colour – brightness	9	574.01	491.68	0.362	0.163
Dewlap colour – hue	9	-2.515	2.072	0.362	0.174
Dewlap colour – RF 365 nm	9	-2.97 × 10⁻⁴	4.79 × 10⁻⁵	0.004	0.846
Dewlap colour – RF 655 nm	9	5.14 × 10⁻⁴	1.23 × 10⁻⁴	0.012	0.714
PC dewlap display	10	0.750	0.238	0.024	0.554
Females (including San Salvador)					
Relative dewlap size	17	-0.002	0.013	0.929	0.002
Dewlap pattern – solid	17	-0.381	0.088	0.005	0.555
Dewlap pattern – marginal	17	0.338	0.086	0.006	0.505
Dewlap pattern – spotted	17	0.059	0.071	0.533	0.045
Dewlap colour – brightness	9	-47.14	513.27	0.929	0.001
Dewlap colour – hue	9	-3.915	2.018	0.195	0.350
Dewlap colour – RF 365 nm	9	-2.00 × 10⁻⁴	8.64 × 10⁻⁵	0.143	0.451
Dewlap colour – RF 655 nm	9	2.83 × 10⁻⁴	1.54 × 10⁻⁴	0.195	0.326
PC dewlap display	10	0.352	0.334	0.483	0.122
Females (excluding San Salvador)					
Relative dewlap size	16	-0.005	0.0148	0.733	0.009
Dewlap pattern – solid	16	-0.454	0.078	< 0.001	0.707
Dewlap pattern – marginal	16	0.444	0.079	< 0.001	0.692
Dewlap pattern – spotted	16	0.045	0.077	0.638	0.024
Dewlap colour – brightness	8	-811.03	472.47	0.246	0.329
Dewlap colour – hue	8	-1.758	2.845	0.638	0.060
Dewlap colour – RF 365 nm	8	-3.35 × 10⁻⁴	8.01 × 10⁻⁵	0.013	0.745
Dewlap colour – RF 655 nm	8	5.41 × 10⁻⁴	1.18 × 10⁻⁴	0.011	0.777
PC dewlap display	9	0.504	0.431	0.42	0.164

Results are shown separately per sex. For females, we additionally provided results from regression analyses excluding the population of San Salvador. *P*-values were corrected for multiple testing using the Benjamini–Hochberg method, and significant results ($P < 0.05$) are shown in bold font.

PC environment only after exclusion of the population sampled in San Salvador. Our results provide clear evidence for a relationship between signal design and prevailing environmental conditions in both sexes of *A. sagrei*, and additionally suggest that the interpopulational variation in signal design is not just the result of random genetic drift or haphazard changes in female preferences. Whether the observed variation in dewlap colour and pattern among *A. sagrei* populations is truly adaptive in terms of optimal signal transmission under disparate environmental conditions remains to be tested. Generally, colour signals should reflect the strongest regions of wavelengths in the ambient light, but at the same time stand out sufficiently against background vegetation to maximize their visibility within certain environments (Endler, 1990). A thorough study of efficacy of the dewlap signal would thus require information on the spectral properties of the ambient light, the reflectance of the background and the visual system of the receiver. Following Ng *et al.*

(2013a), we used global environmental parameters to estimate variation in prevailing environmental conditions and therefore cannot present conclusive data on this matter here. Yet, we have reasons to believe that the observed variation in dewlap colour and design may aid signal efficacy. In his study on the colour of lights in tropical forests around the world, Endler (1993b) distinguished four major light environments when the sun is not blocked by clouds: forest shade, woodland shade, small gaps and large gaps. In forest shade, the greenish leaf radiance dominates the irradiance spectrum because most of the light is transmitted through or reflected from leaves. In woodland shade, bluish sky radiance dominates because much of the light comes from the sky, through canopy holes. Large gaps receive a mixture of yellow-red irradiation (from the sun) and blue radiation (from the sky), combining into a white light. Smaller gaps will see less light from the sun and sky, and more light transmitted through or reflected from leaves, and are therefore characterized

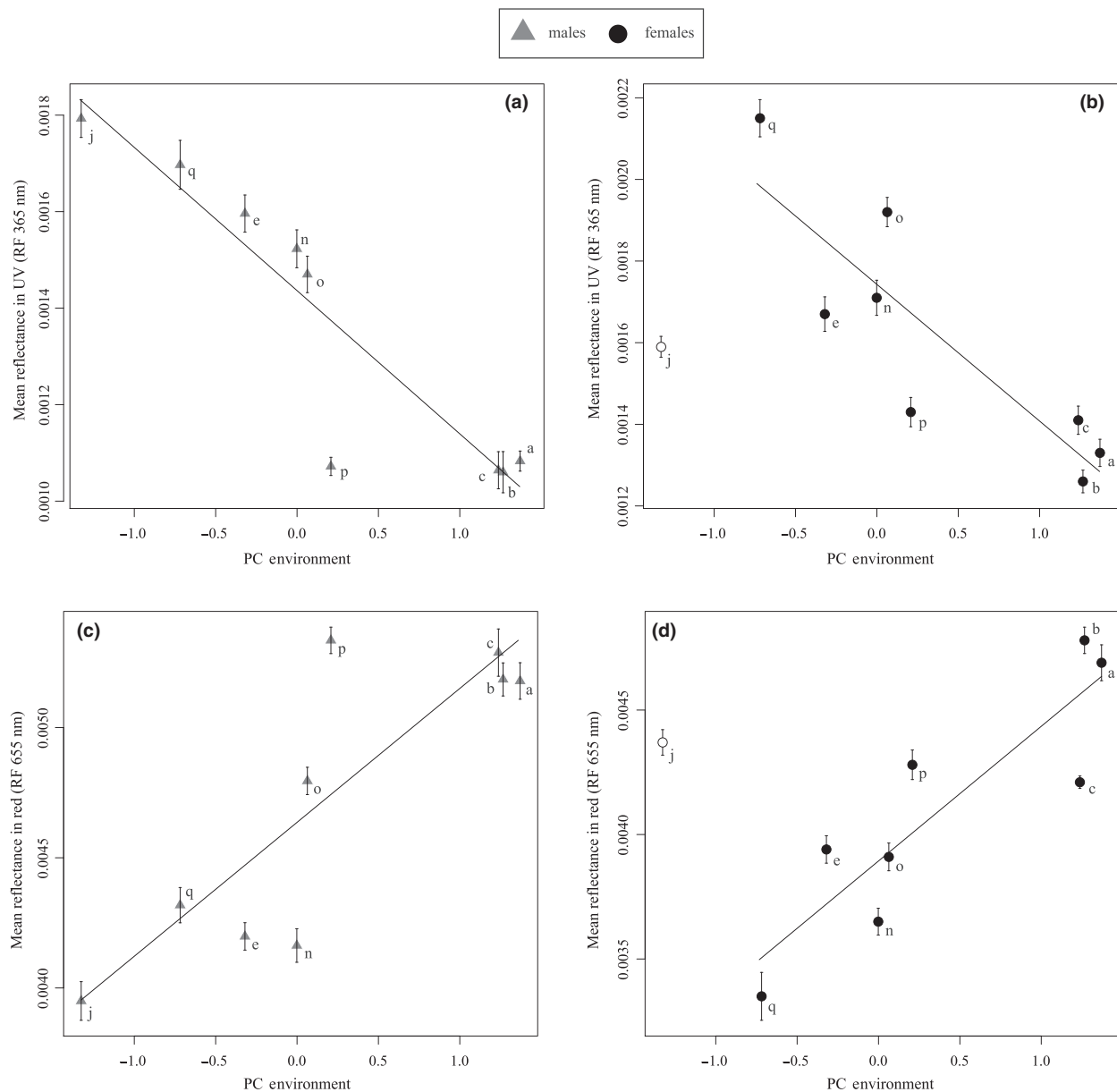


Fig. 4 Relationship between dewlap colour and climate-related environmental conditions. PglS regressions revealed significant associations between PC environment and (a, b) mean (\pm SE) relative reflectance in UV (RF 365 nm), and (c, d) mean relative reflectance in red (RF 655 nm). Data points for males (a, c) are represented by triangles and for females (b, d) by filled dots; letters denote corresponding study populations (see Fig. 3 for abbreviations). For females (b, d), *P*-values were only significant after excluding the San Salvador population and the regression lines were therefore fit discarding this population; the San Salvador data point (j) is represented by the white dot.

by reddish light. Assuming that the 'xeric' environments sampled in our study have light conditions that resemble Endler's 'woodland' and 'large gap' light environments, the relatively high UV reflectance of lizard dewlaps in these populations may enhance their detectability. Likewise, if 'mesic' environments sampled here have light conditions akin to Endler's 'forest shade' and 'small gaps', the reddish colours of the dewlaps would benefit visibility, because they will reflect

the light that is most available there and will additionally appear highly conspicuous against the dense green vegetation backgrounds (Endler, 1992). It should be noted that the evolution of red and UV reflection in *Anolis* dewlaps might be correlated. In *A. sagrei*, the orange-red colours of the dewlap are produced by drosopterin, a pigment that is known to absorb UV (Macedonia *et al.*, 2000; Steffen & McGraw, 2007, 2009). Dewlaps of lizards with high drosopterin concentrations

will therefore show strong reflectance in red, but low reflectance in UV (Steffen & McGraw, 2009). This trade-off is also evident in our measurements, both within and among populations. One could thus argue that low UV reflectance in mesic environments is a pleiotropic effect for selection for red colours, or vice versa. The obtained differences in dewlap pattern among *A. sagrei* populations could be explained along similar lines. In our xeric environments characterized by relatively open sites with high incoming radiation, additional colour contrasts created by patterning may not be required to assure visibility, resulting in a higher proportion of solid dewlaps here. Whereas in our mesic environments characterized by dense green sites with high rainfall and less incoming radiation, additional colour contrasts, such as the adjacency of a yellow margin with a red dewlap centre, are likely essential to ensure signal visibility (especially in 'forest shade' and 'small gaps' light environments; Endler, 1993b).

So far, two other studies have connected differences in *Anolis* dewlap coloration to variation in the physical environment. Ng *et al.* (2013a) showed that *A. distichus* males from environments with more rainfall and a higher percentage tree cover had dewlaps with higher reflectance in red, which corresponds nicely to our results. However, inconsistent to what we report here for *A. sagrei*, Ng *et al.* (2013a) found that precipitation level additionally predicted the brightness, cut-on wavelengths and size of *A. distichus* dewlaps. Also, Ng *et al.* (2013a) failed to explain variation in UV reflectance among *A. distichus* populations, whereas we found a clear association between UV reflectance and prevailing environmental conditions for *A. sagrei*. In the other study, on *A. cristellus*, Leal & Fleishman (2004) also found habitat-related differences in dewlap coloration, but here, the pattern was opposite: males from mesic habitats had bright and UV-reflective dewlaps, whereas males from xeric habitats had duller and redder dewlaps. It is unclear whether the disparity in results between these studies is due to differences in methodology or reflects actual differences between the species.

Alternatively, the relationship between environmental conditions and dewlap design reported here may come about by factors unrelated to light conditions and signal efficacy. For instance, climatic conditions and habitat structure are likely to affect the abundance and composition of prey species. Diet affects the coloration of many animals (Olson & Owens, 1998), including some lizards (Merkling *et al.*, 2016). However, a common garden breeding experiment in *A. distichus* (Ng *et al.*, 2013b) and a two-factor experiment assessing effects of nutritional stress and carotenoid supplementation in *A. sagrei* (Steffen *et al.*, 2010) do not support the idea that differences in dewlap coloration have a dietary origin. Climate and habitat structure may also affect the abundance and range of predators. In this regard, the observed relationship between climate-related

environment and dewlap design among *A. sagrei* populations might be rather attributed to variation in predation pressure (Vanhooydonck *et al.*, 2009). Yet, results from another study using data of the same *A. sagrei* populations in combination with estimates of relative predation pressure do not provide indications in that direction (Driessens, 2016). Finally, the number of syntopic *Anolis* species may largely depend on climate-related environmental conditions and may therefore impact dewlap design via reproductive character displacement (Vanhooydonck *et al.*, 2009). Previously reported results (Driessens, 2016) indeed showed that *A. sagrei* males (but not females) from populations with a higher number of syntopic *Anolis* species are more likely to have a 'spotted' dewlap pattern. Nevertheless, the number of syntopic species did not correlate with the proportion of solid and marginal dewlap patterns (Driessens, 2016), nor did it correlate with climate-related environmental conditions (Spearman's rho test: number of co-occurring *Anolis* species – PC environment, $\rho = 0.19$, $P = 0.467$, $N = 17$). Based on these findings, we believe that the relationship between dewlap design and environmental conditions discussed in this study (i.e. higher proportion of solid dewlaps and more UV reflectance in xeric environments vs. higher proportion of marginal dewlaps and more reflectance in red in mesic environments) is likely not the result of reproductive character displacement.

In addition to dewlap design, we linked display behaviour with variation in climate-related environmental conditions. We found that males use significantly more dewlap displays in mesic compared to xeric environments. For females, in contrast, no significant relation between signal diversity and prevailing environmental conditions was obtained. Previous research has noted that male anoles adjust their signalling behaviour to compete with distractions in their environment decreasing visibility, for example visual background noise and low-light conditions (Fleishman, 1988; Ord *et al.*, 2011). For example, lizards inhabiting poorly lit or windy environments compensate by enhancing the speed of display movements or extending the duration of displays (Ord *et al.*, 2007, 2010). Our finding that males increase dewlap use only in the mesic environments, characterized by a lower visibility due to poor light conditions and complex habitat structures, might thus be a simple strategy to enhance signal transmission. Based on previous results (Driessens, 2016), we believe that the contribution of predation and the number of syntopic species in explaining climate-related variation in dewlap use is limited; see previous paragraph for this discussion on dewlap design.

Dewlap colours of female *A. sagrei* followed a similar pattern of covariation with environmental conditions as seen in males. This may indicate genetic correlation (as expected if the same genes affect dewlap design in males and females). Yet, preliminary analyses on

A. sagrei data provide no support for the genetic by-product hypothesis in explaining female dewlap size. Alternatively, female dewlaps may experience the same selection regime as males (which would be expected if both are selected for efficacy). The interpretation of our results for the female dewlap is hampered by the fact that a relationship was found only after exclusion of the San Salvador data, which were obtained outside the breeding season. This opens the interesting possibility that female dewlap design changes considerably with the reproductive status of the female. In a previous publication, we already hinted at the possible significance of UV reflection as an indicator of receptivity in female *A. sagrei* (Driessens *et al.*, 2015). Alternatively, different selective pressure may be acting on San Salvador. Clearly, the female dewlap is understudied and deserves closer attention.

In the above, we may have overemphasized the possible role of genetic adaptation in producing among-population variation in the signalling system under study. In reality, we have no information on the repeatability and heritability of the traits considered, nor on how much they can change within individuals through time. As a consequence, we cannot ascertain whether the correlations between environmental variables and signalling characteristics arose through genetic adaptation, through adaptive plasticity or both. A number of recent studies have demonstrated the importance of contextual (Ord *et al.*, 2010), seasonal (Lailvaux *et al.*, 2015) and developmental plasticity (Bonneaud *et al.*, 2016) in anole dewlaps.

In conclusion, our observations reveal an association between climate-related environmental conditions and aspects of dewlap design and use in *A. sagrei*. We have presented some indications that this association may result from differential selection on signal efficacy. Yet, detailed measurements of local light conditions and backgrounds, as well as the visual perception of *A. sagrei* are required to further test this idea. Our results additionally invite a closer look at female dewlaps. Testing more species with inclusion of data on the female sex too would thus further enrich our knowledge of the evolution of dewlap diversity and its role in speciation.

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Supporting information

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

Table S1 Genetic populations with corresponding GenBank accession numbers of the specimens used to assess phylogenetic relationships among our 17 *A. sagrei* study populations

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