

Dewlap color variation in *Anolis sagrei* is maintained among habitats within islands of the West Indies

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Abstract

Animal signals evolve in an ecological context. Locally adapting animal sexual signals can be especially important for initiating or reinforcing reproductive isolation during the early stages of speciation. Previous studies have demonstrated that dewlap color in *Anolis* lizards can be highly variable between populations in relation to both biotic and abiotic adaptive drivers at relatively large geographical scales. Here, we investigated differentiation of dewlap coloration among habitat-types at a small spatial scale, within multiple islands of the West Indies, as this may give new insights into the local scale at which adaptation is possible. We explored variation in dewlap coloration in the most widespread species of anole, *Anolis sagrei*, across three characteristic habitats spanning the Bahamas and the Cayman Islands. Using reflectance spectrometry as well as supervised machine learning, we found significant differences in spectral properties of the dewlap between habitats within small islands, sometimes over very short distances. Passive divergence in dewlap phenotype associated with isolation-by-distance did not explain our results. On the other hand, these habitat-specific dewlap differences varied in magnitude and direction across islands, and thus our primary test for adaptation – parallel responses across islands – was not supported. We suggest, however, that selection could be involved in several ways, including sexual selection. Our results shed new light on the scale at which signal color polymorphism can be maintained in the presence of gene flow, and the relative role of local adaptation and other processes in driving these patterns.

Keywords — reflectance, adaptation, sexual signal, machine learning, polymorphism

Introduction

The staggering diversity of animal communication signals has long been of interest to evolutionary biologists. Animals use chemical, mechanical, electromagnetic, and visual signals to communicate in a wide variety of contexts, including, competition for mates, species recognition, aposematism, and cooperation (Bradbury and Vehrencamp, 2011). A primary evolutionary factor shaping communication signals is the sensory system and behavior of their recipients (the sensory drive hypothesis; Endler and McLellan 1988; Endler 1992, 1998). Over the past decades, scientists have established that signals evolve in an ecological context and are dependent on environmental conditions (Endler, 1992, 1993a,b). Just as different habitats may favor different combinations of eco-morphological traits to maximize performance and fitness (Arnold, 1983), they may also shape different forms of a signal, so as to maximize its transmission and detection (e.g. Seehausen 1997), or reduce its detection by unintended recipients such as predators (Endler, 1984, 1990, 1991; Halfwerk et al., 2014). This selective pressure may drive the local adaptation of communication

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37 signals.

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39 One potential barrier to the maintenance of localized signal divergence is the homogenizing effect
40 of gene flow. Population genetics theory suggests that gene flow may counteract local adaptation
41 between localities and prevent divergence altogether, especially at small spatial scales, because
42 of the inflow of maladapted alleles or because of the breaking of linkage between coevolving loci
43 (Felsenstein, 1976; García-Ramos and Kirkpatrick, 1997; Dieckmann and Doebeli, 1999; Lenor-
44 mand, 2002; Hendry et al., 2007a). This genetic homogenization has been confirmed empirically
45 in systems such as stick-insects (Nosil and Crespi, 2004) and sticklebacks (Hendry et al., 2007b).
46 Yet, examples of microgeographic adaptation, i.e. adaptation at smaller scales than the range of
47 dispersal, exist, highlighting the potential of some organisms to respond to selection in the face of
48 gene flow (see Richardson et al. 2014 and references therein). Examples include small scale adap-
49 tation in fragmented areas in Australian fruit flies (Willi and Hoffmann, 2012), or local adaptation
50 to predation pressure in North American salamanders (Richardson and Urban, 2013). Therefore,
51 despite evidence that local adaptation may be particularly difficult at small spatial scales where
52 gene flow tends to cause adjoining populations to remain genetically homogeneous, the potential
53 adaptive response of species traits, in particular communication signals, to localized differences in
54 habitats remains relatively unknown (Richardson et al., 2014).

55
56 Lizards of the neotropical genus *Anolis* are an excellent group for studying the eco-evolutionary
57 dynamics of local adaptation and natural selection (Losos, 2009). A particularly conspicuous trait
58 of anoles is their dewlap; an extensible flap of skin that is typically sexually dimorphic and used as
59 a communication signal in courtship (Sigmund, 1983; Driessens et al., 2014, 2015) and territorial
60 displays (Losos, 1985; Macedonia and Stamps, 1994; Macedonia et al., 2013) as well as in pred-
61 tor deterrence (Leal and Rodríguez-Robles, 1995, 1997; ?). Dewlap characteristics vary widely
62 among the approximately 400 species of the genus (Nicholson et al., 2007). Interspecific variation
63 in dewlap coloration is implicated in species recognition (?Williams, 1969; Williams and Rand,
64 1977; Losos, 1985; Macedonia and Stamps, 1994; Fleishman, 2000; Macedonia et al., 2013), and
65 this function could have had a role in initiating and/or reinforcing reproductive isolation during
66 speciation (Lambert et al., 2013; Geneva et al., 2015; Ng et al., 2017).

67
68 Within species, studies have shown a link between variation in dewlap coloration and differences
69 in habitats or climatic conditions (Macedonia, 2001; Leal and Fleishman, 2002; Thorpe, 2002; ?;
70 Leal and Fleishman, 2004; Vanhooydonck et al., 2009; Ng et al., 2012, 2013, 2016; Vanhooydonck
71 et al., 2009; Driessens et al., 2017). Some studies suggest that those differences may be adap-
72 tive, and that dewlaps may have evolved to maximize detectability given local light conditions
73 (Fleishman and Persons, 2001; Leal and Fleishman, 2002, 2004). Although this claim is further
74 supported by recent findings that dewlap colors are perceived differently under different levels of
75 shading (Fleishman et al., 2020), other studies found conflicting patterns of between-habitat vari-
76 ation that did not support the sensory drive hypothesis (Fleishman et al., 2009; Ng et al., 2012;
77 ?).

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79 Previous studies investigating variation in anole dewlaps compared populations at relatively
80 large geographical scales, e.g. between islands (Vanhooydonck et al., 2009; Driessens et al., 2017)
81 or within large islands such as Puerto Rico (Leal and Fleishman, 2004) or Hispaniola (Ng et al.,
82 2012, 2016). These large scales and marine barriers should reduce gene flow (Ng and Glor, 2011;
83 Lambert et al., 2013; Richardson et al., 2014; Ng et al., 2017). That said, examples do exist of
84 divergence in dewlap coloration at smaller scales or between populations with high degrees of gene
85 flow (Thorpe, 2002; ?; Stapley et al., 2011; Ng et al., 2016).

86
87 The species *Anolis sagrei* is widespread across islands of the West Indies (Reynolds et al.,
88 2020). It has been the subject of numerous studies concerning local adaptation (Losos et al., 1994,
89 1997, 2001; Kolbe et al., 2012), biological invasion (Kolbe et al., 2008), sexual selection (Tokarz,
90 2002; Tokarz et al., 2005; Tokarz, 2006; Driessens et al., 2014; Steffen and Guyer, 2014; Driessens
91 et al., 2015) and many other topics. Between-island variation in the mainly orange-red color of
92 its dewlap was shown to be better explained by climatic variables (Driessens et al., 2017) than by
93 proxies for biotic factors such as sexual selection or predation pressure (Vanhooydonck et al., 2009;
94 Baeckens et al., 2018). How intra-island differences in habitat may contribute to the diversity of

dewlap coloration, however, remains unexplored, and may reveal new insights into the scale of local differentiation despite gene flow.

Here, we analyzed the color characteristics of *A. sagrei* dewlaps within nine islands in the Bahamas and Cayman Islands. These island systems presently, if not historically, comprise relatively small islands, with no major geographic barriers within islands limiting dispersal for this promiscuous species (Kamath and Losos, 2018). These islands all share three characteristic native West Indian habitat-types – beach scrub bush, closed-canopy primary coppice forest, and mangrove forest – that are often spatially intermingled. These habitats contrast in environmental parameters including vegetation community, light irradiance, humidity and temperature (Howard, 1950; Schoener, 1968). The Cayman Islands and the Bahamas have been colonized independently by *A. sagrei* from Cuba (Reynolds et al. 2020, van de Schoot et al. unpubl.), such that these archipelagos constitute an ideal suite of natural replicates to explore within-island dewlap diversity across multiple islands.

Our sampling design included sites in close proximity; the median distance between two sites within an island was 11.2km. Combining reflectance spectrometry and supervised machine learning, we tested for divergence in dewlap phenotype between habitats within islands and between islands across the range of *A. sagrei*. We predicted that if light conditions in the environment indeed drive color evolution, dewlaps should be most similar between beach scrub and mangrove forest, which both have high levels of light irradiance, compared to the darker, closed-canopy coppice forest. Similar, if detectability is maximized given the local conditions, we expected darker and more contrasting dewlaps in high irradiance habitats. Finally, if habitat characteristics are strong determinants of dewlap color variation, similar patterns should be observed across multiple islands (Losos, 2011).

120 **Methods**

121 **Data collection**

122 We sampled 466 male *Anolis sagrei* from seven islands in the Bahamas Archipelago – Abaco,
123 North Andros, South Andros, South Bimini, Eleuthera, Long Island, Ragged Island – and two
124 in the Cayman Islands – Cayman Brac and Little Cayman (Figure S1A). These islands were
125 chosen to span the breadth of the West Indian range of *A. sagrei*, because they have highly similar
126 habitat types, and because the *A. sagrei* on each island group are derived from ancient and distinct
127 colonization events from Cuba (i.e. relatively evolutionarily independent, Reynolds et al. 2020).
128 Three habitats were sampled on each island based on characterizations by Howard (1950) and
129 Schoener (1968). Each habitat is clearly distinguishable by its dominant vegetation type — xeric
130 coastal scrub (open, relatively dry habitat consisting of low vegetation or isolated trees), primary
131 coppice forest (closed-canopy forest) and mangrove forest (wet coastal habitat with trees growing
132 in brackish water and high light penetration). Sample sizes are given in Table S1. Our sampling
133 design enabled us to test for differences between habitats at a coarse and fine geographical scale.
134 The median distance between two localities within an island was ~ 11km, with some islands being
135 sampled at smaller or larger scales (Figure S1B), and 80.3% of all pairwise distances within islands
136 were less than 50km. Additionally, there are no major barriers to dispersal (such as mountains or
137 grassland) on any of the islands that we sampled.

138 **Reflectance measurements**

139 We measured reflectance between 300 and 700nm wavelength, a range that encompasses the colors
140 visible to most lizards and vertebrates in general (Lazareva et al., 2012). Measurements were taken
141 with an Ocean Optics USB4000 spectrometer, a pulsed Xenon light source (PX-2, Ocean Optics,
142 Largo, FL, USA) and a reflectance probe protected by a black anodized aluminum sheath. Mea-
143 surements were taken with a 45-degree inclination to prevent specular reflection (Endler, 1990).
144 The device was regularly standardized with a Spectralon white standard (Labsphere, North Sutton,
145 NH, USA). Reflectance was measured at the center of the dewlap. Reflectance curves were
146 smoothed using the R package pavo (Maia et al., 2013) as well as with custom R functions, down
147 to one reflectance value at each nanometer in wavelength from 300 to 700nm.

148 **Analysis**

149 We tested for detectable differences in dewlap coloration between populations from different habi-
150 tats across islands by following an analytic pipeline in several steps. First, we used multivariate
151 analyses of variance to assess the relative contributions of islands, habitats and habitat-by-island
152 interactions on the partitioning of variation in color space. Second, and provided that habitat-by-
153 island interactions were found, we investigated habitat-differences in dewlap color for each island
154 separately using machine learning classification. Third, for each island where multivariate differ-
155 ences were detected using our machine learning pipeline, we used univariate analyses of variance
156 to decompose the signal among the different dimensions of color space. Fourth, for each significant
157 between-habitat variation found in univariate analyses, we used posthoc tests to determine which
158 habitats were involved. Last, to get insights into the spatial scale of phenotypic variation, for each
159 significant contrast between two habitats detected along a given dimension on a given island we
160 performed multiple pairwise Wilcoxon tests to assess differences in dewlap coloration among the
161 sites involved in that significant contrast, and recorded the geographical distance between sites
162 that were found significant. In parallel, we tested a possible effect of isolation-by-distance, an al-
163 ternative cause of phenotypic divergence between localities based on diffusion approximation and
164 dispersal distance, irrespective of habitat-types. We did so using a permutation test to assess the
165 significance of the correlation between geographical distances and phenotypic distances among sites
166 within each island.

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168 All analyses in this study were performed in R 3.6.1 (R Core Team, 2019) and the source code
169 can be found at <https://github.com/rscherrer/dewlap>, presently private.

Dimensionality reduction

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Because neighboring wavelengths are highly collinear and redundant in reflectance, we reduced the dimensionality of the data using principal component analysis (PCA), as per Cuthill et al. (1999) and Leal and Fleishman (2002). We performed PCA on data from all islands combined, as well as on each island separately and systematically retained the first four principal components (PC), which together always explained more than 88.8% of the variance across islands (Table S2). PCs need not represent the same wavelengths across islands because they are fitted on different datasets. Nevertheless, PC1 was highly collinear with brightness for all islands (Figure S9), while the other PCs captured the chromatic variation (i.e. irrespective of brightness) in dewlap color.

Among-island variance partitioning

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We performed a two-way nonparametric multivariate analysis of variance (PERMANOVA, Anderson 2001, R package vegan, Oksanen et al. 2019) to identify differences in coloration between islands, habitats and habitats within islands, using principal components fitted on data from all islands together. We used a nonparametric test because although no multivariate outliers were detected based on the Mahalanobis distance, the assumption of multivariate normality was violated in several habitats on several islands (Henze-Zirkler's test, Henze and Zirkler 1990, R package MVN, Korkmaz et al. 2014, $P < 0.05$, Table S3).

Within-island machine learning

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We performed a machine learning classification analysis on the first four principal components within each island separately, using random forests (Breiman, 2001). Random forests are a versatile, intuitive and powerful algorithm commonly used in machine learning, using decision trees to predict the labels of particular observations, based on their multivariate coordinates, i.e. variables, passed through a series of successive decision nodes, each examining a given variable of any given observation (James et al., 2013). The prediction for each observation is an aggregate over a large number of decision trees, each being trained on a subset of observations sampled with replacement from the dataset, and each allowed to examine only a subset of the variables. This allows the random forest to overcome the individual errors of all trees in the predictions it makes.

To detect differences in dewlap coloration between habitats, we measured the success of random forests in reassigning individual lizards to their correct habitat of origin, based solely on their principal component scores. In machine learning, this so-called cross-validation procedure is typically done in two steps (James et al., 2013). First, a random forest is trained in recognizing features of dewlap coloration most associated with the different habitats, by being presented multiple observations, making predictions about them and updating its own decision rules based on whether the prediction deviates from the truth. Then, once trained, the patterns that the random forest has learned to recognize are tested by presenting new, previously unseen observations to the random forest, and measuring the proportion of correct predictions. This proportion, or success score, can then be statistically assessed against random guessing using a binomial test.

The cross-validation procedure requires that the data be split into a training set and a testing set. To remove any bias due to the set that is being sampled for training, it is common practice to use k-fold cross-validation (James et al., 2013), where the data is split into k random bins and k independent machines are trained, each taking one of the bins as a testing set and the rest for training, and where classification success is measured by summing all correct classifications from the k machines.

Here, we used a k-fold cross-validation procedure with $k = 5$, where each training set consisted of 80% of the data and the machine was tested on the remaining 20%. Each training set was conditioned on containing at least 5 lizards from each of the three habitats. We also down-sampled the training set to the sample size of the least represented habitat, to ensure that the different habitats were equally represented. To further remove any bias due to the specific random split into the different bins, we replicated each k-fold cross-validation five times. We then averaged the five resulting confusion matrices across replicates, where each confusion matrix shows the number of lizards from each habitat reassigned into each habitat. For each island, we then used the aver-

age proportion of correctly reassigned lizards (i.e. the proportion of observations on the diagonal of the average confusion matrix) as an estimate of classification success. This score was tested against random guessing by comparing it to a binomial distribution with number of trials being the number of lizards on that island and success probability 1/3, representing the rate of successful classification by chance when three habitats are involved.

We used the machine learning fitting functions in the R package rminer ([Cortez, 2020](#)), which call random forest routines from the randomForest package ([Liaw and Wiener 2002](#), implementation from the original random forest algorithm, [Breiman, 2001](#)). For each random forest, we optimized the number of trees in the forest and the number of variables examined by each tree using the grid hyperparameter search procedure implemented in rminer, to choose between two numbers of trees (500 or 1,000) and four numbers of principal components examined per tree (1 to 4), using rminer's ordered holdout validation method with 2/3 of the data used for training.

We validated the results of our analysis by using two other widely used machine learning classification methods: linear discriminant analysis and support vector machines ([Cristianini and Shawe-Taylor, 2000](#); [James et al., 2013](#)), both accessible in rminer ([Cortez, 2020](#)).

To know which wavelengths were most used to assign data points to each habitat, we trained another set of random forests, this time directly on reflectance data (taken every 5nm from 300 to 700nm) instead of principal components. We recorded the relative importance of each wavelength for each habitat, as measured by the mean decrease in accuracy during wavelength permutation, implemented in the randomForest package ([Liaw and Wiener, 2002](#)).

Univariate analyses

For each island where significant differences in dewlap coloration were detected between habitats, we used multiple univariate analyses of variance (ANOVA) to identify possible principal components underlying the observed differences. We constructed our ANOVA models in two steps, as per [Zuur \(2009\)](#). In a first step, we accounted for heterogeneity of variances across groups by systematically comparing the goodness-of-fit of an ANOVA model estimated with ordinary least squares (OLS) with that of a model estimated with generalized least squares (GLS), which allowed one estimate of residual variance per habitat (using the R package nlme, [Pinheiro and Bates 2000](#); [Pinheiro et al. 2020](#)). Both models were fitted with restricted maximum likelihood (REML). Goodness-of-fit was estimated using Akaike's Information Criterion corrected for small sample sizes (AICc, R package MuMIn, [Bartoń 2019](#)), and the estimation method yielding the lowest AICc was retained. In a second step, we re-fitted the retained model with maximum likelihood (ML) to test for the effect of habitat-type using likelihood ratio tests (LRT) between a model including a habitat-term and a null model lacking the habitat-term.

We evaluated the normality of the standardized residuals (residuals divided by their standard error, which can differ among habitats in a GLS model) of each fitted ANOVA model using Shapiro-Wilk's test, with P-values adjusted for multiple testing using the Benjamini-Hochberg correction ([Benjamini and Hochberg, 1995](#)). In cases where significant deviations from normality were detected ($P_{adj} < 0.05$, Table S4) we performed Kruskal-Wallis's nonparametric test to back up the ANOVA results.

To know which habitat-populations were different from which in dewlap coloration, we performed different post-hoc multiple comparison tests (all implemented in the PMCMRplus package, [Pohlert, 2020](#)), depending on which assumptions were met. In cases where normality and homoskedasticity were met (i.e. OLS-ANOVA was the best fit), we used Tukey's honest significant difference test. When normality was met but not homoskedasticity (i.e. GLS-ANOVA was the best fit), we used Dunnett's T3 test. Finally, whenever we used Kruskal-Wallis's test because the ANOVA residuals were not normally distributed, we used Nemenyi's test for post-hoc comparisons.

Spatial autocorrelation

We tested for within-island spatial autocorrelation between the geographical distances among sampling sites and their Euclidean distances in multivariate color space (mean PC1 to PC4 per site,

Table S5), regardless of habitat-type. Because often only a few sites were sampled per island, we
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could not get meaningful results from tests that use sites as units of observation, such as Moran's
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I test (Gittleman and Kot, 1990). Instead, we designed a permutation test where we randomly
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reshuffled individual lizards across sites within islands 1,000 times each, and systematically re-
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calculated Pearson's correlation coefficient between geographic distances (computed as geodesic
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distances in the R package geosphere; Hijmans 2019) and phenotypic distances. We used the re-
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sulting null distributions of correlation coefficients to assess the significance of the observed spatial
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autocorrelation for each island.
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Site differences

In this study, we were interested in the minimum spatial scale at which significant differences
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between habitats could be detected within islands. We performed multiple pairwise nonparametric
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Wilcoxon-Mann-Whitney tests (Hollander et al., 2013) to compare dewlap coloration between
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sites with different habitat-types, for each pair of habitats and each variable where significant
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differences were detected with our analyses of variances. The P-values were adjusted using a
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Benjamini-Hochberg correction for multiple testing (Benjamini and Hochberg, 1995).
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294 **Results**

295 We tested for variation in *A. sagrei* dewlap coloration between populations living in three char-
296 acteristic habitat types across nine islands that span the West Indian range of the brown anole
297 (coastal scrub, primary coppice and mangroves). We found that most of the variation in coloration
298 was partitioned between islands (two-way PERMANOVA, approx. $F(df = 8) = 43.7, P = 0.0001$,
299 Fig. ??, explained variance $R^2 = 40.9\%$). Nonetheless, we did find evidence for differences in
300 dewlap coloration between habitat-types, and those were mostly island-specific (habitat-by-island
301 interaction term, approx. $F(16) = 3.53, P = 0.0001, R^2 = 6.6\%$), with a small but significant
302 portion of the variation explained by an archipelago-wide habitat effect (approx. $F(2) = 4.7,$
303 $P = 0.0001, R^2 = 1.1\%$).

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305 We subsequently tested for differences in dewlap coloration between habitat-populations within
306 each island, using within-island principal component scores, that is, computed from the data spe-
307 cific to each island (to maximize the variation captured for each island). Our within-island random
308 forest classification analyses revealed detectable differences in dewlap coloration on seven out of
309 the nine islands in our sample: Abaco, Bimini, Cayman Brac, Eleuthera, Little Cayman, Long
310 Island and South Andros. On these islands, our classifiers could reassign individual dewlaps to
311 their correct habitat more often than expected by chance (Table 1). We obtained nearly identical
312 results using support vector machines (Table S7) and linear discriminant analysis (Table S8) for
313 classification. We did not find evidence of spatial autocorrelation in dewlap coloration between
314 the sites within islands, except for Eleuthera (Table 2). We now describe the specific differences
315 detected on each island.

316
317 On Abaco, we detected the strongest differences between the coastal and mangrove habitats,
318 while dewlaps from the coppice habitat were more difficult to classify (Fig. 1A). Importance anal-
319 ysis revealed that coastal and mangrove lizards mostly differed in reflectance in the UV-end of the
320 spectrum (below 400nm, Fig. 1B), where mangrove dewlaps had higher UV reflectance relative
321 to coastal lizards, and coppice lizards had an intermediate UV reflectance between the two other
322 habitats (Fig. 1C). Consistent with this, our analyses of variance detected significantly lower PC2
323 scores in mangrove lizards than in the two other habitats, representing a higher UV-reflectance
324 relative to red (Fig. 1D, E, Table 3). Coastal lizards also scored lower on PC3, indicating less
325 curvature of the reflectance profile and relatively higher reflectance at intermediate wavelengths
326 (blue-to-yellow) than at the ends of the range (Fig. 1D, E). Differences were detected between
327 sites both at large ($\sim 100\text{km}$) and short ($< 1\text{km}$) distances (Fig. 1F, G).

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329 On Bimini, the random forests mostly correctly classified lizards from the coastal habitat (Fig.
330 S2A), with a relatively flat importance profile suggesting that brightness was used instead of a
331 particular wavelength (Fig. S2B). Indeed, some coastal dewlaps were substantially brighter than
332 the rest (Fig. S2C), a pattern that was captured by our analysis of variance along PC1 (i.e. bright-
333 ness, Fig. S2D, E, Table 3). Coastal lizards were also characterized by elevated red reflectance
334 relative to UV (as represented by PC2, (Fig. S2D, E)), and coastal and mangrove lizards were
335 characterized by a more even distribution of the reflectance along the spectrum (as represented
336 by PC3, (Fig. S2D, E)), in contrast to coppice lizards which harbored a stronger curvature at
337 intermediate wavelengths (Fig. S2D, E). On this island, the coastal and coppice habitats were
338 separated by a few hundred meters, making this contrast the smallest geographical scale at which
339 differences in coloration were found in our study (Fig. S2F, G).

340
341 On Cayman Brac, all three habitats could be fairly well discriminated against each other (Fig.
342 S3A), with UV reflectance appearing to be again an important variable (Fig. S3B). Coastal and
343 mangrove lizards were the best differentiated habitats (Fig. S3A). At a distance between 2 and
344 3km (Fig. S3F, G), dewlaps in the coastal habitat reflected more red light (as represented by
345 PC2, Fig. S3D, E) and more UV (as represented by PC3) than in the mangrove habitat. Coppice
346 lizards were characterized by darker dewlaps than the rest (represented by PC1), and a higher UV
347 reflectance than mangrove dewlaps (PC3, Fig. S3D, E, Table 3).

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349 Eleuthera was the only island where we detected significant spatial autocorrelation (Table 2),
350 that is, sites that were closer geographically tended to have populations of lizards with more simi-

lar dewlap colors. The strongest identified differences were between coastal and mangrove lizards (Fig. S4A), where coastal lizards had higher levels of red reflectance and mangrove lizards higher levels of UV reflectance (as represented by PC2, Fig. S4D, E, Table 3). 351
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Little Cayman was also characterized by a good discrimination of all three habitats, particularly of mangrove lizards (Fig. S5A). These lizards differed in short wavelengths from the rest (Fig. S5B), with significantly lower UV reflectance (as represented by PC2, Fig. S5D, E, Table 3). Coastal lizards were characterized by brighter dewlaps than coppice lizards (PC1), and also more convex curves, i.e. slightly higher UV and red reflectance (as represented by higher PC3 scores), than lizards from the other two habitats (Fig. S5D, E, Table 3). 355
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On Long Island the three habitats were well discriminated too, with the largest differences between coastal and mangrove lizards (Fig. S6A). Coastal lizards had more curved reflectance profiles than in either of the two other habitats, with higher levels of UV and red reflectance relative to intermediate wavelengths (PC3, Fig. S6D, E, Table 3). Coastal lizards also differed from mangrove lizards along PC4 (Fig. S6D), which represents a rather small portion of the variance not already explained by the first three principal components, and is therefore difficult to interpret (Fig. S6E). Coppice lizards were significantly darker than mangrove and coastal lizards (PC1, Fig. S6D, E, Table 3). 362
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On North Andros, although the random forest classification was significant ($P = 0.0216$, Table 1) and the average confusion matrix indicated that lizards from beach scrub were particularly well predicted (Fig. S7A), no significant univariate differences were detected along any of the four PCs (Fig. S7D, Table 3). Importance analysis of full-spectrum random forests showed higher importance scores near the UV-end of the spectrum in discriminating beach scrub dewlaps from the rest (Fig. S7B). Besides, reflectance curves of beach scrub dewlaps appeared more similar to each other in the UV range than dewlaps from other habitats (Fig. S7C), suggesting that the machines may have used this low within-habitat variance, as opposed to between-habitat differences in means, to correctly classify beach scrub lizards. A small sample size on this island may also have contributed to a lack of power in detecting univariate differences using analyses of variance (Table S1). 382
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On South Andros classification was not as successful as on other islands, but coastal and coppice dewlaps could be discriminated better than expected by chance (Fig. S8A). Coppice lizards had more curved reflectance profiles than coastal lizards (PC3), and lizards from both habitats differed along PC4, which is again more difficult to interpret (Fig. S8D, E, Table 3). Coastal lizards also differed from mangrove lizards in PC4 (Fig. S8D, E, Table 3). 382
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Classification success was not significantly better than expected by chance on Ragged Island (Table 1) where nearly no habitat could be differentiated from any other based on reflectance. 382
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390 **Discussion**

391 Two main insights follow from our results. First, excluding North Andros where the follow-up
392 univariate analyses were not significant, we detected significant differences in dewlap coloration
393 between habitats within seven out of the nine islands investigated, suggesting a putatively high
394 potential for local differentiation of dewlap coloration in *Anolis sagrei*. Second, we found differ-
395 ences in coloration along different dimensions of color space, and in different directions, on different
396 islands.

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398 Detectable differences in dewlap color between habitat-populations are surprising, as habitats
399 were often in close geographical proximity to each other (as close as a few hundred meters on Bimini
400 and most of the time within ten kilometers). Indeed, given that (1) the populations were contin-
401 uously distributed between the habitats, (2) different habitat-populations were not monophyletic
402 with respect to mitochondrial haplotypes (van de Schoot, unpublished thesis), and (3) *A. sagrei*
403 have been shown to be a highly mobile species within these islands (Kamath and Losos, 2018), we
404 would have expected more homogeneous distributions of color phenotypes within islands due to
405 extensive gene flow, with fewer differences between populations, especially those in close proximity.

406

407 Several scenarios could account for these findings. One explanation is an adaptive one. Indeed,
408 populations living in different habitats could be phenotypically adapted to their local environmen-
409 tal conditions. Given that the brightly colored dewlap of *A. sagrei* is used as a communication
410 signal, its color may be a target for selection if the transmission or perception of the signal differs
411 from one habitat to another, for example because of differences in ambient light, according to the
412 sensory-drive hypothesis (Endler and McLellan, 1988; Endler, 1992, 1998). The sensory-drive hy-
413 pothesis has been tested multiple times for dewlap coloration in *Anolis* lizards, with mixed results.
414 Some authors found support for it (Leal and Fleishman, 2002, 2004), while others did find differ-
415 ences in dewlap coloration between habitats, but those were inconsistent with the sensory-drive
416 hypothesis (Fleishman et al., 2009; Ng et al., 2012).

417

418 If our results were an example of sensory drive, we would have expected to see consistent dif-
419 ferences between habitat-populations across islands (a pattern that would have been a compelling
420 indicator of adaptation at all, Losos 2011). This is because environmental conditions that may be
421 relevant to color signal detectability such as light, temperature, moisture and vegetation, are con-
422 sistent within the three main and clearly distinct habitat-types found across the sampled islands,
423 i.e. beach scrub, primary coppice and mangroves (Howard, 1950; Schoener, 1968). Moreover, the
424 patterns of divergence expected under a sensory drive scenario should be consistent with increased
425 detectability given the local light conditions, such as the high contrasts with background vegetation
426 found in the UV-range by Leal and Fleishman (2002) and Leal and Fleishman (2004).

427

428 Instead, we found differences in the way dewlap color differs between habitats across islands.
429 While short-wavelengths (UV reflectance) were often involved in color differences, they were not
430 involved on all islands where significant differences were detected. On some islands, other or ad-
431 ditional variables differed, such as brightness, red reflectance or the reflectance at the ends of the
432 spectrum visible to *Anolis* lizards (UV and red, Lazareva et al. 2012) relative to intermediate
433 wavelengths (blue-to-yellow). Similar portions of the spectrum were sometimes involved in oppo-
434 site directions on different islands, such as on Abaco and Cayman Brac, where mangrove lizards
435 had a higher UV-reflectance than coastal lizards on the former, but a lower UV-reflectance on the
436 latter. Under a sensory-drive scenario, we would have expected the same variables to be consis-
437 tently divergent between habitats, or at least in a consistent direction.

438

439 Not only consistent patterns across islands would have been a good clue for a sensory-drive ex-
440 planation, but in particular consistent differences between habitats that are most different in their
441 local conditions regarding the ecological function of the dewlap, such as ambient light. For exam-
442 ple, if ambient light is an important factor shaping dewlap coloration, we would expect mangrove
443 and beach scrub lizards, both inhabiting areas with high light penetration, to harbor more similar
444 dewlaps, and to differ significantly from lizards from the coppice habitat, where irradiance is low.
445 Overall, the observed heterogeneity of divergence patterns across islands provides no support to a
446 sensory-drive explanation.

Phenotypic plasticity could be another cause for dewlap color variation between habitats, where different conditions would favor different phenotypes in different habitats, without genetic changes. Indeed, the yellow, orange and red colors in anoline dewlaps are produced by pterins and carotenoids (Ortiz, 1962; Ortiz et al., 1962; Ortiz and Williams-Ashman, 1963; Ortiz and Maldonado, 1966; Macedonia et al., 2000; Steffen and McGraw, 2007, 2009). Animals can be synthesize 447
pterins from nucleotides, but lack the ability to synthesize carotenoids (Goodwin, 1984; Hill et al.,
448
2002; Hill and McGraw, 2006). Different food qualities across sites within islands could therefore
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potentially cause detectable differences in coloration. Alternatively, more subtle effects on dewlap
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color could arise from developmental plasticity and depend, e.g. on differences in egg-rearing con-
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ditions. However, more data are needed to test these hypotheses, and although some work has
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shown plastic responses of dewlap color in response to parasites in *A. sagrei* (Cook et al., 2013), we
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find it unlikely to account for the widespread habitat differences we found. Besides, studies testing
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the effect of carotenoid deprivation (Steffen et al., 2010; Ng et al., 2013) and heritability (Cox
455
et al., 2017) of dewlap coloration in *A. sagrei* and another species with a carotenoid-based dewlap,
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A. distichus, found little support for phenotypic and developmental plasticity in dewlap coloration.
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Genetic drift is another process that can account for differences in phenotype between localities, especially in small populations. One way this could proceed is through isolation-by-distance, where more distant populations accumulate more differences through time because of the reduced effect of gene flow at larger geographical scales relative to the dispersal range of the species (Rousset, 2004). Here, we only found a significant correlation between phenotypic and geographical distances on Eleuthera to support this scenario. On all the other islands, in contrast, populations from closer sites were not phenotypically more similar, which argues against isolation-by-distance. That said, there were often few sampling sites per island in our study, whose locations were not uniformly chosen within the islands, and so the true extent of isolation-by-distance may be difficult to test. Other, less trivial forms of drift may be at play than isolation-by-distance, but nevertheless, we did find significant differences in color phenotype at relatively small spatial scales, sometimes in neighboring habitats, on islands where gene flow is probably highly pervasive, as suggested by high rates of encounter between males and females (Kamath and Losos, 2018), making the divergence of habitat-populations by drift in relative genetic isolation an unlikely scenario.

A number of alternative explanations remain. First, there could sexual selection for different dewlap colors in different locations. Indeed, although the sensory-drive hypothesis may include a sexual selection aspect, e.g. if the optimal male phenotype in a given habitat is a function of female perception, sexual preferences may also be arbitrary and independent of the habitat (Andersson, 1994), and so could differ across habitats and islands. However, one previous study has found no link between dewlap coloration and body size dimorphism in *A. sagrei* (a proxy for the intensity of sexual selection) in an among-island comparison (Baeckens et al., 2018), and although within-island data are lacking, this scenario may have little plausibility.

Alternatively, selective pressures may be different in similar habitats from one island to another, because of other environmental variables not accounted for by the habitat-type classification we used (Howard, 1950; Schoener, 1968). The islands we sampled indeed exhibit variation in some climatic variables but also in densities of predators and anole congeners, which have all been shown to correlate with variation in *A. sagrei* mean dewlap coloration among islands (Vanhooijdonck et al., 2009; Baeckens et al., 2018).

Finally, different island-populations could also respond differently to similar selective pressures, resulting in various between-habitat divergence patterns across islands. Several factors could account for this. For example, the founder populations of each island, which we know colonized the islands independently (van de Schoot, unpublished thesis; Driessens et al. 2017; Reynolds et al. 2020), could have exhibited different dewlap colors at the time of colonization, as may be suggested by the larger differences we observed between than within islands. In turn, different initial phenotypes could have led to different ways in which populations would have diverged between habitats. Moreover, the different founding populations may have also consisted in different subsets of the standing genetic variation of their Cuban ancestor due to potential bottlenecks (Reynolds et al., 2020), which may have constrained the way they would later respond to the local selective

505 pressures of their respective islands.

506

507 Altogether, our results show that dewlap color of *A. sagrei* commonly varies between habitat-
508 types, even in close geographical proximity, within islands of the West Indies, and that coloration
509 differs in different ways from one island to another. We discussed several non-mutually exclusive
510 mechanisms that could explain these observations, but more data are needed to thoroughly test
511 each of these. Nevertheless, heterogeneous patterns of divergence across islands do not support an
512 adaptive sensory-drive scenario, and we propose that within-island dewlap color variation may
513 be underlain by a more subtle mosaic of factors.

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Figures

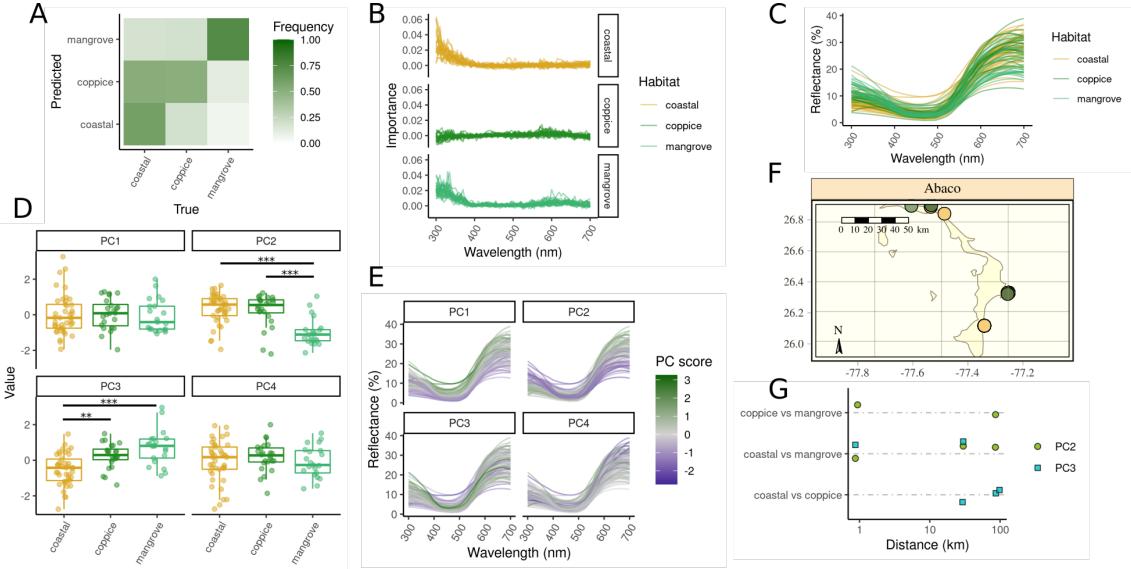


Figure 1: Comparison of dewlap coloration across habitats on Abaco. (A) Confusion matrix showing the proportion of lizards from each (true) habitat reassigned to each (predicted) habitat by the random forests, based on the first four within-island principal components and averaged across replicates. Each column sums to one. (B) One-dimensional sensitivity analysis showing the relative importance (mean decrease in accuracy) of the various wavelengths in random forest classification of the whole spectrum. (C) Reflectance profiles of all the dewlaps on the island. (D) Within-island principal component scores across habitats. Bars indicate significant contrasts. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (E) How reflectance profiles map onto the within-island principal components. (F) Map of the island with the sampling sites colored by habitat. (G) Geographical distance between sites where significant differences were detected in within-island principal component scores (Wilcoxon test, Benjamini-Hochberg correction, $P < 0.05$), including only pairs of sites whose habitats were involved in between-habitat dewlap differences.

Tables

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Table 1: Random forest classification results. For each island are shown the sample size (N) and the proportion of correctly reassigned observations (or success score). P-values were computed using a binomial test and assess the significance of the observed success score relative to the score expected under random guessing. *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$.

Island	N	Score	P
Abaco	86	0.612	< 0.0001 ***
Bimini	67	0.510	0.0011 **
Cayman Brac	50	0.728	< 0.0001 ***
Eleuthera	56	0.493	0.0072 **
Little Cayman	45	0.649	< 0.0001 ***
Long Island	53	0.634	< 0.0001 ***
North Andros	28	0.507	0.0216 *
Ragged Island	50	0.368	0.2874
South Andros	31	0.484	0.0270 *

Table 2: Test of spatial autocorrelation. For each island are shown the correlation (Pearson's ρ) between the matrix of phenotypic distances between populations from each site and the matrix of geographic distances between sites, where phenotypic distances are Euclidean distances between the mean phenotypes of each site in the multivariate space consisting of the first four within-island principal components. P-values assess the significance of the observed correlation against the correlation expected if lizards were randomly permuted among sites (1,000 permutations). *, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$.

Island	ρ	P
Abaco	0.448	0.065
Bimini	0.810	0.137
Cayman Brac	-0.737	0.754
Eleuthera	0.844	0.006 **
Little Cayman	-0.042	0.625
Long Island	0.367	0.183
North Andros	0.051	0.505
Ragged Island	-0.363	0.620
South Andros	-0.979	0.904

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Supplementary Figures

765

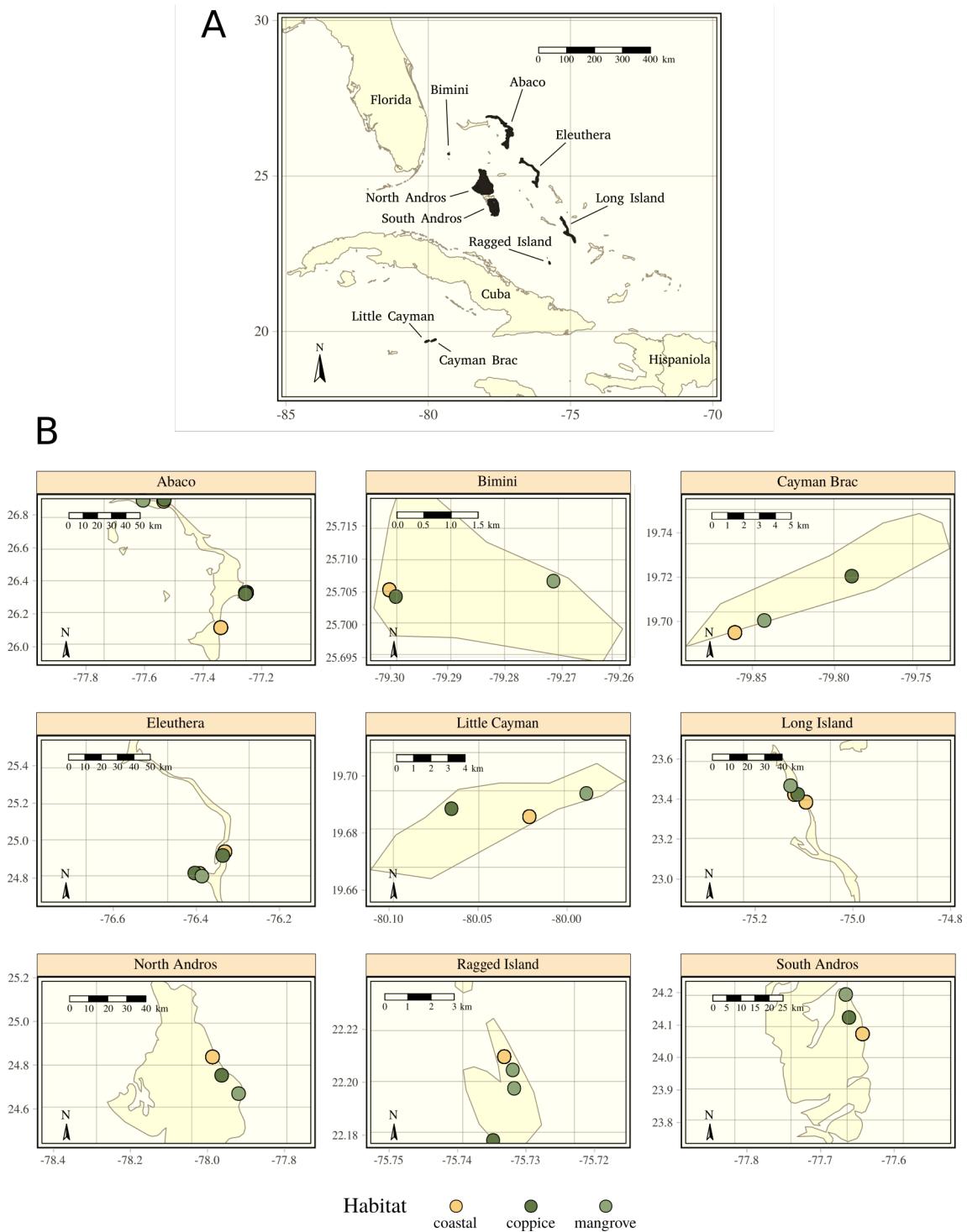


Figure S1: Maps of the islands. (A) Map of the West Indies with sampled islands highlighted in black. (B) Sampling sites within islands colored after their respective habitat-types.

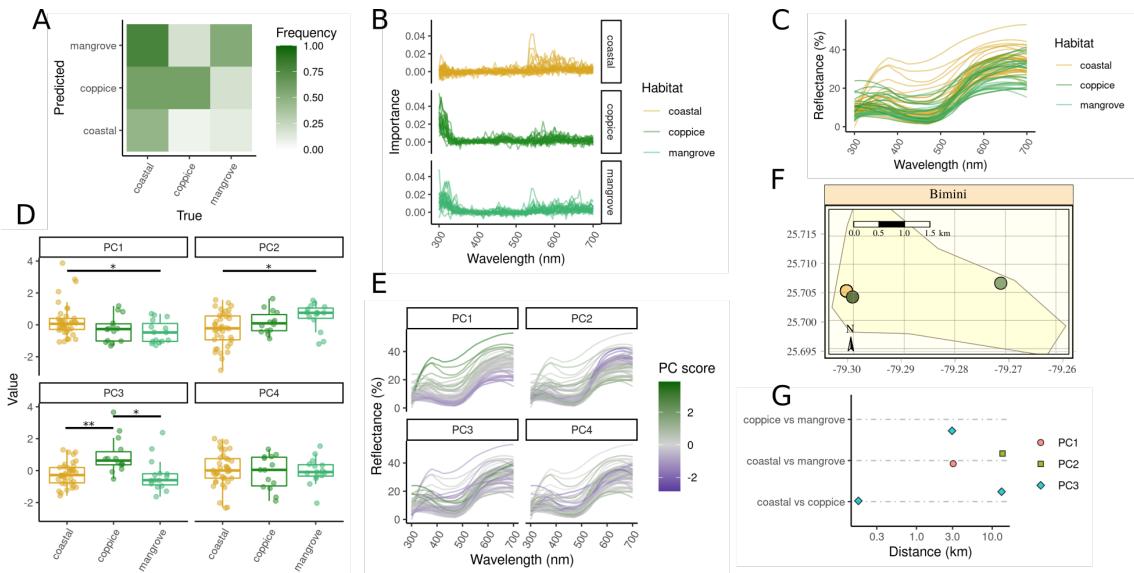


Figure S2: Comparison of dewlap coloration across habitats on Bimini. Legend is as per Figure 1.

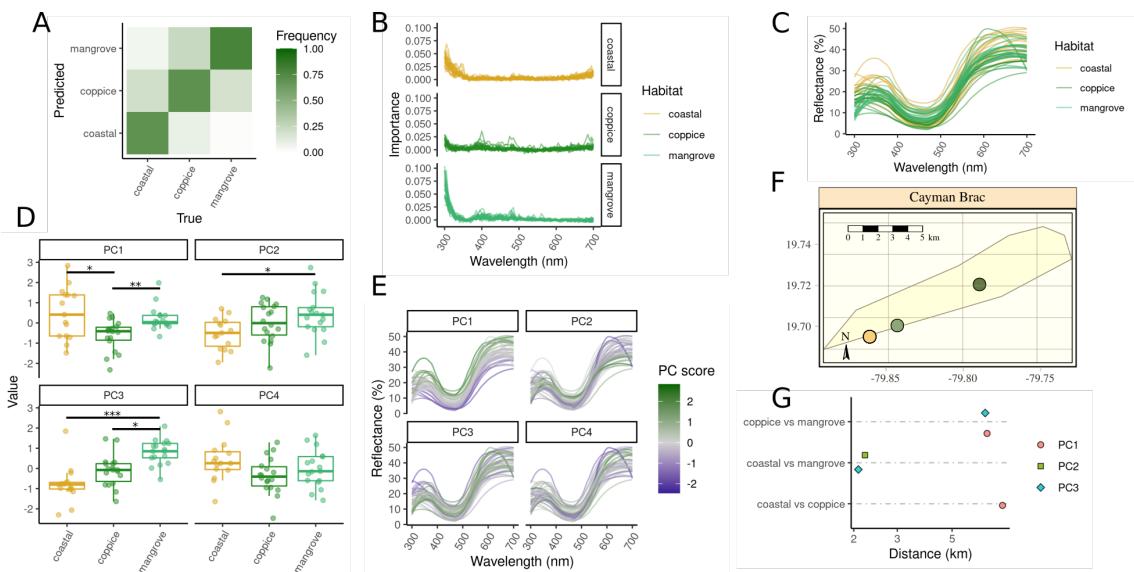


Figure S3: Comparison of dewlap coloration across habitats on Cayman Brac. Legend is as per Figure 1.

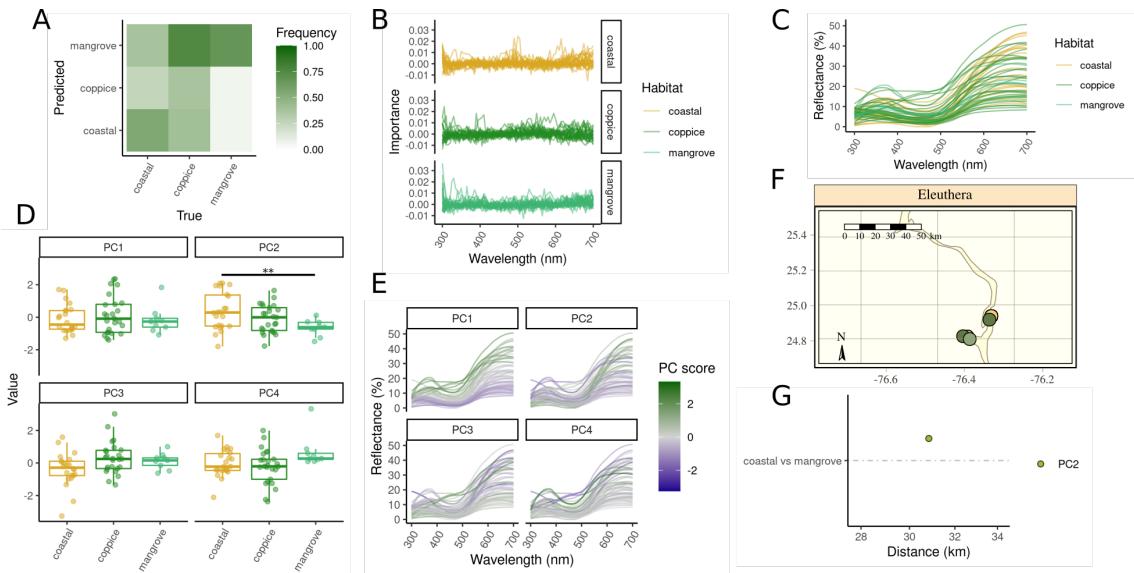


Figure S4: Comparison of dewlap coloration across habitats on Eleuthera. Legend is as per Figure 1.

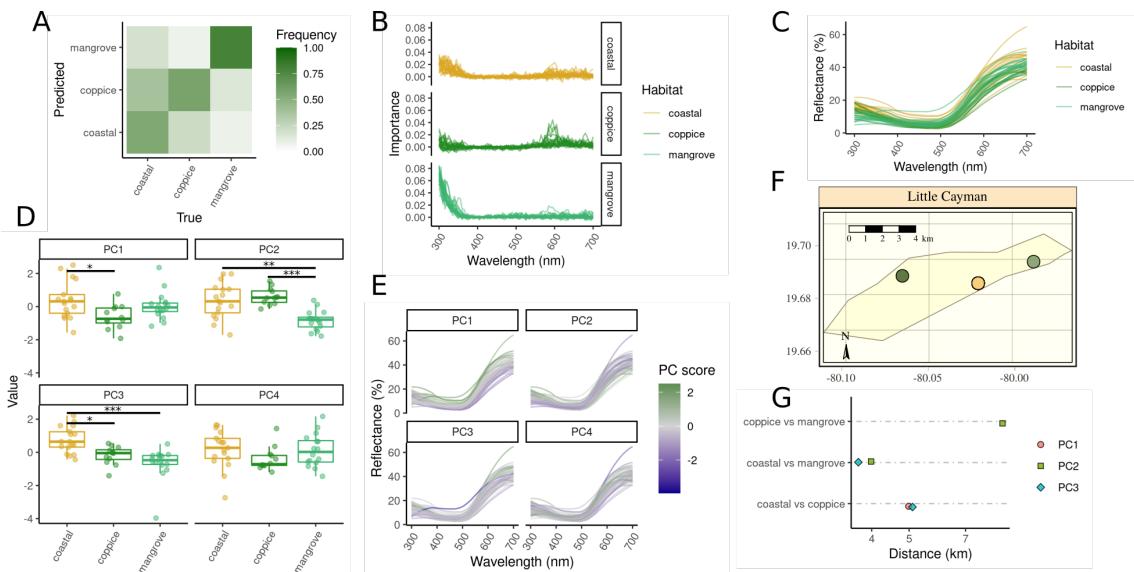


Figure S5: Comparison of dewlap coloration across habitats on Little Cayman. Legend is as per Figure 1.

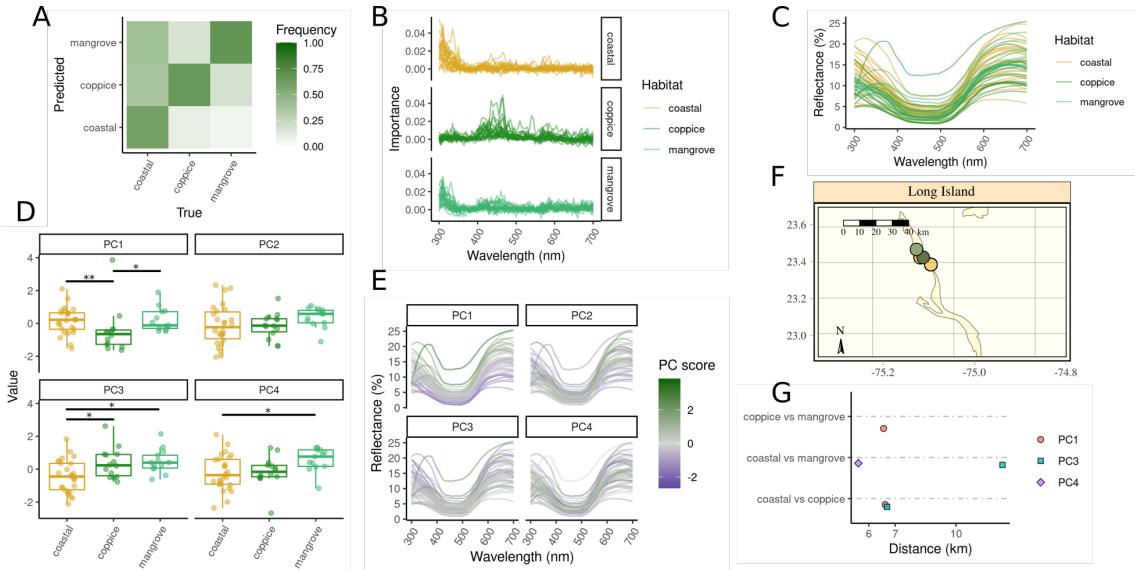


Figure S6: Comparison of dewlap coloration across habitats on Long Island. Legend is as per Figure 1.

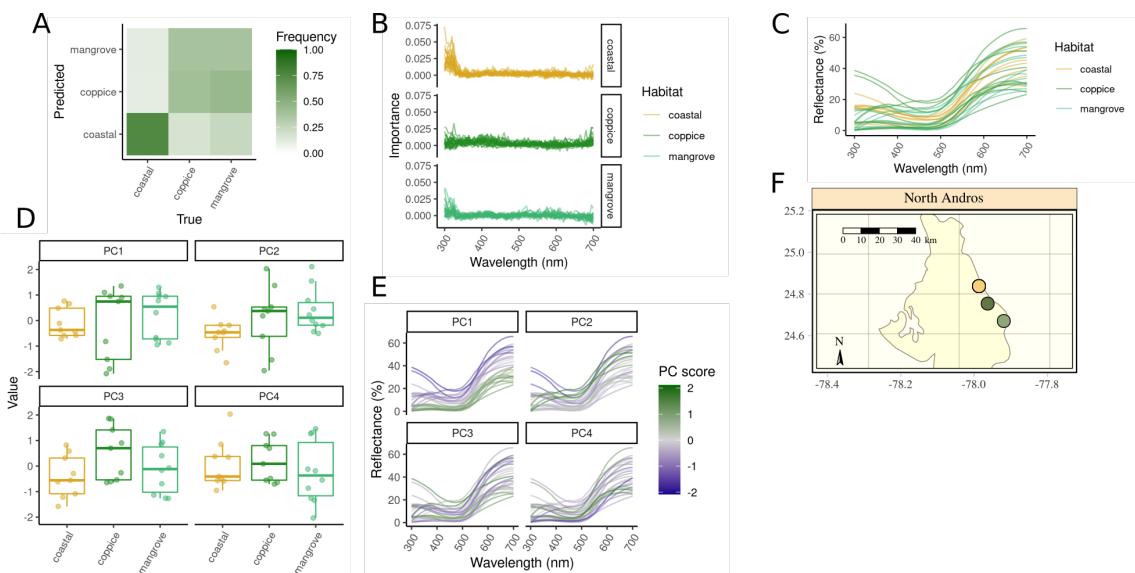


Figure S7: Comparison of dewlap coloration across habitats on North Andros. Legend is as per Figure 1.

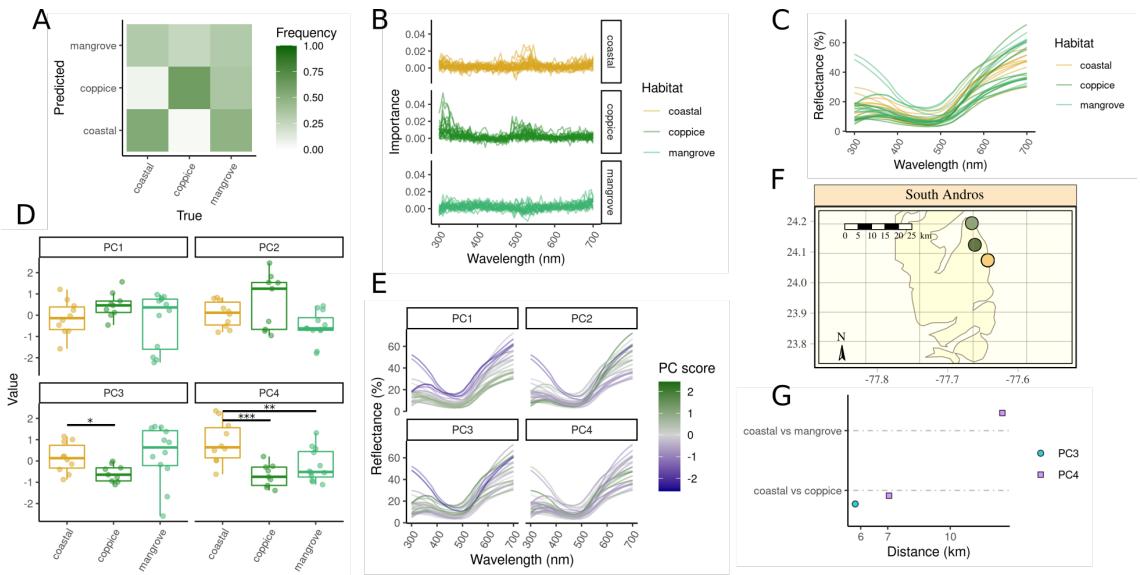


Figure S8: Comparison of dewlap coloration across habitats on South Andros. Legend is as per Figure 1.

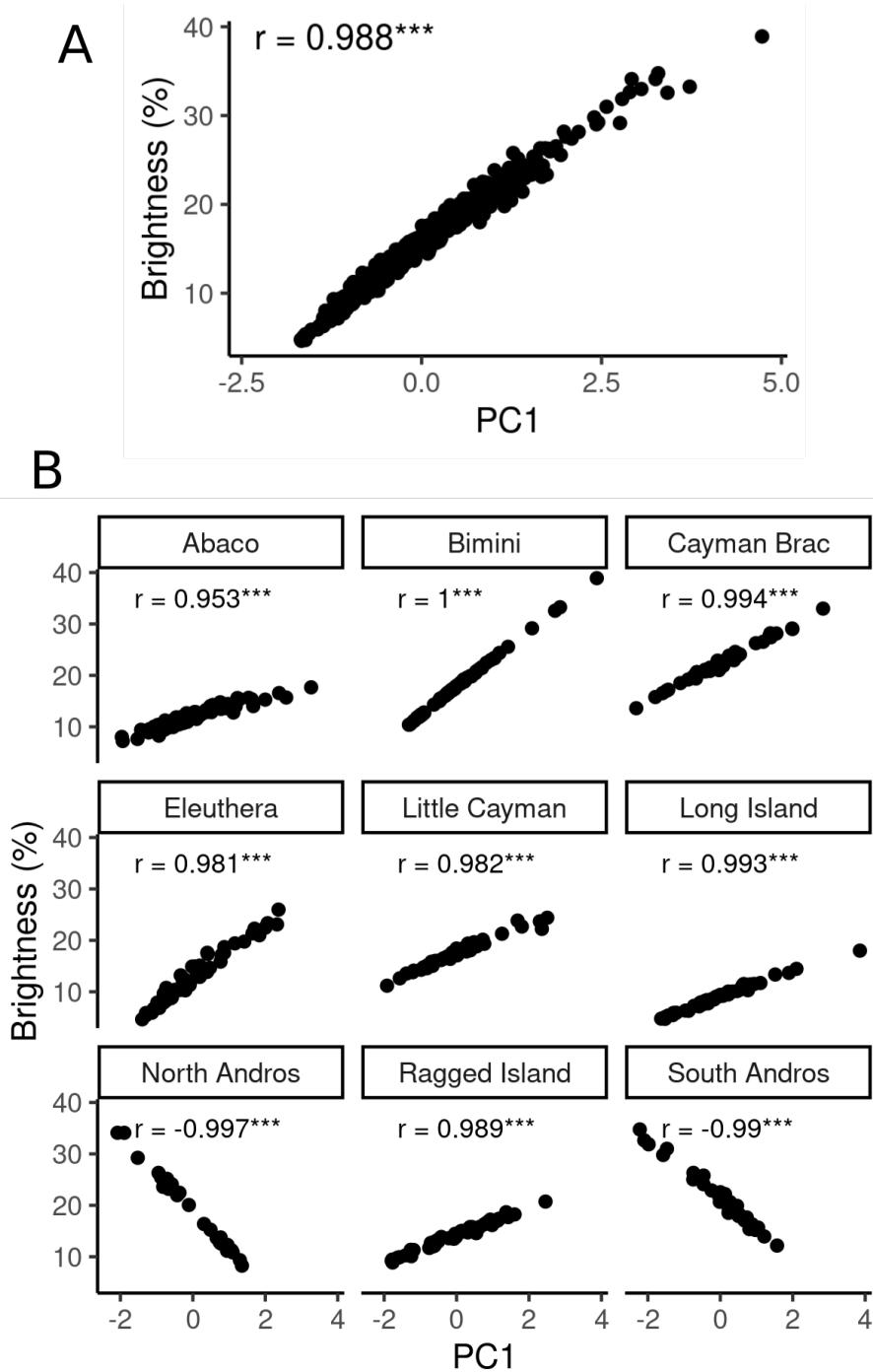


Figure S9: PC1 captures brightness across all islands. (A) Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across the whole archipelago. (B) Correlation between brightness and PC1 for each island. Pearson's correlation coefficients are reported. ***, $P < 0.001$.

Supplementary Tables

766

Table S1: Number of lizards sampled in each habitat on each island.

	coastal	coppice	mangrove
Abaco	41	24	21
Bimini	38	14	15
Cayman Brac	15	18	17
Eleuthera	22	25	9
Little Cayman	17	12	16
Long Island	26	14	13
North Andros	9	9	10
Ragged Island	18	15	17
South Andros	10	9	12

Table S2: Proportion of variance explained by the first four principal components on each island, as well as across the whole archipelago.

Island	Total	PC1	PC2	PC3	PC4
Abaco	0.906	0.400	0.279	0.147	0.079
Bimini	0.921	0.502	0.208	0.160	0.051
Cayman Brac	0.888	0.438	0.190	0.155	0.105
Eleuthera	0.926	0.490	0.233	0.138	0.066
Little Cayman	0.907	0.441	0.212	0.176	0.078
Long Island	0.925	0.515	0.205	0.161	0.043
North Andros	0.937	0.560	0.170	0.152	0.054
Ragged Island	0.907	0.483	0.226	0.127	0.072
South Andros	0.948	0.488	0.247	0.146	0.067

Table S3: Henze-Zirkler's test of multivariate normality, performed on global principal components (i.e. fitted on data from all islands together) in each habitat and on each island. The number of outlier points detected based on the Mahalanobis distance is reported. HZ , test statistic. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Island	Habitat	Outliers	HZ	P
Abaco	coastal	0	1.099	0.0027 **
Abaco	coppice	0	1.074	0.0022 **
Abaco	mangrove	0	1.063	0.0023 **
Bimini	coastal	0	1.281	< 0.0001 ***
Bimini	coppice	0	0.850	0.0482 *
Bimini	mangrove	0	1.191	0.0001 ***
Cayman Brac	coastal	0	0.647	0.5311
Cayman Brac	coppice	0	0.701	0.3940
Cayman Brac	mangrove	0	0.657	0.5357
Eleuthera	coastal	0	1.614	< 0.0001 ***
Eleuthera	coppice	0	1.481	< 0.0001 ***
Eleuthera	mangrove	0	0.729	0.1423
Little Cayman	coastal	0	0.624	0.6599
Little Cayman	coppice	0	0.638	0.4867
Little Cayman	mangrove	0	0.873	0.0413 *
Long Island	coastal	0	0.824	0.1468
Long Island	coppice	0	0.923	0.0150 *
Long Island	mangrove	0	0.773	0.1289
North Andros	coastal	0	0.658	0.3174
North Andros	coppice	0	0.763	0.0900
North Andros	mangrove	0	0.668	0.3185
Ragged Island	coastal	0	0.756	0.2268
Ragged Island	coppice	0	0.797	0.1115
Ragged Island	mangrove	0	0.542	0.9022
South Andros	coastal	0	0.660	0.3451
South Andros	coppice	0	0.659	0.3154
South Andros	mangrove	0	0.911	0.0144 *

Table S4: Shapiro-Wilk's test of univariate normality of the standardized residuals from OLS and GLS-ANOVAs performed on each island where significant differences were detected by random forest classification. W , test statistic. *, $P_{adj} < 0.05$; **, $P_{adj} < 0.01$; ***, $P_{adj} < 0.001$.

Island	Variable	W	P	P_{adj}
Abaco	PC1	0.961	0.0109	0.0497 *
Abaco	PC2	0.960	0.0089	0.0473 *
Abaco	PC3	0.988	0.5938	0.7308
Abaco	PC4	0.982	0.2709	0.4816
Bimini	PC1	0.890	0.0000	0.0008 ***
Bimini	PC2	0.984	0.5179	0.6906
Bimini	PC3	0.959	0.0281	0.0997
Bimini	PC4	0.980	0.3386	0.5418
Cayman Brac	PC1	0.986	0.8236	0.8785
Cayman Brac	PC2	0.989	0.9299	0.9299
Cayman Brac	PC3	0.934	0.0079	0.0473 *
Cayman Brac	PC4	0.981	0.5926	0.7308
Eleuthera	PC1	0.930	0.0031	0.0330 *
Eleuthera	PC2	0.975	0.3090	0.5204
Eleuthera	PC3	0.981	0.4989	0.6906
Eleuthera	PC4	0.970	0.1788	0.4082
Little Cayman	PC1	0.955	0.0821	0.2539
Little Cayman	PC2	0.982	0.6854	0.7833
Little Cayman	PC3	0.891	0.0005	0.0081 **
Little Cayman	PC4	0.977	0.4858	0.6906
Long Island	PC1	0.937	0.0074	0.0473 *
Long Island	PC2	0.989	0.9039	0.9299
Long Island	PC3	0.971	0.2170	0.4341
Long Island	PC4	0.983	0.6261	0.7420
North Andros	PC1	0.937	0.0952	0.2539
North Andros	PC2	0.978	0.8075	0.8785
North Andros	PC3	0.905	0.0147	0.0587
North Andros	PC4	0.949	0.1913	0.4082
South Andros	PC1	0.941	0.0879	0.2539
South Andros	PC2	0.946	0.1199	0.2952
South Andros	PC3	0.965	0.3950	0.6019
South Andros	PC4	0.957	0.2480	0.4668

Table S7: Support vector machine classification results. Legend is as per Table 1.

Island	<i>N</i>	Score	<i>P</i>	
Abaco	86	0.581	< 0.0001	***
Bimini	67	0.555	< 0.0001	***
Cayman Brac	50	0.708	< 0.0001	***
Eleuthera	56	0.443	0.0513	
Little Cayman	45	0.711	< 0.0001	***
Long Island	53	0.664	< 0.0001	***
North Andros	28	0.429	0.1039	
Ragged Island	50	0.424	0.0756	
South Andros	31	0.574	0.0040	**

Table S8: Linear discriminant analysis classification results. Legend is as per Table 1.

Island	<i>N</i>	Score	<i>P</i>	
Abaco	86	0.644	< 0.0001	***
Bimini	67	0.585	< 0.0001	***
Cayman Brac	50	0.780	< 0.0001	***
Eleuthera	56	0.425	0.0871	
Little Cayman	45	0.738	< 0.0001	***
Long Island	53	0.574	0.0001	***
North Andros	28	0.414	0.1911	
Ragged Island	50	0.400	0.1259	
South Andros	31	0.587	0.0013	**