

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

RAPHAËL SCHERRER<sup>1,3</sup> \*, COLIN M. DONIHUE<sup>1,4</sup>,  
R. GRAHAM REYNOLDS<sup>2</sup>, JONATHAN B. LOSOS<sup>1,4</sup> AND ANTHONY J. GENEVA<sup>1,5</sup>

<sup>1</sup> Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology  
Harvard University, Cambridge, MA, USA

<sup>2</sup> Department of Biology, University of North Carolina Asheville, Asheville, NC, USA

<sup>3</sup> Current address: Groningen Institute for Evolutionary Life Sciences,  
University of Groningen, Groningen, The Netherlands

<sup>4</sup> Current address: Department of Biology, Washington University, St. Louis, MO, USA

<sup>5</sup> Current address: Department of Biology, Center for Computational and Integrative Biology,  
Rutgers University–Camden, Camden, NJ, USA

## 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

\*Corresponding author: r.scherrer@rug.nl

37 signals.

38  
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 ?).

78  
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.

167  
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

170

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

179

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

187

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  
279  
could not get meaningful results from tests that use sites as units of observation, such as Moran's  
280  
I test (Gittleman and Kot, 1990). Instead, we designed a permutation test where we randomly  
281  
reshuffled individual lizards across sites within islands 1,000 times each, and systematically re-  
282  
calculated Pearson's correlation coefficient between geographic distances (computed as geodesic  
283  
distances in the R package geosphere; Hijmans 2019) and phenotypic distances. We used the re-  
284  
sulting null distributions of correlation coefficients to assess the significance of the observed spatial  
285  
autocorrelation for each island.  
286

### Site differences

In this study, we were interested in the minimum spatial scale at which significant differences  
287  
between habitats could be detected within islands. We performed multiple pairwise nonparametric  
288  
Wilcoxon-Mann-Whitney tests (Hollander et al., 2013) to compare dewlap coloration between  
289  
sites with different habitat-types, for each pair of habitats and each variable where significant  
290  
differences were detected with our analyses of variances. The P-values were adjusted using a  
291  
Benjamini-Hochberg correction for multiple testing (Benjamini and Hochberg, 1995).  
292

294 **Results**

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

304 We subsequently tested for differences in dewlap coloration between habitat-populations within  
305 each island, using within-island principal component scores, that is, from components computed  
306 from the data specific to each island (to maximize the variation captured for each island, see  
307 Methods). Our within-island random forest classification analyses revealed detectable differences  
308 in dewlap coloration on seven out of the nine islands in our sample: Abaco, Bimini, Cayman Brac,  
309 Eleuthera, Little Cayman, Long Island, North Andros and South Andros. On these islands, our  
310 classifiers could reassign individual dewlaps to their correct habitat more often than expected by  
311 chance (Table 1). We obtained similar results using other machine learning approaches such as  
312 support vector machines (Table S7) and linear discriminant analysis (Table S8), except that these  
313 methods did not detect significant differences on Eleuthera and North Andros. We did not find  
314 evidence of spatial autocorrelation in dewlap coloration between the sites within islands, except  
315 for Eleuthera (Table 2). We now describe the specific differences detected on each island.  
316

317 On Abaco, dewlaps from the mangrove habitat were the best discriminated, while dewlaps from  
318 the coastal habitat were often mistaken for dewlaps from the coppice habitat (Fig. 1A). Importance  
319 analysis revealed that coastal and mangrove lizards mostly differed in reflectance in the UV-end of  
320 the 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).  
328

329 On Bimini, the random forests mostly correctly classified lizards from the coppice and man-  
330 grove habitats while often misclassifying lizards from the coastal habitat (Fig. S2A). Relatively flat  
331 importance profiles for coastal lizards suggested that brightness was used instead of a particular  
332 wavelength to identify some of the coastal dewlaps (Fig. S2B). Indeed, some coastal dewlaps were  
333 substantially brighter than the rest (Fig. S2C), a pattern that was captured by our analysis of  
334 variance along PC1 (i.e. brightness, Fig. S2D, E, Table 3). The random forests also used UV  
335 reflectance to discriminate between coppice and mangrove dewlaps (Fig. S2B), which could reflect  
336 the significant difference we detected along PC3 between these two habitats (Fig. S2D, Table 3).  
337 Coastal lizards were characterized by elevated red reflectance relative to UV (as represented by  
338 PC2, (Fig. S2D, E)), and coastal and mangrove lizards were characterized by a more even distri-  
339 bution of the reflectance along the spectrum (as represented by PC3, (Fig. S2D, E)), in contrast  
340 to coppice lizards which harbored a stronger curvature at intermediate wavelengths (Fig. S2D, E).  
341 On this island, the coastal and coppice habitats were separated by a few hundred meters, making  
342 this contrast the smallest geographical scale at which differences in coloration were found in our  
343 study (Fig. S2F, G).  
344

345 On Cayman Brac, all three habitats could be well discriminated against each other (Fig. S3A),  
346 with UV reflectance appearing to be an important variable differentiating coastal and mangrove  
347 dewlaps (Fig. S3B). In contrast, coppice dewlaps had a relatively flat importance profile, suggest-  
348 ing that brightness made them more distinct rather than any particular wavelength (Fig. S3B).  
349 Consistent with this, coppice dewlaps were significantly different from all other dewlaps along PC1  
350 (Fig. S3D, Table 3). At a distance between 2 and 3km (Fig. S3F, G), dewlaps in the coastal habi-

tat reflected more red light (as represented by PC2, Fig. S3D, E) and more UV (as represented by PC3) than in the mangrove habitat. Coppice lizards were also characterized by a higher UV reflectance than mangrove dewlaps (PC3, Fig. S3D, E, Table 3).

Eleuthera was the only island where we detected significant spatial autocorrelation (Table 2), that is, sites that were closer geographically tended to have populations of lizards with more similar dewlap colors. Although random forests detected between-habitat differences in dewlap color, other approaches did not (Tables S8 and S7), suggesting that the differences may be small. Consistent with this, the only significant univariate difference detected was for PC2 between coastal and mangrove lizards, where coastal lizards had higher levels of red reflectance and mangrove lizards higher levels of UV reflectance (Fig. S4D, E, Table 3).

Little Cayman was characterized by a better discrimination of mangrove lizards from the rest than between coastal and coppice lizards (Fig. S5A). Mangrove dewlaps were possibly most distinct with respect to their reflectance in short wavelengths (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).

On Long Island the three habitats were relatively well discriminated (Fig. S6A). Importance profiles indicated that short wavelengths were used to discriminate between coastal and mangrove lizards (Fig. S6B). 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 represented 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).

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 islands may also have contributed to a lack of power in detecting univariate differences using analyses of variance (Table S1).

On South Andros coastal and coppice dewlaps could be discriminated better against each other than with mangrove dewlaps (Fig. S8A), with importance profiles supported UV-reflectance as a predictor of coppice lizards (Fig. S8B). 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).

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.

## 400 Discussion

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

407

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

416

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

427

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

437

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

448

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

456

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 pterins from nucleotides, but lack the ability to synthesize carotenoids (Goodwin, 1984; Hill et al., 2002; Hill and McGraw, 2006). Different food qualities across sites within islands could therefore potentially cause detectable differences in coloration. Alternatively, more subtle effects on dewlap color could arise from developmental plasticity and depend, e.g. on differences in egg-rearing conditions. However, more data are needed to test these hypotheses, and although some work has shown plastic responses of dewlap color in response to parasites in *A. sagrei* (Cook et al., 2013), we find it unlikely to account for the widespread habitat differences we found. Besides, studies testing the effect of carotenoid deprivation (Steffen et al., 2010; Ng et al., 2013) and heritability (Cox et al., 2017) of dewlap coloration in *A. sagrei* and another species with a carotenoid-based dewlap, *A. distichus*, found little support for phenotypic and developmental plasticity in dewlap coloration.

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

<sup>515</sup> pressures of their respective islands.

<sup>516</sup>  
<sup>517</sup> Altogether, our results show that dewlap color of *A. sagrei* commonly varies between habitat-  
<sup>518</sup> types, even in close geographical proximity, within islands of the West Indies, and that coloration  
<sup>519</sup> differs in different ways from one island to another. We discussed several non-mutually exclusive  
<sup>520</sup> mechanisms that could explain these observations, but more data are needed to thoroughly test  
<sup>521</sup> each of these. Nevertheless, heterogeneous patterns of divergence across islands do not support an  
<sup>522</sup> adaptive sensory-drive scenario, and we propose that within-island dewlap color variation may  
be underlain by a more subtle mosaic of factors.

<sup>523</sup>

## Acknowledgements

524

Collection permission was granted by the Bahamas Environment, Science and Technology Commission, the Bahamas National Trust, the Bahamas Ministry of Agriculture, and the Cayman Islands Department of the Environment. The authors thank Sofia Prado-Irwin, Pavitra Muralidhar, Nicholas Herrmann, Richard E. Glor, Alberto R. Puente-Rolón, Kevin Aviles-Rodriguez, Kristin Winchell, Jason Fredette and Melissa Kemp for assistance in the field and Pratik Gupte, Max Lambert and James Stroud for helpful discussions. Funding for this work was provided by the Templeton Foundation (to JBL), NSF DEB #1927194 (to JBL and AJG), NSF DEB #1500761 (to AJG), NSF DBI #1609284 (to CMD), and a Harvard Museum of Comparative Zoology Putnam Expedition Grant (to RGR).

525

526

527

528

529

530

531

532

533

## Figures

534

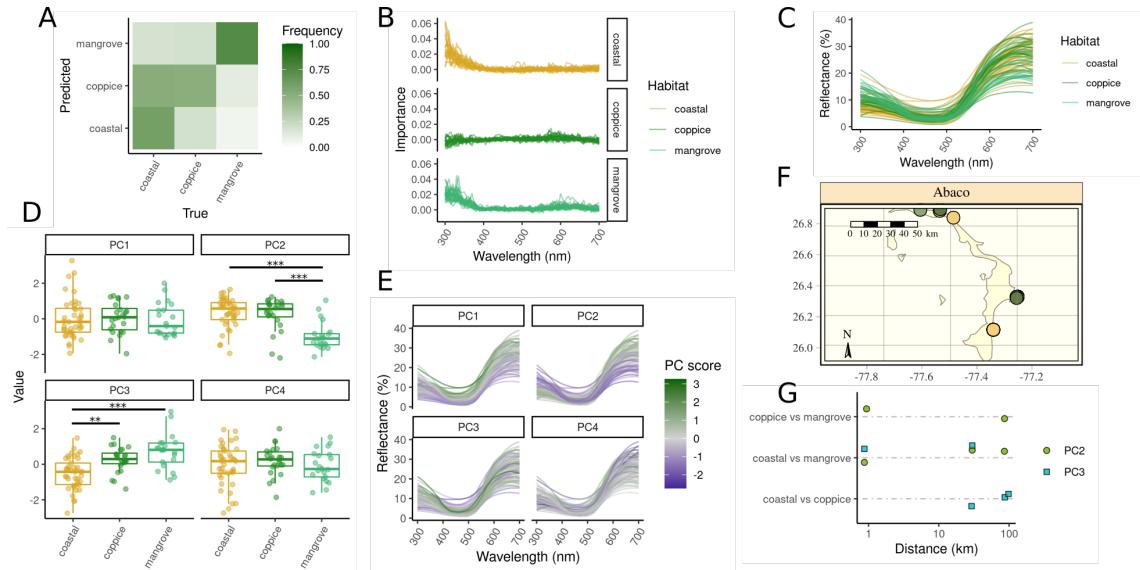


Figure 1: Comparison of dewlap coloration across habitats on Abaco. (A) Confusion matrix showing the proportion of lizards from each (true) habitat reassigned to each (predicted) habitat by the random forests, based on the first four within-island principal components and averaged across replicates. 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

535

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  $\rho$ ) 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	$\rho$	$P$
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

Table 3: Significance of habitat differences in dewlap coloration, using ANOVA for all islands where significant multivariate differences in dewlap coloration were detected by random forests. Model, best-fitting model (either OLS or GLS). AICc, corrected AIC score of the best-fitting model.  $\Delta\text{AICc}$ , difference in AICc between the best-fitting model and the OLS-model. AICcw, AICc weight. Log-lik., log-likelihood.  $\chi^2$ , likelihood ratio. df, degrees of freedom. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Island	Variable	AICc	$\Delta\text{AICc}$	AICcw	Model	Log-lik.	$\chi^2$	df	P
Abaco	PC1	255.81	2.16	0.746	OLS	-121.45	0.14	2	0.9308
Abaco	PC2	225.32	4.02	0.882	OLS	-105.66	31.74	2	< 0.0001 ***
Abaco	PC3	229.53	2.01	0.732	OLS	-107.84	27.37	2	< 0.0001 ***
Abaco	PC4	254.64	0.78	0.596	OLS	-120.85	1.36	2	0.5070
Bimini	PC1	194.16	0.77	0.595	OLS	-90.87	7.40	2	0.0248 *
Bimini	PC2	193.49	1.29	0.656	OLS	-90.52	8.09	2	0.0175 *
Bimini	PC3	184.22	-0.23	0.529	GLS	-83.46	10.39	2	0.0056 **
Bimini	PC4	200.91	3.54	0.854	OLS	-94.40	0.33	2	0.8499
Cayman Brac	PC1	136.64	-4.05	0.884	GLS	-59.29	13.81	2	0.0010 **
Cayman Brac	PC2	144.75	3.51	0.853	OLS	-66.24	8.41	2	0.0149 *
Cayman Brac	PC3	127.13	2.77	0.800	OLS	-56.86	27.16	2	< 0.0001 ***
Cayman Brac	PC4	147.37	4.33	0.897	OLS	-67.63	5.63	2	0.0600
Eleuthera	PC1	168.72	2.42	0.770	OLS	-78.46	1.00	2	0.6074
Eleuthera	PC2	160.03	-2.20	0.750	GLS	-70.89	11.34	2	0.0034 **
Eleuthera	PC3	163.49	-0.20	0.525	GLS	-72.69	5.57	2	0.0617
Eleuthera	PC4	164.08	3.49	0.852	OLS	-76.01	5.89	2	0.0525
Little Cayman	PC1	130.60	2.50	0.777	OLS	-59.26	8.18	2	0.0167 *
Little Cayman	PC2	112.66	-3.61	0.859	GLS	-46.74	29.76	2	< 0.0001 ***
Little Cayman	PC3	118.32	1.41	0.669	OLS	-52.68	21.34	2	< 0.0001 ***
Little Cayman	PC4	135.58	2.53	0.780	OLS	-61.92	2.85	2	0.2410
Long Island	PC1	154.54	-2.09	0.740	GLS	-68.62	2.91	2	0.2331
Long Island	PC2	155.80	-3.08	0.823	GLS	-68.92	4.52	2	0.1043
Long Island	PC3	150.54	3.67	0.862	OLS	-69.08	11.24	2	0.0036 **
Long Island	PC4	155.05	2.38	0.767	OLS	-71.47	6.46	2	0.0395 *
North Andros	PC1	88.64	0.27	0.534	OLS	-38.84	0.75	2	0.6864
North Andros	PC2	85.36	2.17	0.748	OLS	-37.01	4.42	2	0.1100
North Andros	PC3	85.31	5.82	0.948	OLS	-36.98	4.48	2	0.1065
North Andros	PC4	88.45	4.83	0.918	OLS	-38.74	0.96	2	0.6194
South Andros	PC1	95.12	0.44	0.554	OLS	-41.93	3.10	2	0.2125
South Andros	PC2	89.93	-0.05	0.506	GLS	-35.84	7.76	2	0.0206 *
South Andros	PC3	87.21	-6.14	0.956	GLS	-34.05	10.35	2	0.0056 **
South Andros	PC4	83.01	2.94	0.813	OLS	-35.23	16.51	2	0.0003 ***

## References

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. <i>Austral Ecology</i> , 26(1):32–46.	536 537 538
Andersson, M. B. (1994). <i>Sexual Selection</i> . Monographs in Behavior and Ecology. Princeton University Press, Princeton, N.J.	539 540
Arnold, S. J. (1983). Morphology, Performance and Fitness. <i>American Zoologist</i> , 23(2):347–361.	541
Baeckens, S., Driessens, T., and Van Damme, R. (2018). The brown anole dewlap revisited: Do predation pressure, sexual selection, and species recognition shape among-population signal diversity? <i>PeerJ</i> , 6:e4722.	542 543 544
Bartoń, K. (2019). MuMIn: Multi-Model Inference.	545
Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. <i>Journal of the Royal Statistical Society Series B</i> , 57:289–300.	546 547 548
Bradbury, J. W. and Vehrencamp, S. L. (2011). <i>Principles of Animal Communication</i> . Sinauer Associates, Sunderland, Mass, 2nd ed edition.	549 550
Breiman, L. (2001). Random forests. <i>Machine Learning</i> , 45(1):5–32.	551
Cook, E. G., Murphy, T. G., and Johnson, M. A. (2013). Colorful displays signal male quality in a tropical anole lizard. <i>Naturwissenschaften</i> , 100(10):993–996.	552 553
Cortez, P. (2020). Rminer: Data Mining Classification and Regression Methods.	554
Cox, R. M., Costello, R. A., Camber, B. E., and McGlothlin, J. W. (2017). Multivariate genetic architecture of the <i>Anolis</i> dewlap reveals both shared and sex-specific features of a sexually dimorphic ornament. <i>Journal of Evolutionary Biology</i> , 30(7):1262–1275.	555 556 557
Cristianini, N. and Shawe-Taylor, J. (2000). <i>An Introduction to Support Vector Machines and Other Kernel-Based Learning Methods</i> . Cambridge University Press, first edition.	558 559
Cuthill, I. C., Bennett, A. T. D., Partridge, J. C., and Maier, E. J. (1999). Plumage Reflectance and the Objective Assessment of Avian Sexual Dichromatism. <i>The American Naturalist</i> , 153(2):183–200.	560 561 562
Dieckmann, U. and Doebeli, M. (1999). On the origin of species by sympatric speciation. <i>Nature</i> , 400(6742):354–357.	563 564
Driessens, T., Baeckens, S., Balzarolo, M., Vanhooydonck, B., Huyghe, K., and Van Damme, R. (2017). Climate-related environmental variation in a visual signalling device: The male and female dewlap in <i>Anolis sagrei</i> lizards. <i>Journal of Evolutionary Biology</i> , 30(10):1846–1861.	565 566 567
Driessens, T., Huyghe, K., Vanhooydonck, B., and Van Damme, R. (2015). Messages conveyed by assorted facets of the dewlap, in both sexes of <i>Anolis sagrei</i> . <i>Behavioral Ecology and Sociobiology</i> , 69(8):1251–1264.	568 569 570
Driessens, T., Vanhooydonck, B., and Van Damme, R. (2014). Deterring predators, daunting opponents or drawing partners? Signaling rates across diverse contexts in the lizard <i>Anolis sagrei</i> . <i>Behavioral Ecology and Sociobiology</i> , 68(2):173–184.	571 572 573
Endler, J. (1998). Sensory ecology, receiver biases and sexual selection. <i>Trends in Ecology &amp; Evolution</i> , 13(10):415–420.	574 575
Endler, J. A. (1984). Natural and sexual selection on color patterns in poeciliid fishes. In Balon, E. K. and Zaret, T. M., editors, <i>Evolutionary Ecology of Neotropical Freshwater Fishes</i> , volume 3, pages 95–111. Springer Netherlands, Dordrecht.	576 577 578
Endler, J. A. (1990). On the measurement and classification of colour in studies of animal colour patterns. <i>Biological Journal of the Linnean Society</i> , 41(4):315–352.	579

- 581 Endler, J. A. (1991). Variation in the appearance of guppy color patterns to guppies and their  
582 predators under different visual conditions. *Vision Research*, 31(3):587–608.
- 583 Endler, J. A. (1992). Signals, Signal Conditions, and the Direction of Evolution. *The American  
584 Naturalist*, 139:S125–S153.
- 585 Endler, J. A. (1993a). The Color of Light in Forests and Its Implications. *Ecological Monographs*,  
586 63(1):1–27.
- 587 Endler, J. A. (1993b). Some general comments on the evolution and design of animal communi-  
588 cation systems. *Philosophical Transactions of the Royal Society of London. Series B: Biological  
589 Sciences*, 340(1292):215–225.
- 590 Endler, J. A. and McLellan, T. (1988). The Processes of Evolution: Toward a Newer Synthesis.  
591 *Annual Review of Ecology and Systematics*, 19(1):395–421.
- 592 Felsenstein, J. (1976). The Theoretical Population Genetics of Variable Selection and Migration.  
593 *Annual Review of Genetics*, 10(1):253–280.
- 594 Fleishman, L. J. (2000). *Signal Function, Signal Efficiency and the Evolution of Anoline Lizard  
595 Dewlap Color*, pages 209–236. Tapir Academic, Trondheim.
- 596 Fleishman, L. J., Leal, M., and Persons, M. H. (2009). Habitat light and dewlap color diversity in  
597 four species of Puerto Rican anoline lizards. *Journal of Comparative Physiology A*, 195(11):1043–  
598 1060.
- 599 Fleishman, L. J. and Persons, M. (2001). The influence of stimulus and background colour on  
600 signal visibility in the lizard *Anolis cristatellus*. *The Journal of Experimental Biology*, 204(Pt  
601 9):1559–1575.
- 602 Fleishman, L. J., Wadman, C. S., and Maximov, K. J. (2020). The interacting effects of total  
603 light intensity and chromatic contrast on visual signal visibility in an *Anolis* lizard. *Animal  
604 Behaviour*, page S0003347220302037.
- 605 García-Ramos, G. and Kirkpatrick, M. (1997). Genetic Models of Adaptation and Gene Flow in  
606 Peripheral Populations. *Evolution*, 51(1):21–28.
- 607 Geneva, A. J., Hilton, J., Noll, S., and Glor, R. E. (2015). Multilocus phylogenetic analyses of  
608 Hispaniolan and Bahamian trunk anoles (*distichus* species group). *Molecular Phylogenetics and  
609 Evolution*, 87:105–117.
- 610 Gittleman, J. L. and Kot, M. (1990). Adaptation: Statistics and a Null Model for Estimating  
611 Phylogenetic Effects. *Systematic Zoology*, 39(3):227.
- 612 Goodwin, T. W. (1984). *The Biochemistry of the Carotenoids*. Springer Netherlands, Dordrecht.
- 613 Halfwerk, W., Jones, P. L., Taylor, R. C., Ryan, M. J., and Page, R. A. (2014). Risky Ripples Allow  
614 Bats and Frogs to Eavesdrop on a Multisensory Sexual Display. *Science*, 343(6169):413–416.
- 615 Hendry, A. P., Day, T., and Taylor, E. B. (2007a). Population Mixing and the Adaptive Divergence  
616 of Quantitative Traits in Discrete Populations: A Theoretical Framework for Empirical Tests.  
617 *Evolution*, 55(3):459–466.
- 618 Hendry, A. P., Taylor, E. B., and McPhail, J. D. (2007b). Adaptive Divergence and the Balance  
619 Between Selection and Gene Flow: Lake and Stream Stickleback in the Misty System. *Evolution*,  
620 56(6):1199–1216.
- 621 Henze, N. and Zirkler, B. (1990). A class of invariant consistent tests for multivariate normality.  
622 *Communications in Statistics - Theory and Methods*, 19(10):3595–3617.
- 623 Hijmans, R. J. (2019). Geosphere: Spherical Trigonometry.
- 624 Hill, G. E., Inouye, C. Y., and Montgomerie, R. (2002). Dietary carotenoids predict plumage  
625 coloration in wild house finches. *Proceedings of the Royal Society of London. Series B: Biological  
Sciences*, 269(1496):1119–1124.

Hill, G. E. and McGraw, K. J., editors (2006). <i>Bird Coloration</i> . Harvard University Press, Cambridge, Mass.	627 628
Hollander, M., Wolfe, D. A., and Chicken, E. (2013). <i>Nonparametric Statistical Methods</i> . Wiley Series in Probability and Statistics. John Wiley & Sons, Inc, Hoboken, New Jersey, third edition edition.	629 630 631
Howard, R. A. (1950). Vegetation of the Bimini Island Group: Bahamas, B. W. I. <i>Ecological Monographs</i> , 20(4):317–349.	632 633
James, G., Witten, D., Hastie, T., and Tibshirani, R. (2013). <i>An Introduction to Statistical Learning</i> , volume 103 of <i>Springer Texts in Statistics</i> . Springer New York, New York, NY.	634 635
Kamath, A. and Losos, J. B. (2018). Estimating encounter rates as the first step of sexual selection in the lizard <i>Anolis sagrei</i> . <i>Proceedings of the Royal Society B: Biological Sciences</i> , 285(1873):20172244.	636 637 638
Kolbe, J. J., Larson, A., Losos, J. B., and de Queiroz, K. (2008). Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species. <i>Biology Letters</i> , 4(4):434–437.	639 640 641
Kolbe, J. J., Leal, M., Schoener, T. W., Spiller, D. A., and Losos, J. B. (2012). Founder Effects Persist Despite Adaptive Differentiation: A Field Experiment with Lizards. <i>Science</i> , 335(6072):1086–1089.	642 643 644
Korkmaz, S., Goksuluk, D., and Zararsiz, G. (2014). MVN: An R Package for Assessing Multivariate Normality. <i>The R Journal</i> , 6(2):151–162.	645 646
Lambert, S. M., Geneva, A. J., Luke Mahler, D., and Glor, R. E. (2013). Using genomic data to revisit an early example of reproductive character displacement in Haitian <i>Anolis</i> lizards. <i>Molecular Ecology</i> , 22(15):3981–3995.	647 648 649
Lazareva, O. F., Shimizu, T., and Wasserman, E. A. (2012). <i>How Animals See the WorldComparative Behavior, Biology, and Evolution of Vision</i> . Oxford University Press.	650 651
Leal, M. and Fleishman, L. J. (2002). Evidence for habitat partitioning based on adaptation to environmental light in a pair of sympatric lizard species. <i>Proceedings of the Royal Society of London. Series B: Biological Sciences</i> , 269(1489):351–359.	652 653 654
Leal, M. and Fleishman, L. J. (2004). Differences in Visual Signal Design and Detectability between Allopatric Populations of <i>Anolis</i> Lizards. <i>The American Naturalist</i> , 163(1):26–39.	655 656
Leal, M. and Rodríguez-Robles, J. A. (1995). Antipredator Responses of <i>Anolis cristatellus</i> (Sauria: Polychrotidae). <i>Copeia</i> , 1995(1):155–161.	657 658
Leal, M. and Rodríguez-Robles, J. A. (1997). Signalling displays during predator–prey interactions in a Puerto Rican anole, <i>Anolis cristatellus</i> . <i>Animal Behaviour</i> , 54(5):1147–1154.	659 660
Lenormand, T. (2002). Gene flow and the limits to natural selection. <i>Trends in Ecology &amp; Evolution</i> , 17(4):183–189.	661 662
Liaw, A. and Wiener, M. (2002). Classification and Regression by randomForest. <i>R News</i> , 2(3):18–22.	663 664
Losos, J. B. (1985). An Experimental Demonstration of the Species-Recognition Role of <i>Anolis</i> Dewlap Color. <i>Copeia</i> , 1985(4):905–910.	665 666
Losos, J. B. (2009). <i>Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles</i> . University of California Press.	667 668
Losos, J. B. (2011). Convergence, Adaptation, and Constraint. <i>Evolution</i> , 65(7):1827–1840.	669
Losos, J. B., Irschick, D. J., and Schoener, T. W. (1994). Adaptation and Constraint in the Evolution of Specialization of Bahamian <i>Anolis</i> Lizards. <i>Evolution</i> , 48(6):1786–1798.	670

- 672 Losos, J. B., Schoener, T. W., Warheit, K. I., and Creer, D. (2001). Experimental studies of  
673 adaptive differentiation in Bahamian *Anolis* lizards. *Genetica*, 112-113:399–415.
- 674 Losos, J. B., Warheit, K. I., and Schoener, T. W. (1997). Adaptive differentiation following  
675 experimental island colonization in *Anolis* lizards. *Nature*, 387(6628):70–73.
- 676 Macedonia, J. M. (2001). Habitat light, colour variation, and ultraviolet reflectance in the Grand  
677 Cayman anole, *Anolis conspersus*. *Biological Journal of the Linnean Society*, 73(3):299–320.
- 678 Macedonia, J. M., Clark, D. L., Riley, R. G., and Kemp, D. J. (2013). Species recognition of color  
679 and motion signals in *Anolis grahami*: Evidence from responses to lizard robots. *Behavioral  
680 Ecology*, 24(4):846–852.
- 681 Macedonia, J. M., James, S., Witte, L. W., and Clark, D. L. (2000). Skin Pigments and Coloration  
682 in the Jamaican Radiation of *Anolis* Lizards. *Journal of Herpetology*, 34(1):99–109.
- 683 Macedonia, J. M. and Stamps, J. A. (1994). Species Recognition in *Anolis grahami* (Sauria,  
684 Iguanidae): Evidence from Responses to Video Playbacks of Conspecific and Heterospecific  
685 Displays. *Ethology*, 98(3-4):246–264.
- 686 Maia, R., Eliason, C. M., Bitton, P.-P., Doucet, S. M., and Shawkey, M. D. (2013). Pavo: An  
687 R package for the analysis, visualization and organization of spectral data. *Methods in Ecology  
688 and Evolution*, pages n/a–n/a.
- 689 Ng, J., Geneva, A. J., Noll, S., and Glor, R. E. (2017). Signals and Speciation: *Anolis* Dewlap  
690 Color as a Reproductive Barrier. *Journal of Herpetology*, 51(3):437–447.
- 691 Ng, J. and Glor, R. E. (2011). Genetic differentiation among populations of a Hispaniolan trunk  
692 anole that exhibit geographical variation in dewlap colour. *Molecular Ecology*, 20(20):4302–4317.
- 693 Ng, J., Kelly, A. L., MacGuigan, D. J., and Glor, R. E. (2013). The Role of Heritable and Dietary  
694 Factors in the Sexual Signal of a Hispaniolan *Anolis* Lizard, *Anolis distichus*. *Journal of Heredity*,  
695 104(6):862–873.
- 696 Ng, J., Landeen, E. L., Logsdon, R. M., and Glor, R. E. (2012). Correlation Between *Anolis*  
697 Lizard Dewlap Phenotype and Environmental Variation Indicates Adaptive Divergence of a  
698 Signal Important to Sexual Selection and Species Recognition. *Evolution*, 67(2):573–582.
- 699 Ng, J., Ossip-Klein, A. G., and Glor, R. E. (2016). Adaptive signal coloration maintained in the  
700 face of gene flow in a Hispaniolan *Anolis* Lizard. *BMC Evolutionary Biology*, 16(1):193.
- 701 Nicholson, K. E., Harmon, L. J., and Losos, J. B. (2007). Evolution of *Anolis* Lizard Dewlap  
702 Diversity. *PLoS ONE*, 2(3):e274.
- 703 Nosil, P. and Crespi, B. J. (2004). Does Gene Flow Constrain Adaptive Divergence or Vice Versa? A  
704 Test Using Ecomorphology and Sexual Isolation in *Timema cristinae* Walking-Sticks. *Evolution*,  
705 58(1):102–112.
- 706 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R.,  
707 O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H.  
708 (2019). Vegan: Community Ecology Package. R package version 2.5-6.
- 709 Ortiz, E. (1962). Drosopterins in the dewlap of some anoline lizards. *American Zoologist*, 2:545–546.
- 710 Ortiz, E. and Maldonado, A. A. (1966). Pteridine accumulation in lizards of the genus *Anolis*.  
711 *Caribbean Journal of Science*, 6:9–13.
- 712 Ortiz, E., Throckmorton, L. H., and Williams-Ashman, H. G. (1962). Drosopterins in the Throat-  
713 Fans of Some Puerto Rican Lizards. *Nature*, 196(4854):595–596.
- 714 Ortiz, E. and Williams-Ashman, H. (1963). Identification of skin pteridines in the pasture lizard  
715 *Anolis pulchellus*. *Comparative Biochemistry and Physiology*, 10(3):181–190.
- 716 Pinheiro, J. and Bates, D. (2000). *Mixed-Effects Models in S and S-PLUS*. Statistics and Com-  
puting. Springer-Verlag, New York.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and Team, R. C. (2020). Nlme: Linear and Nonlinear Mixed Effects Models.	718 719
Pohlert, T. (2020). PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended. R package version 1.4.4.	720 721
R Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.	722 723
Reynolds, R. G., Kolbe, J. J., Glor, R. E., López-Darias, M., Gómez Pourroy, C. V., Harrison, A. S., Queiroz, K., Revell, L. J., and Losos, J. B. (2020). Phylogeographic and phenotypic outcomes of brown anole colonization across the Caribbean provide insight into the beginning stages of an adaptive radiation. <i>Journal of Evolutionary Biology</i> , 33(4):468–494.	724 725 726 727
Richardson, J. L. and Urban, M. C. (2013). Strong Selection Barriers Explain Microgeographic Adaptation in Wild Salamander Populations. <i>Evolution</i> , 67(6):1729–1740.	728 729
Richardson, J. L., Urban, M. C., Bolnick, D. I., and Skelly, D. K. (2014). Microgeographic adaptation and the spatial scale of evolution. <i>Trends in Ecology &amp; Evolution</i> , 29(3):165–176.	730 731
Rousset, F. (2004). <i>Genetic Structure and Selection in Subdivided Populations</i> . Number 40 in Monographs in Population Biology. Princeton University Press, Princeton.	732 733
Schoener, T. W. (1968). The <i>Anolis</i> Lizards of Bimini: Resource Partitioning in a Complex Fauna. <i>Ecology</i> , 49(4):704–726.	734 735
Seehausen, O. (1997). Cichlid Fish Diversity Threatened by Eutrophication That Curbs Sexual Selection. <i>Science</i> , 277(5333):1808–1811.	736 737
Sigmund, W. R. (1983). Female Preference for <i>Anolis carolinensis</i> Males as a Function of Dewlap Color and Background Coloration. <i>Journal of Herpetology</i> , 17(2):137–143.	738 739
Stapley, J., Wordley, C., and Slate, J. (2011). No Evidence of Genetic Differentiation Between Anoles With Different Dewlap Color Patterns. <i>Journal of Heredity</i> , 102(1):118–124.	740 741
Steffen, J. E. and Guyer, C. C. (2014). Display behaviour and dewlap colour as predictors of contest success in brown anoles: Dewlap Colour and Behaviour in Contests. <i>Biological Journal of the Linnean Society</i> , 111(3):646–655.	742 743 744
Steffen, J. E., Hill, G. E., and Guyer, C. (2010). Carotenoid Access, Nutritional Stress, and the Dewlap Color of Male Brown Anoles. <i>Copeia</i> , 2010(2):239–246.	745 746
Steffen, J. E. and McGraw, K. J. (2007). Contributions of pterin and carotenoid pigments to dewlap coloration in two anole species. <i>Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology</i> , 146(1):42–46.	747 748 749
Steffen, J. E. and McGraw, K. J. (2009). How dewlap color reflects its carotenoid and pterin content in male and female brown anoles ( <i>Norops sagrei</i> ). <i>Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology</i> , 154(3):334–340.	750 751 752
Thorpe, R. S. (2002). Analysis of Color Spectra in Comparative Evolutionary Studies: Molecular Phylogeny and Habitat Adaptation in the St. Vincent Anole ( <i>Anolis trinitatis</i> ). <i>Systematic Biology</i> , 51(4):554–569.	753 754 755
Tokarz, R. R. (2002). An Experimental Test of the Importance of the Dewlap in Male Mating Success in the Lizard <i>Anolis sagrei</i> . <i>Herpetologica</i> , 58(1):87–94.	756 757
Tokarz, R. R. (2006). Importance of Prior Physical Contact with Familiar Females in the Development of a Male Courtship and Mating Preference for Unfamiliar Females in the Lizard <i>Anolis Sagrei</i> . <i>Herpetologica</i> , 62(2):115–124.	758 759 760
Tokarz, R. R., Paterson, A. V., and McMann, S. (2005). Importance of Dewlap Display in Male Mating Success in Free-Ranging Brown Anoles ( <i>Anolis sagrei</i> ). <i>Journal of Herpetology</i> , 39(1):174–177.	761 762

- 764 Vanhooydonck, B., Herrel, A., Meyers, J. J., and Irschick, D. J. (2009). What determines dewlap  
765 diversity in *Anolis* lizards? An among-island comparison. *Journal of Evolutionary Biology*,  
766 22(2):293–305.
- 767 Willi, Y. and Hoffmann, A. A. (2012). Microgeographic adaptation linked to forest fragmentation  
768 and habitat quality in the tropical fruit fly *Drosophila birchii*. *Oikos*, 121(10):1627–1637.
- 769 Williams, E. E. (1969). The Ecology of Colonization as Seen in the Zoogeography of Anoline  
770 Lizards on Small Islands. *The Quarterly Review of Biology*, 44(4):345–389.
- 771 Williams, E. E. and Rand, A. S. (1977). Species Recognition, Dewlap Function and Faunal Size.  
772 *American Zoologist*, 17(1):261–270.
- 773 Zuur, A. F., editor (2009). *Mixed Effects Models and Extensions in Ecology with R*. Statistics for  
Biology and Health. Springer, New York, NY.

774

## Supplementary Figures

775

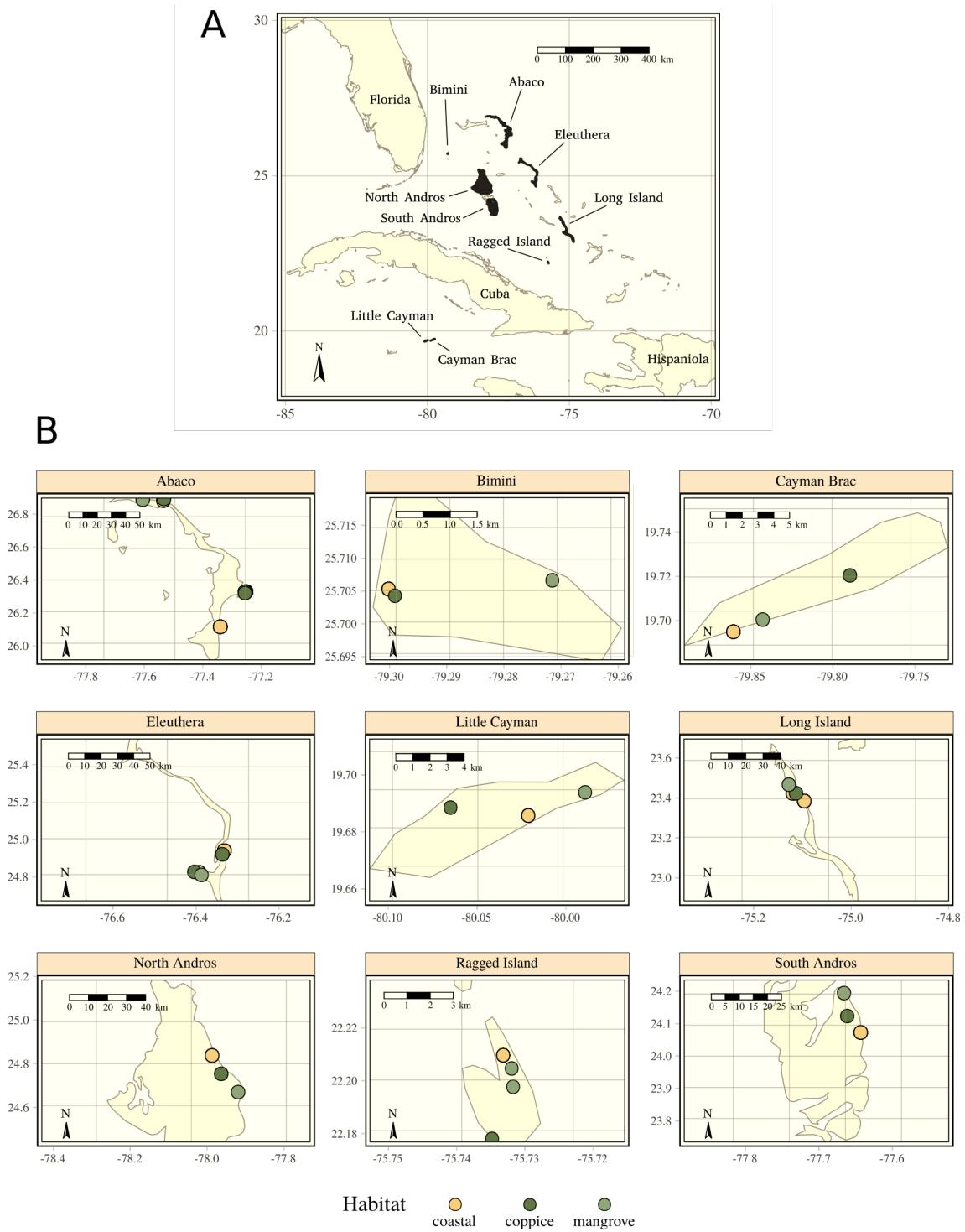


Figure S1: Maps of the islands. (A) Map of the West Indies with sampled islands highlighted in black. (B) Sampling sites within islands colored after their respective habitat-types.

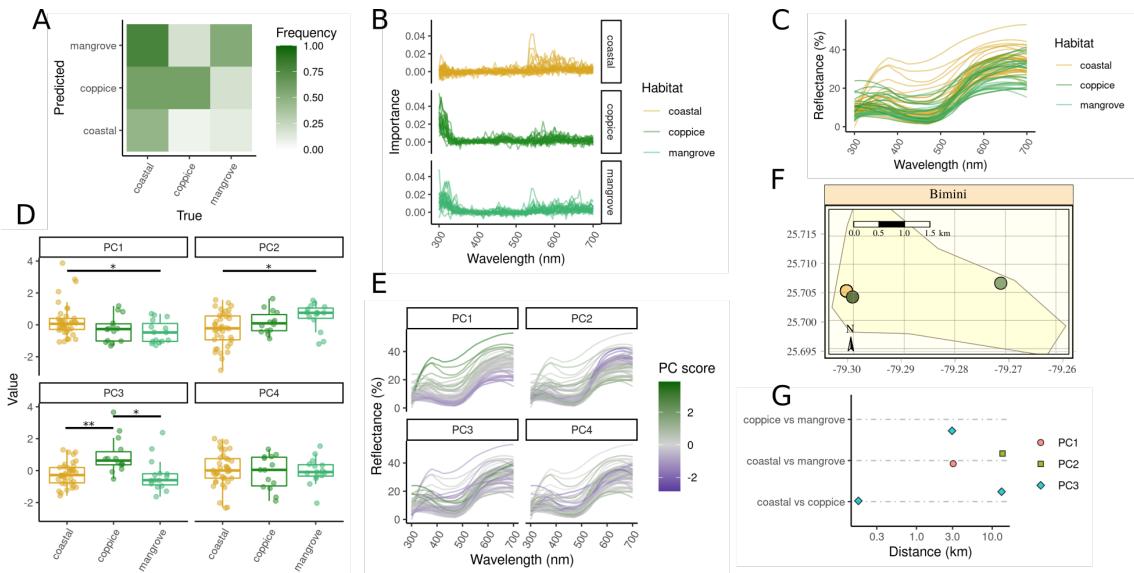


Figure S2: Comparison of dewlap coloration across habitats on Bimini. Legend is as per Figure 1.

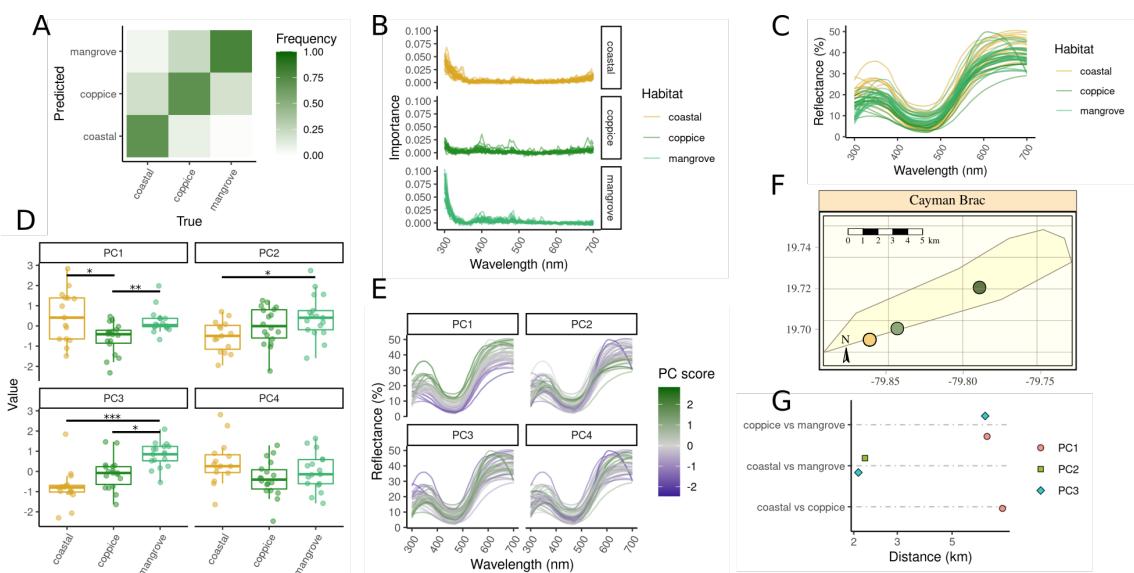


Figure S3: Comparison of dewlap coloration across habitats on Cayman Brac. Legend is as per Figure 1.

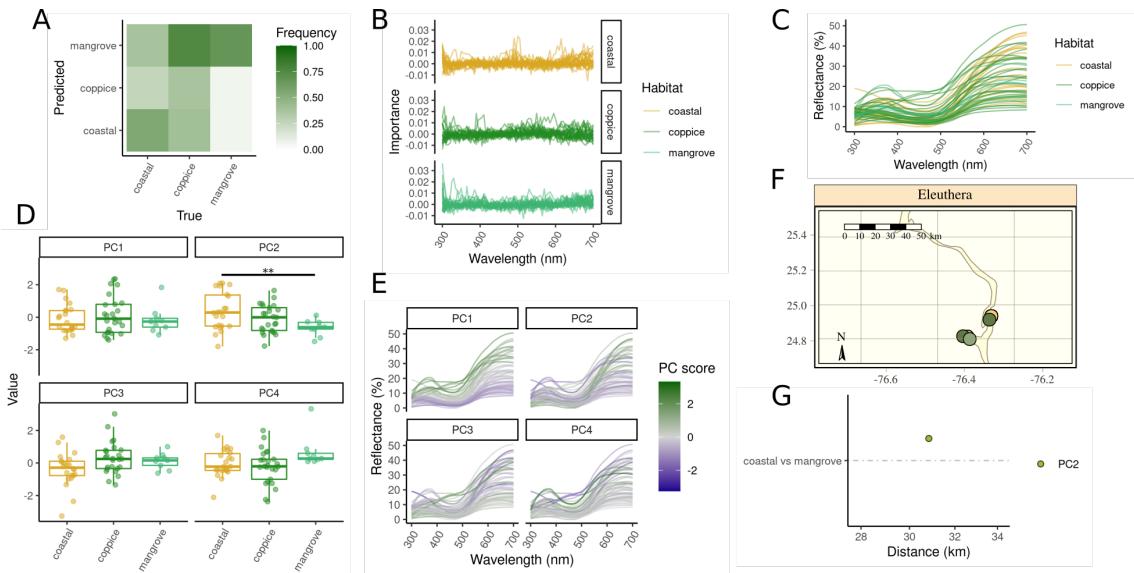


Figure S4: Comparison of dewlap coloration across habitats on Eleuthera. Legend is as per Figure 1.

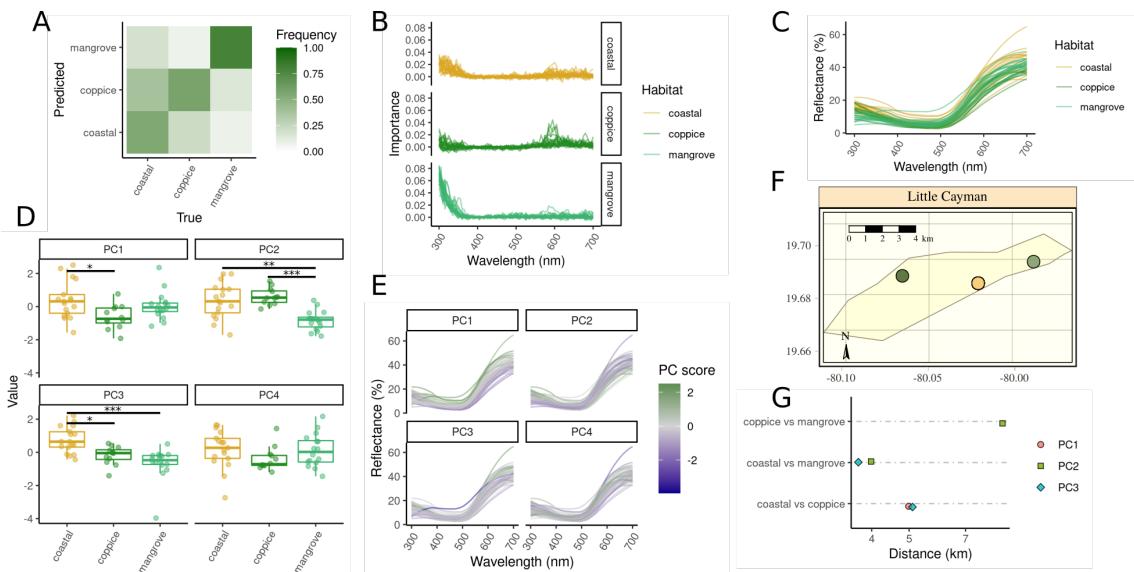


Figure S5: Comparison of dewlap coloration across habitats on Little Cayman. Legend is as per Figure 1.

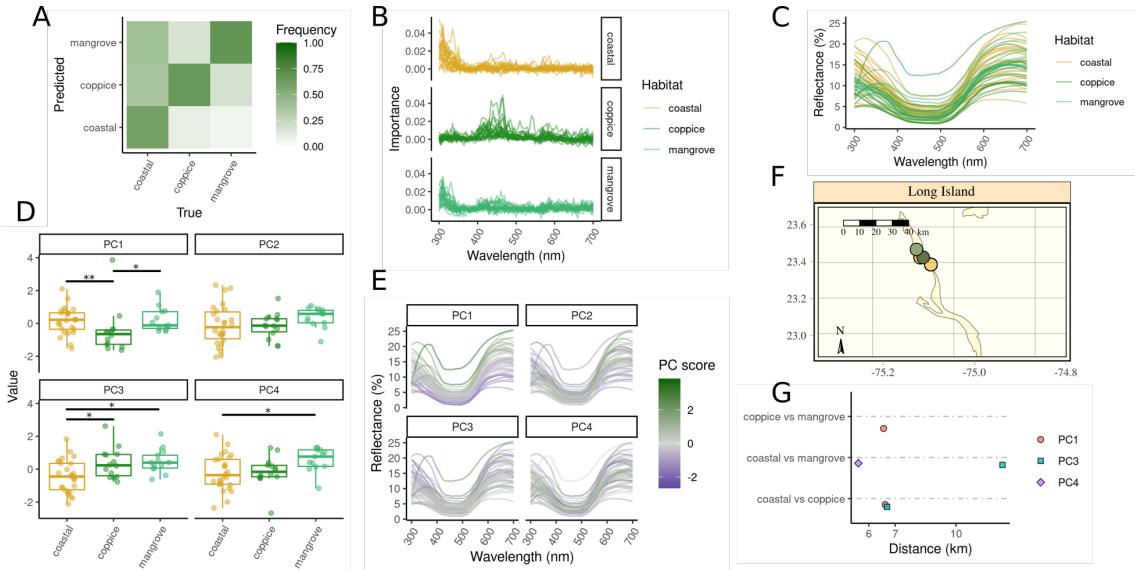


Figure S6: Comparison of dewlap coloration across habitats on Long Island. Legend is as per Figure 1.

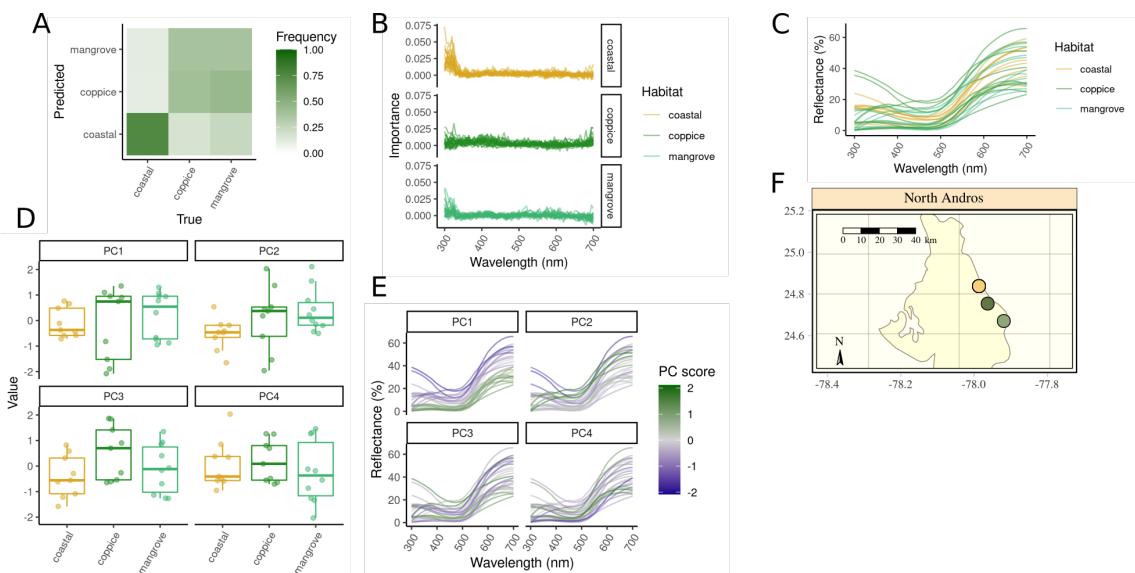


Figure S7: Comparison of dewlap coloration across habitats on North Andros. Legend is as per Figure 1.

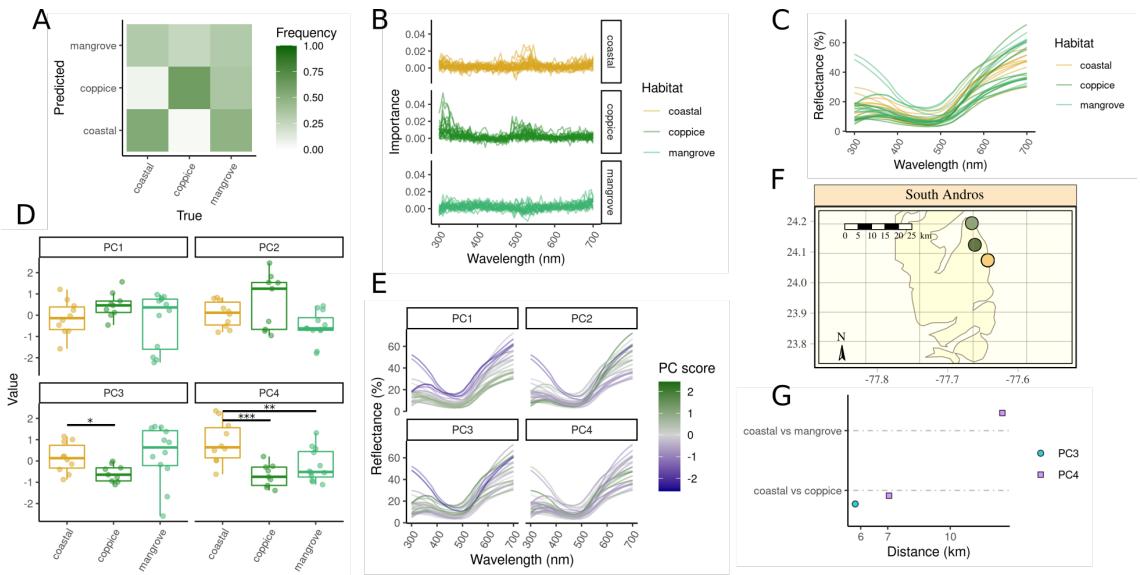


Figure S8: Comparison of dewlap coloration across habitats on South Andros. Legend is as per Figure 1.

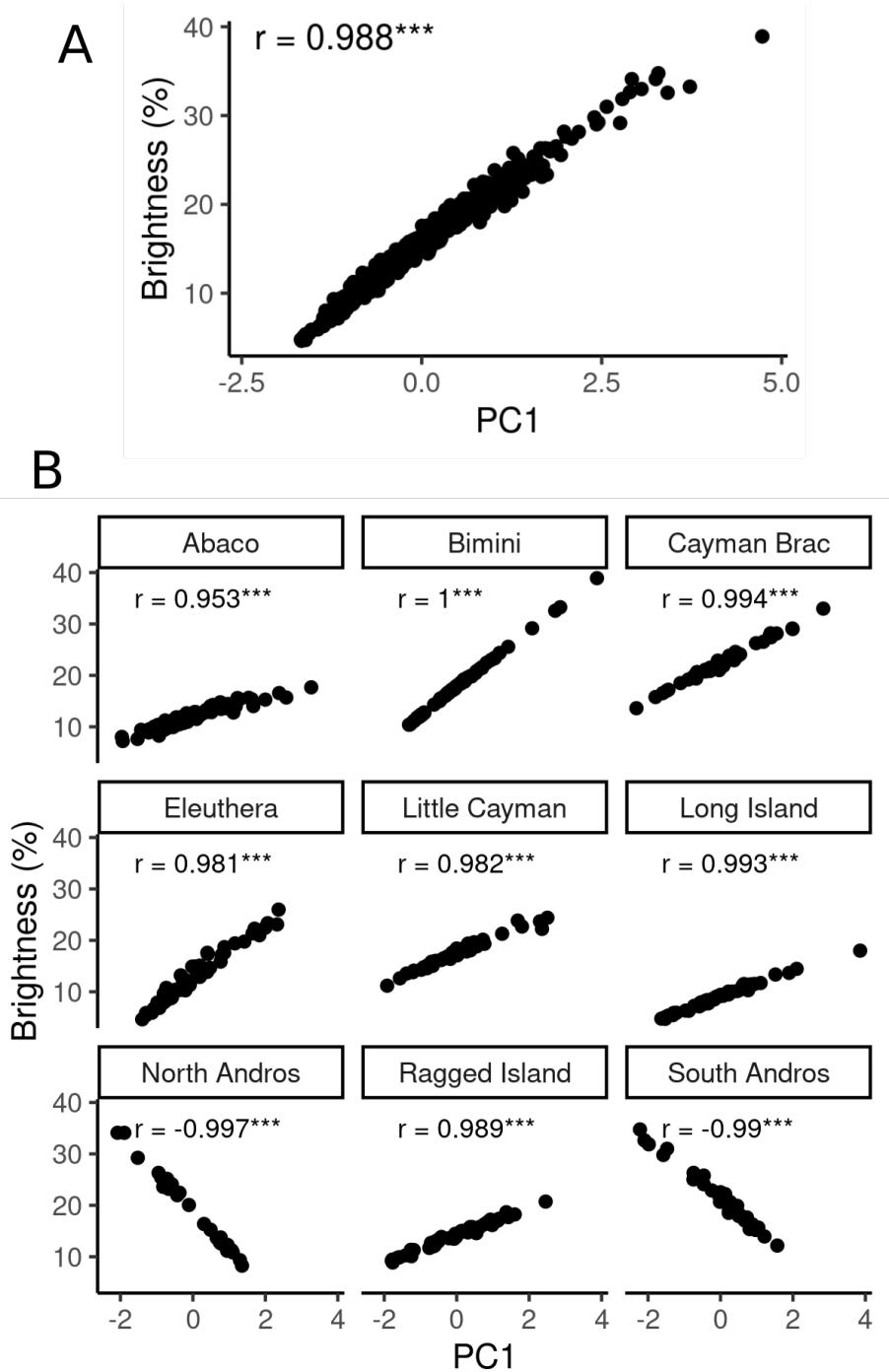


Figure S9: PC1 captures brightness across all islands. (A) Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across all islands. (B) Correlation between brightness and within-island PC1, for each island. Pearson's correlation coefficients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

## Supplementary Tables

776

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 in the PCA performed on all islands together (last row).

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
All islands	0.913	0.473	0.197	0.164	0.079

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 S5: Locations of the sampling sites across islands, with mean within-island principal component scores per site.

Island	Longitude	Latitude	Habitat	PC1	PC2	PC3	PC4
Abaco	-77.7	26.9	mangrove	1.006	0.117	-0.057	-1.259
Abaco	-77.6	26.9	coastal	0.155	0.532	0.079	-2.046
Abaco	-77.6	26.9	coppice	-0.084	0.033	0.636	-0.652
Abaco	-77.2	26.1	coastal	0.304	-0.093	-0.987	-0.133
Abaco	-77.0	26.3	mangrove	-0.316	-1.224	0.899	0.185
Abaco	-77.0	26.3	coppice	0.092	0.314	0.124	0.483
Abaco	-77.0	26.3	coastal	-0.337	0.750	-0.207	0.540
Bimini	-79.3	25.6	coastal	-0.263	-1.270	-0.185	0.164
Bimini	-79.3	25.7	coastal	0.468	0.083	-0.242	0.011
Bimini	-79.3	25.7	coppice	-0.270	0.171	0.926	-0.124
Bimini	-79.3	25.7	mangrove	-0.447	0.533	-0.288	-0.014
Cayman Brac	-79.9	19.7	coastal	0.483	-0.523	-0.781	0.443
Cayman Brac	-79.8	19.7	mangrove	0.219	0.453	0.810	-0.015
Cayman Brac	-79.8	19.7	coppice	-0.610	0.008	-0.114	-0.355
Eleuthera	-76.3	24.8	coppice	-0.123	-0.857	0.045	0.117
Eleuthera	-76.3	24.8	coastal	-0.520	-0.642	-0.492	-0.538
Eleuthera	-76.3	24.8	mangrove	-0.163	-0.651	0.126	0.685
Eleuthera	-76.2	24.9	coppice	0.391	0.602	0.540	-0.539
Eleuthera	-76.1	24.9	coastal	0.254	1.228	-0.315	0.402
Little Cayman	-80.1	19.7	coppice	-0.621	0.614	-0.179	-0.404
Little Cayman	-80.0	19.7	coastal	0.395	0.355	0.743	0.175
Little Cayman	-80.0	19.7	mangrove	0.047	-0.838	-0.655	0.118
Long Island	-75.2	23.5	mangrove	0.207	0.366	0.484	0.587
Long Island	-75.2	23.4	coastal	-0.097	-0.798	-0.140	-0.347
Long Island	-75.2	23.4	coppice	-0.485	-0.133	0.366	-0.148
Long Island	-75.1	23.4	coastal	0.316	0.317	-0.626	-0.130
North Andros	-77.9	24.8	coastal	-0.098	-0.516	-0.403	0.066
North Andros	-77.8	24.8	coppice	-0.139	0.090	0.519	0.192
North Andros	-77.8	24.7	mangrove	0.213	0.384	-0.104	-0.232
Ragged Island	-75.7	22.2	coppice	-0.269	0.440	-0.088	-0.360
Ragged Island	-75.7	22.2	coastal	0.049	0.076	-0.221	0.029
Ragged Island	-75.7	22.2	mangrove	0.246	-0.273	0.118	0.933
Ragged Island	-75.7	22.2	mangrove	0.099	-0.749	0.588	-0.636
South Andros	-77.6	24.2	mangrove	-0.250	-0.539	0.316	-0.211
South Andros	-77.6	24.1	coppice	0.466	0.657	-0.586	-0.687
South Andros	-77.5	24.1	coastal	-0.120	0.055	0.149	0.872

Table S6: Nonparametric Kruskal-Wallis tests performed on each variable on each island where deviations from normality were detected. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Island	Variable	$\chi^2$	df	$P$
Abaco	PC1	0.74	2	0.6924
Abaco	PC2	23.13	2	< 0.0001 ***
Bimini	PC1	7.38	2	0.0250 *
Cayman Brac	PC3	22.46	2	< 0.0001 ***
Eleuthera	PC1	0.29	2	0.8666
Little Cayman	PC3	19.95	2	< 0.0001 ***
Long Island	PC1	10.98	2	0.0041 **

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	**