

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

RAPHAËL SCHERRER^{1,3} *, COLIN M. DONIHUE^{1,4},
R. GRAHAM REYNOLDS², JONATHAN B. LOSOS^{1,4} AND ANTHONY J. GENEVA^{1,5}

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

² Department of Biology, University of North Carolina Asheville, Asheville, NC, USA

³ Current address: Groningen Institute for Evolutionary Life Sciences,
University of Groningen, Groningen, The Netherlands

⁴ Current address: Department of Biology, Washington University, St. Louis, MO, USA

⁵ 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, to test the hypothesis that similar local adaptive processes occur over smaller spatial scales. 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, namely beach scrub, primary coppice forest and mangrove forest. Using reflectance spectrometry paired with 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 that neutral processes or 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 Vehrenkamp, 2011). A primary evolutionary factor shaping communication signals is the sensory system and behavior of 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 ecomorphological 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.,

*Corresponding author: r.scherrer@rug.nl

37 This selective pressure may drive the local adaptation of communication 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 stickleback (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 adapta-
49 tion in fragmented areas in Australian fruit flies (Willi and Hoffmann, 2012), and 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). Lizards of the neotropical genus
55 *Anolis* are an excellent group for studying the eco-evolutionary dynamics of local adaptation and
56 natural selection (Losos, 2009). A particularly conspicuous trait of anoles is their dewlap, an
57 extensible flap of skin that is typically sexually dimorphic and used as a communication signal
58 in courtship (Sigmund, 1983; Driessens et al., 2014, 2015) and territorial displays (Losos, 1985;
59 Macedonia and Stamps, 1994; Macedonia et al., 2013) as well as in predator deterrence (Leal
60 and Rodríguez-Robles, 1995, 1997; Leal and Rodriguez-Robles, 1997). Dewlap characteristics vary
61 widely among the approximately 400 species of the genus (Nicholson et al., 2007). Interspecific
62 variation in dewlap coloration is implicated in species recognition (Rand and Williams, 1970;
63 Williams, 1969; Williams and Rand, 1977; Losos, 1985; Macedonia and Stamps, 1994; Fleishman,
64 2000; Macedonia et al., 2013), and this function could have had a role in initiating or reinforcing
65 reproductive isolation during speciation (Lambert et al., 2013; Geneva et al., 2015; Ng et al., 2017).

66
67 Within species, studies have shown a link between variation in dewlap coloration and differ-
68 ences in habitats or climatic conditions (Macedonia, 2001; Leal and Fleishman, 2002; Thorpe and
69 Stenson, 2002; Thorpe, 2002; Leal and Fleishman, 2004; Vanhooydonck et al., 2009; Ng et al., 2012,
70 2013, 2016; Vanhooydonck et al., 2009; Driessens et al., 2017). Some studies suggest that those
71 differences may be adaptive and that dewlaps may have evolved to maximize detectability given
72 local light conditions (Fleishman and Persons, 2001; Leal and Fleishman, 2002, 2004). Although
73 this claim is further supported by recent findings that dewlap colors are perceived differently un-
74 der different levels of shading (Fleishman et al., 2020), other studies found conflicting patterns
75 of between-habitat variation that did not support the sensory drive hypothesis (Fleishman et al.,
76 2009; Ng et al., 2012; Macedonia et al., 2014).

77
78 Previous studies investigating variation in anole dewlaps compared populations at relatively
79 large geographical scales, e.g. between islands (Vanhooydonck et al., 2009; Driessens et al., 2017)
80 or within large islands such as Puerto Rico (Leal and Fleishman, 2004) or Hispaniola (Ng et al.,
81 2012, 2016). These large scales and marine barriers should reduce gene flow (Ng and Glor, 2011;
82 Lambert et al., 2013; Richardson et al., 2014; Ng et al., 2017). That said, examples do exist of
83 divergence in dewlap coloration at smaller scales or between populations with high degrees of gene
84 flow (Thorpe and Stenson, 2002; Thorpe, 2002; Stapley et al., 2011; Ng et al., 2016).

85
86 The species *Anolis sagrei* is widespread across islands of the West Indies (Reynolds et al., 2020).
87 It has been the subject of numerous studies concerning local adaptation (Losos et al., 1994, 1997,
88 2001; Kolbe et al., 2012), biological invasion (Kolbe et al., 2008), and sexual selection (Tokarz,
89 2002; Tokarz et al., 2005; Tokarz, 2006; Driessens et al., 2014; Steffen and Guyer, 2014; Driessens
90 et al., 2015) among many other topics. Between-island variation in the mainly orange-red color of
91 its dewlap was shown to be better explained by climatic variables (Driessens et al., 2017) than by
92 proxies for biotic factors such as sexual selection or predation pressure (Vanhooydonck et al., 2009;
93 Baeckens et al., 2018). How intra-island differences in habitat may contribute to the diversity of
94 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 small-island 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 (van de Schoot 2016 unpublished thesis; Reynolds et al. 2020), 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 part of 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).

Methods

119

Data collection

120

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

136 Reflectance measurements

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

147 Analysis

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

166
167 All analyses in this study were performed in R 3.6.1 (R Core Team, 2019) and the source code
168 can be found at <https://github.com/rscherrer/dewlap>, presently private.

169 **Dimensionality reduction**

170 Because neighboring wavelengths are highly collinear and redundant in reflectance, we reduced
171 the dimensionality of the data using principal component analysis (PCA), as per Cuthill et al.
172 (1999) and Leal and Fleishman (2002). We performed PCA on data from all islands combined, as
173 well as on each island separately and systematically retained the first four principal components
174 (PC), which together always explained more than 88.8% of the variance across islands (Table S2).
175 PCs need not represent the same wavelengths across islands because they are fitted on different
176 datasets. Nevertheless, PC1 was highly collinear with brightness for all islands (Figure S11), while
177 the other PCs captured the chromatic variation (i.e. irrespective of brightness) in dewlap color.

178 **Among-island variance partitioning**

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

186 **Within-island machine learning**

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

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

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

213 Here, we used a k-fold cross-validation procedure with $k = 5$, where each training set consisted
214 of 80% of the data and the machine was tested on the remaining 20%. Each training set was con-
215 ditioned on containing at least five lizards from each of the three habitats. We also down-sampled
216 the training set to the sample size of the least represented habitat, to ensure that the different
217 habitats were equally represented. To further remove any bias due to the specific random split
218 into the different bins, we replicated each k-fold cross-validation five times. We then averaged the
219 five resulting confusion matrices across replicates, where each confusion matrix shows the number
220 of correctly classified lizards for each habitat. We used the random forest function in the R package
221 randomForest (R Core Team 2019) to fit the random forests, and calculated the success rate using
222 the confusionMatrix function in the R package caret (Kuhn 2020).

of lizards from each habitat reassigned into each habitat. For each island, we then used the average 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 calls 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 homoscedasticity were met (i.e. OLS-ANOVA was the best fit), we used Tukey's honest significant difference test. When normality was met but not homoscedasticity (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.

276 Spatial autocorrelation

277 We tested for within-island spatial autocorrelation between the geographical distances among sam-
278 pling 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

287 Site differences

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

Results

294

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

295

296

We subsequently tested for differences in dewlap coloration between habitat-populations within each island, using within-island principal component scores (to maximize the variation captured for each island, see Methods). Our within-island random forest classification analyses revealed detectable differences in dewlap coloration on eight out of the nine islands in our sample: Abaco, Bimini, Cayman Brac, Eleuthera, Little Cayman, Long Island, North Andros and South Andros. The accuracy of random forest classification exceeded random expectation more often than expected by chance for all these islands (Table 1). Accuracy was as high as 73% for Cayman Brac. We obtained similar results using other machine learning approaches such as support vector machines (Table S7) and linear discriminant analysis (Table S8), except that these methods did not detect significant differences on Eleuthera and North Andros. We did not find evidence of spatial autocorrelation in dewlap coloration between the sites within islands, except for Eleuthera (Table 2). We now describe the specific differences detected on each island.

On Abaco, dewlaps from the mangrove habitat were the best discriminated, while dewlaps from the beach scrub habitat were often mistaken for dewlaps from the coppice habitat (Fig. 2D). Importance analysis revealed that beach scrub and mangrove lizards mostly differed in reflectance in the ultraviolet (UV) end of the spectrum (below 400nm, Fig. S2F), where mangrove dewlaps had higher UV reflectance relative to beach scrub lizards, and coppice lizards had an intermediate UV reflectance between the two other habitats (Fig. 2B). Consistent with this, our analyses of variance detected significantly lower PC2 scores in mangrove lizards than in the two other habitats (Fig. 2E, Table 3), representing a higher UV-reflectance relative to red (Fig. 2C). Beach scrub lizards also scored lower on PC3 (Fig. 2E, Table 3), indicating less curvature of the reflectance profile and relatively higher reflectance at intermediate wavelengths (blue-to-yellow) than at the ends of the range (Fig. 2C). Differences were detected between sites both at large ($\sim 100\text{km}$) and short ($< 1\text{km}$) distances (Fig. S2G).

On Bimini, the random forests mostly correctly classified lizards from the coppice and mangrove habitats while often misclassifying lizards from the beach scrub habitat (Fig. S3D). Relatively flat importance profiles for beach scrub lizards suggested that brightness was used instead of a particular wavelength to identify some of the beach scrub dewlaps (Fig. S3F). Indeed, some beach scrub dewlaps were substantially brighter than the rest (Fig. S3B, C), a pattern that was captured by our analysis of variance along PC1 (i.e. brightness, Fig. S3C, E, Table 3). The random forests also used UV reflectance to discriminate between coppice and mangrove dewlaps (Fig. S3F), which could reflect the significant difference we detected along PC3 between these two habitats (Fig. S3C, E, Table 3). Beach scrub lizards were characterized by elevated red reflectance relative to UV (as represented by PC2, (Fig. S3C, E)), and beach scrub and mangrove lizards were characterized by a more even distribution of the reflectance along the spectrum (as represented by PC3, (Fig. S3C, E)), in contrast to coppice lizards which harbored a stronger curvature at intermediate wavelengths (Fig. S3C, E). On this island, the beach scrub and coppice habitats were separated by a few hundred meters, making this contrast the smallest geographical scale at which differences in coloration were found in our study (Fig. S3G).

On Cayman Brac, all three habitats could be well discriminated against each other (Fig. S4D), with UV reflectance appearing to be an important variable differentiating beach scrub and mangrove dewlaps (Fig. S4F). In contrast, coppice dewlaps had a relatively flat importance profile, suggesting that brightness made them more distinct rather than any particular wavelength (Fig.

351 S4F). Consistent with this, coppice dewlaps were significantly different from all other dewlaps
352 along PC1 (Fig. S4E, Table 3). At a distance between 2 and 3km (Fig. S4G), dewlaps in the
353 beach scrub habitat reflected more red light (as represented by PC2, Fig. S4C, E) and more UV
354 (as represented by PC3, Fig. S4C, E) than in the mangrove habitat. Coppice lizards were also
355 characterized by a higher UV reflectance than mangrove dewlaps (PC3, Fig. S4C, E, Table 3).

356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402

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 beach scrub and mangrove lizards, where beach scrub lizards had higher levels of red reflectance and mangrove lizards higher levels of UV reflectance (Fig. S5C, E, Table 3).

Little Cayman was characterized by a better discrimination of mangrove lizards from the rest than between beach scrub and coppice lizards (Fig. S6D). Mangrove dewlaps were most distinct with respect to their reflectance in short wavelengths (Fig. S6F), with significantly lower UV reflectance (as represented by PC2, Fig. S6C, E, Table 3). Beach scrub 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. S6C, E, Table 3).

On Long Island the three habitats were relatively well discriminated (Fig. S7D). Importance profiles indicated that short wavelengths were used to discriminate between beach scrub and mangrove lizards (Fig. S7F). Beach scrub 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. S7C, E, Table 3). Beach scrub lizards also differed from mangrove lizards along PC4 (Fig. S7E), 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. S7C). Coppice lizards were significantly darker than mangrove and beach scrub lizards (PC1, Fig. S7C, 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. S8D), no significant univariate differences were detected along any of the four PCs (Fig. S8E, 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. S8F). Besides, reflectance curves of beach scrub dewlaps appeared more similar to each other in the UV range than dewlaps from other habitats (Fig. S8B), 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).

On South Andros beach scrub and coppice dewlaps could be discriminated better against each other than with mangrove dewlaps (Fig. S10D), with importance profiles supporting UV-reflectance as a predictor of coppice lizards (Fig. S10F). Coppice lizards had more curved reflectance profiles than beach scrub lizards (PC3), and lizards from both habitats differed along PC4, which is again more difficult to interpret (Fig. S10C, E, Table 3). Beach scrub lizards also differed from mangrove lizards in PC4 (Fig. S10C, 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.

Discussion

403

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

404

405

Detectable differences in dewlap color between populations are surprising, as habitats were often in close geographical proximity to each other (as close as a few hundred meters on Bimini and most of the time within ten kilometers). Indeed, given that (1) the populations were continuously distributed between the habitats, (2) populations from different habitats were not monophyletic with respect to mitochondrial haplotypes (van de Schoot 2016 unpubl.), and (3) *A. sagrei* has been shown to be a highly mobile species (Kamath and Losos, 2018), we would have expected more homogeneous distributions of color phenotypes within islands due to extensive gene flow, with fewer differences between populations, especially those in close proximity.

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

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

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

Consistent patterns across islands would have been a good clue for a sensory-drive explanation, but in particular consistent differences between habitats that are most different in their local conditions regarding the ecological function of the dewlap, such as ambient light. For example, if ambient light is an important factor shaping dewlap coloration, we would expect mangrove and beach scrub lizards, both inhabiting areas with high light penetration, to harbor more similar dewlaps, and to differ significantly from lizards from the coppice habitat, where irradiance is low. Overall, the observed heterogeneity of divergence patterns across islands provides no support to a sensory-drive explanation.

459

460 Phenotypic plasticity could be another cause for dewlap color variation between habitats, where
461 different environmental conditions produce different phenotypes in different habitats. The yellow,
462 orange and red colors in anoline dewlaps are produced by pterins and carotenoids (Ortiz, 1962;
463 Ortiz et al., 1962; Ortiz and Williams-Ashman, 1963; Ortiz and Maldonado, 1966; Macedonia
464 et al., 2000; Steffen and McGraw, 2007, 2009). Animals can synthesize pterins from nucleotides,
465 but lack the ability to synthesize carotenoids (Goodwin, 1984; Hill et al., 2002; Hill and McGraw,
466 2006). Different food qualities across sites within islands could therefore potentially cause de-
467 tectable differences in coloration. Alternatively, more subtle effects on dewlap color could arise
468 from developmental plasticity and depend, e.g., on differences in egg-rearing conditions. However,
469 more data are needed to test these hypotheses, and although Cook et al. (2013) have shown plastic
470 responses of dewlap color in response to parasites, we find it unlikely to account for the widespread
471 habitat differences we found. Besides, studies testing the effect of carotenoid deprivation (Steffen
472 et al., 2010; Ng et al., 2013) and heritability (Cox et al., 2017) of dewlap coloration in *A. sagrei* and
473 another species with a carotenoid-based dewlap, *A. distichus*, found little support for phenotypic
474 and developmental plasticity in dewlap coloration.

475 Genetic drift is another process that can account for differences in phenotype between localities,
476 especially in small populations. One way this could proceed is through isolation-by-distance,
477 where more distant populations accumulate more differences through time because of the reduced
478 effect of gene flow at larger geographical scales relative to the dispersal range of the species (Rous-
479 set, 2004). Here, we only found a significant correlation between phenotypic and geographical
480 distances on Eleuthera to support this scenario. On all the other islands, in contrast, populations
481 from closer sites were not phenotypically more similar, which argues against isolation-by-distance.
482 That said, there were often few sampling sites per island in our study, whose locations were not
483 uniformly chosen within the islands, and so the true extent of isolation-by-distance may be diffi-
484 cult to test. Other forms of drift may be at play than isolation-by-distance, but nevertheless, we
485 did find significant differences in color phenotype at relatively small spatial scales, sometimes in
486 neighboring habitats, on islands where gene flow is probably highly pervasive, as suggested by high
487 rates of encounter between males and females (Kamath and Losos, 2018), making the divergence
488 of habitat-populations by drift in relative genetic isolation an unlikely scenario.

489 A number of alternative explanations remain. First, there could be sexual selection for different
490 dewlap colors in different locations. Indeed, although the sensory-drive hypothesis may include a
491 sexual selection aspect, e.g., if the optimal male phenotype in a given habitat is a function of female
492 perception, sexual preferences may also be arbitrary and independent of the habitat (Andersson,
493 1994), and so could differ across habitats and islands. Although one previous study has found no
494 link between dewlap coloration and body size dimorphism in *A. sagrei* (a proxy for the intensity
495 of sexual selection) in an among-island comparison (Baeckens et al., 2018), within-island data are
496 lacking to test the plausibility of this scenario.

497 Alternatively, selective pressures may be different in similar habitats from one island to another,
498 because of other environmental variables not accounted for by the habitat-type classification we
499 used (Howard, 1950; Schoener, 1968). The islands we sampled exhibit variation in some climatic
500 variables, but also in densities of predators and anole congeners, which have all been shown to
501 correlate with variation in *A. sagrei* mean dewlap coloration among islands (Vanhooydonck et al.,
502 2009; Baeckens et al., 2018).

503 Finally, different island-populations could also respond differently to similar selective pressures,
504 resulting in various between-habitat divergence patterns across islands. Several factors could ac-
505 count for this. For example, the founder populations of each island, which colonized the islands
506 independently (van de Schoot, unpublished thesis; Driessens et al. 2017; Reynolds et al. 2020),
507 could have exhibited different dewlap colors at the time of colonization, as may be suggested by
508 the larger differences we observed between than within islands. In turn, different initial pheno-
509 types could have led to different ways in which populations would have diverged between habitats.
510 Moreover, the different founding populations may have also consisted in different subsets of the
511 standing genetic variation of their Cuban ancestor due to potential bottlenecks (Reynolds et al.,
512 2020), which may have constrained the way they would later respond to the local selective pressures
513 of their respective islands.

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

526 Acknowledgements

527 Collection permission was granted by the Bahamas Environment, Science and Technology Com-
528 mission, the Bahamas National Trust, the Bahamas Ministry of Agriculture, and the Cayman Is-
529 lands 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, Quyhn Quach, Wendy Jesse, Inbar Maayan, Alexis Harrison and Melissa
Kemp for assistance in the field and Pratik Gupte, Max Lambert and James Stroud for helpful
discussions. This project was made possible through the support of a grant from the John Tem-
pleton Foundation (to JBL). The opinions expressed in this publication are those of the author(s)
and do not necessarily reflect the views of the John Templeton Foundation. Additional funding
for this work was provided by 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).

530
531
532
533
534
535
536
537
538

Figures

539

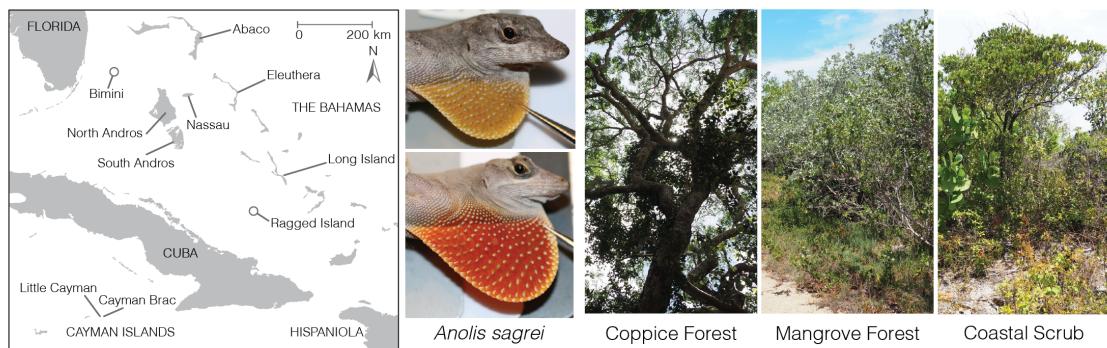


Figure 1: Overview of our study design, including a map of the Bahamas and the Cayman Islands, on which are indicated the nine islands we sampled, two representatives of our study species *Anolis sagrei* with their dewlaps deployed, and the three types of habitats we considered on each island.

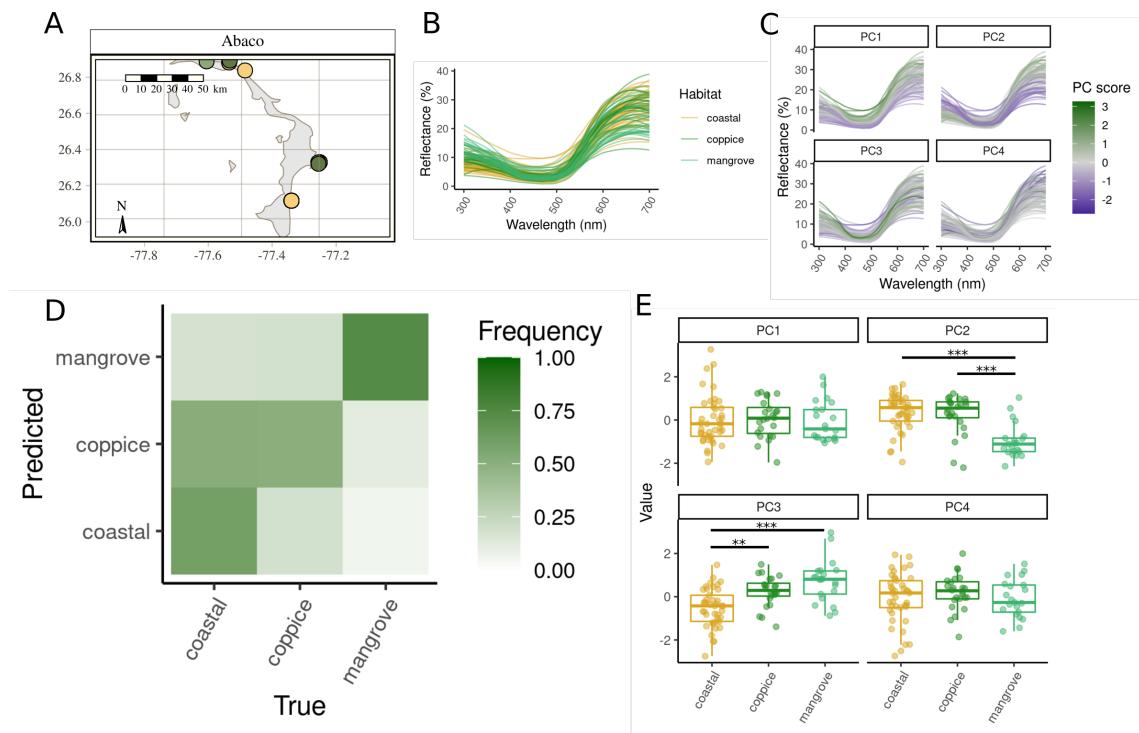


Figure 2: Comparison of dewlap coloration across habitats on Abaco. (A) Map of the island with the sampling sites colored by habitat. (B) Reflectance profiles of all the dewlaps on the island. (C) How reflectance profiles map onto the within-island principal components. (D) Confusion matrix showing the 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. (E) Within-island principal component scores across habitats. Bars indicate significant contrasts. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

540 **Tables**

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

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. ΔAICc , difference in AICc between the best-fitting model and the OLS-model. AICcw, AICc weight. Log-lik., log-likelihood. χ^2 , likelihood ratio. df, degrees of freedom. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Island	Variable	AICc	ΔAICc	AICcw	Model	Log-lik.	χ^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. *Austral Ecology*, 26(1):32–46. 541
542
543
- Andersson, M. B. (1994). *Sexual Selection*. Monographs in Behavior and Ecology. Princeton University Press, Princeton, N.J. 544
545
- Arnold, S. J. (1983). Morphology, Performance and Fitness. *American Zoologist*, 23(2):347–361. 546
- 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? *PeerJ*, 6:e4722. 547
548
549
- Bartoń, K. (2019). MuMIn: Multi-Model Inference. 550
- Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B*, 57:289–300. 551
552
553
- Bradbury, J. W. and Vehrencamp, S. L. (2011). *Principles of Animal Communication*. Sinauer Associates, Sunderland, Mass, 2nd ed edition. 554
555
- Breiman, L. (2001). Random forests. *Machine Learning*, 45(1):5–32. 556
- Cook, E. G., Murphy, T. G., and Johnson, M. A. (2013). Colorful displays signal male quality in a tropical anole lizard. *Naturwissenschaften*, 100(10):993–996. 557
558
- Cortez, P. (2020). Rminer: Data Mining Classification and Regression Methods. 559
- Cox, R. M., Costello, R. A., Camber, B. E., and McGlothlin, J. W. (2017). Multivariate genetic architecture of the *Anolis* dewlap reveals both shared and sex-specific features of a sexually dimorphic ornament. *Journal of Evolutionary Biology*, 30(7):1262–1275. 560
561
562
- Cristianini, N. and Shawe-Taylor, J. (2000). *An Introduction to Support Vector Machines and Other Kernel-Based Learning Methods*. Cambridge University Press, first edition. 563
564
- 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. *The American Naturalist*, 153(2):183–200. 565
566
567
- Dieckmann, U. and Doebeli, M. (1999). On the origin of species by sympatric speciation. *Nature*, 400(6742):354–357. 568
569
- 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 *Anolis sagrei* lizards. *Journal of Evolutionary Biology*, 30(10):1846–1861. 570
571
572
- Driessens, T., Huyghe, K., Vanhooydonck, B., and Van Damme, R. (2015). Messages conveyed by assorted facets of the dewlap, in both sexes of *Anolis sagrei*. *Behavioral Ecology and Sociobiology*, 69(8):1251–1264. 573
574
575
- Driessens, T., Vanhooydonck, B., and Van Damme, R. (2014). Deterring predators, daunting opponents or drawing partners? Signaling rates across diverse contexts in the lizard *Anolis sagrei*. *Behavioral Ecology and Sociobiology*, 68(2):173–184. 576
577
578
- Endler, J. (1998). Sensory ecology, receiver biases and sexual selection. *Trends in Ecology & Evolution*, 13(10):415–420. 579
580
- Endler, J. A. (1984). Natural and sexual selection on color patterns in poeciliid fishes. In Balon, E. K. and Zaret, T. M., editors, *Evolutionary Ecology of Neotropical Freshwater Fishes*, volume 3, pages 95–111. Springer Netherlands, Dordrecht. 581
582
583
- Endler, J. A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society*, 41(4):315–352. 584
585

- Endler, J. A. (1991). Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Research*, 31(3):587–608. 586
- Endler, J. A. (1992). Signals, Signal Conditions, and the Direction of Evolution. *The American Naturalist*, 139:S125–S153. 588
- Endler, J. A. (1993a). The Color of Light in Forests and Its Implications. *Ecological Monographs*, 63(1):1–27. 590
- Endler, J. A. (1993b). Some general comments on the evolution and design of animal communication systems. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 340(1292):215–225. 592
- Endler, J. A. and McLellan, T. (1988). The Processes of Evolution: Toward a Newer Synthesis. *Annual Review of Ecology and Systematics*, 19(1):395–421. 595
- Felsenstein, J. (1976). The Theoretical Population Genetics of Variable Selection and Migration. *Annual Review of Genetics*, 10(1):253–280. 597
- Fleishman, L. J. (2000). *Signal Function, Signal Efficiency and the Evolution of Anoline Lizard Dewlap Color*, pages 209–236. Tapir Academic, Trondheim. 599
- Fleishman, L. J., Leal, M., and Persons, M. H. (2009). Habitat light and dewlap color diversity in four species of Puerto Rican anoline lizards. *Journal of Comparative Physiology A*, 195(11):1043–1060. 601
- Fleishman, L. J. and Persons, M. (2001). The influence of stimulus and background colour on signal visibility in the lizard *Anolis cristatellus*. *The Journal of Experimental Biology*, 204(Pt 9):1559–1575. 604
- Fleishman, L. J., Wadman, C. S., and Maximov, K. J. (2020). The interacting effects of total light intensity and chromatic contrast on visual signal visibility in an *Anolis* lizard. *Animal Behaviour*, page S0003347220302037. 607
- García-Ramos, G. and Kirkpatrick, M. (1997). Genetic Models of Adaptation and Gene Flow in Peripheral Populations. *Evolution*, 51(1):21–28. 610
- Geneva, A. J., Hilton, J., Noll, S., and Glor, R. E. (2015). Multilocus phylogenetic analyses of Hispaniolan and Bahamian trunk anoles (*distichus* species group). *Molecular Phylogenetics and Evolution*, 87:105–117. 612
- Gittleman, J. L. and Kot, M. (1990). Adaptation: Statistics and a Null Model for Estimating Phylogenetic Effects. *Systematic Zoology*, 39(3):227. 615
- Goodwin, T. W. (1984). *The Biochemistry of the Carotenoids*. Springer Netherlands, Dordrecht. 617
- Halfwerk, W., Jones, P. L., Taylor, R. C., Ryan, M. J., and Page, R. A. (2014). Risky Ripples Allow Bats and Frogs to Eavesdrop on a Multisensory Sexual Display. *Science*, 343(6169):413–416. 618
- Hendry, A. P., Day, T., and Taylor, E. B. (2007a). Population Mixing and the Adaptive Divergence of Quantitative Traits in Discrete Populations: A Theoretical Framework for Empirical Tests. *Evolution*, 55(3):459–466. 620
- Hendry, A. P., Taylor, E. B., and McPhail, J. D. (2007b). Adaptive Divergence and the Balance Between Selection and Gene Flow: Lake and Stream Stickleback in the Misty System. *Evolution*, 56(6):1199–1216. 623
- Henze, N. and Zirkler, B. (1990). A class of invariant consistent tests for multivariate normality. *Communications in Statistics - Theory and Methods*, 19(10):3595–3617. 626
- Hijmans, R. J. (2019). Geosphere: Spherical Trigonometry. 628
- Hill, G. E., Inouye, C. Y., and Montgomerie, R. (2002). Dietary carotenoids predict plumage coloration in wild house finches. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269(1496):1119–1124. 629

632	Hill, G. E. and McGraw, K. J., editors (2006). <i>Bird Coloration</i> . Harvard University Press, Cambridge, Mass.	633
	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.	634
		635
		636
	Howard, R. A. (1950). Vegetation of the Bimini Island Group: Bahamas, B. W. I. <i>Ecological Monographs</i> , 20(4):317–349.	637
		638
	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.	639
		640
	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.	641
		642
		643
	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.	644
		645
		646
	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.	647
		648
		649
	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.	650
		651
	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.	652
		653
		654
	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.	655
		656
	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.	657
		658
		659
	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.	660
		661
	Leal, M. and Rodríguez-Robles, J. A. (1995). Antipredator Responses of <i>Anolis cristatellus</i> (Sauria: Polychrotidae). <i>Copeia</i> , 1995(1):155–161.	662
		663
	Leal, M. and Rodriguez-Robles, J. A. (1997). Antipredator Responses of the Puerto Rican Giant Anole, <i>Anolis cuvieri</i> (Squamata: Polychrotidae). <i>Biotropica</i> , 29(3):372–375.	664
		665
	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.	666
		667
	Lenormand, T. (2002). Gene flow and the limits to natural selection. <i>Trends in Ecology & Evolution</i> , 17(4):183–189.	668
		669
	Liaw, A. and Wiener, M. (2002). Classification and Regression by randomForest. <i>R News</i> , 2(3):18–22.	670
		671
	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.	672
		673
	Losos, J. B. (2009). <i>Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles</i> . University of California Press.	674
		675
	Losos, J. B. (2011). Convergence, Adaptation, and Constraint. <i>Evolution</i> , 65(7):1827–1840.	676

- 678 Losos, J. B., Irschick, D. J., and Schoener, T. W. (1994). Adaptation and Constraint in the 677
Evolution of Specialization of Bahamian *Anolis* Lizards. *Evolution*, 48(6):1786–1798.
- 679 Losos, J. B., Schoener, T. W., Warheit, K. I., and Creer, D. (2001). Experimental studies of 680
adaptive differentiation in Bahamian *Anolis* lizards. *Genetica*, 112-113:399–415.
- 681 Losos, J. B., Warheit, K. I., and Schoener, T. W. (1997). Adaptive differentiation following 682
experimental island colonization in *Anolis* lizards. *Nature*, 387(6628):70–73.
- 683 Macedonia, J. M. (2001). Habitat light, colour variation, and ultraviolet reflectance in the Grand 684
Cayman anole, *Anolis conspersus*. *Biological Journal of the Linnean Society*, 73(3):299–320.
- 685 Macedonia, J. M., Clark, D. L., Riley, R. G., and Kemp, D. J. (2013). Species recognition of color 686
and motion signals in *Anolis grahami*: Evidence from responses to lizard robots. *Behavioral 687
Ecology*, 24(4):846–852.
- 688 Macedonia, J. M., Clark, D. L., and Tamasi, A. L. (2014). Does Selection Favor Dewlap Colors that 689
Maximize Detectability? A Test with Five Species of Jamaican *Anolis* Lizards. *Herpetologica*, 690
70(2):157–170.
- 691 Macedonia, J. M., James, S., Witte, L. W., and Clark, D. L. (2000). Skin Pigments and Coloration 692
in the Jamaican Radiation of *Anolis* Lizards. *Journal of Herpetology*, 34(1):99–109.
- 693 Macedonia, J. M. and Stamps, J. A. (1994). Species Recognition in *Anolis grahami* (Sauria, 694
Iguanidae): Evidence from Responses to Video Playbacks of Conspecific and Heterospecific 695
Displays. *Ethology*, 98(3-4):246–264.
- 696 Maia, R., Eliason, C. M., Bitton, P.-P., Doucet, S. M., and Shawkey, M. D. (2013). Pavo: An 697
R package for the analysis, visualization and organization of spectral data. *Methods in Ecology 698
and Evolution*, pages n/a–n/a.
- 699 Ng, J., Geneva, A. J., Noll, S., and Glor, R. E. (2017). Signals and Speciation: *Anolis* Dewlap 700
Color as a Reproductive Barrier. *Journal of Herpetology*, 51(3):437–447.
- 701 Ng, J. and Glor, R. E. (2011). Genetic differentiation among populations of a Hispaniolan trunk 702
anole that exhibit geographical variation in dewlap colour. *Molecular Ecology*, 20(20):4302–4317.
- 703 Ng, J., Kelly, A. L., MacGuigan, D. J., and Glor, R. E. (2013). The Role of Heritable and Dietary 704
Factors in the Sexual Signal of a Hispaniolan *Anolis* Lizard, *Anolis distichus*. *Journal of Heredity*, 705
104(6):862–873.
- 706 Ng, J., Landeen, E. L., Logsdon, R. M., and Glor, R. E. (2012). Correlation Between *Anolis* 707
Lizard Dewlap Phenotype and Environmental Variation Indicates Adaptive Divergence of a 708
Signal Important to Sexual Selection and Species Recognition. *Evolution*, 67(2):573–582.
- 709 Ng, J., Ossip-Klein, A. G., and Glor, R. E. (2016). Adaptive signal coloration maintained in the 710
face of gene flow in a Hispaniolan *Anolis* Lizard. *BMC Evolutionary Biology*, 16(1):193.
- 711 Nicholson, K. E., Harmon, L. J., and Losos, J. B. (2007). Evolution of *Anolis* Lizard Dewlap 712
Diversity. *PLoS ONE*, 2(3):e274.
- 713 Nosil, P. and Crespi, B. J. (2004). Does Gene Flow Constrain Adaptive Divergence or Vice Versa? A 714
Test Using Ecomorphology and Sexual Isolation in *Timema cristinae* Walking-Sticks. *Evolution*, 715
58(1):102–112.
- 716 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., 717
O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H. 718
(2019). Vegan: Community Ecology Package. R package version 2.5-6.
- 719 Ortiz, E. (1962). Drosopterins in the dewlap of some anoline lizards. *American Zoologist*, 2:545–546.
- 720 Ortiz, E. and Maldonado, A. A. (1966). Pteridine accumulation in lizards of the genus *Anolis*. 721
Caribbean Journal of Science, 6:9–13.

- ⁷²² Ortiz, E., Throckmorton, L. H., and Williams-Ashman, H. G. (1962). Drosopterins in the Throat-Fans of Some Puerto Rican Lizards. *Nature*, 196(4854):595–596. ⁷²³
- Ortiz, E. and Williams-Ashman, H. (1963). Identification of skin pteridines in the pasture lizard *Anolis pulchellus*. *Comparative Biochemistry and Physiology*, 10(3):181–190. ⁷²⁴
- Pinheiro, J. and Bates, D. (2000). *Mixed-Effects Models in S and S-PLUS*. Statistics and Computing. Springer-Verlag, New York. ⁷²⁵
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and Team, R. C. (2020). Nlme: Linear and Nonlinear Mixed Effects Models. ⁷²⁶
- Pohlert, T. (2020). PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended. R package version 1.4.4. ⁷²⁷
- R Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. ⁷²⁸
- Rand, A. S. and Williams, E. E. (1970). An Estimation of Redundancy and Information Content of Anole Dewlaps. *The American Naturalist*, 104(935):99–103. ⁷²⁹
- 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. *Journal of Evolutionary Biology*, 33(4):468–494. ⁷³⁰
- Richardson, J. L. and Urban, M. C. (2013). Strong Selection Barriers Explain Microgeographic Adaptation in Wild Salamander Populations. *Evolution*, 67(6):1729–1740. ⁷³¹
- Richardson, J. L., Urban, M. C., Bolnick, D. I., and Skelly, D. K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology & Evolution*, 29(3):165–176. ⁷³²
- Rousset, F. (2004). *Genetic Structure and Selection in Subdivided Populations*. Number 40 in Monographs in Population Biology. Princeton University Press, Princeton. ⁷³³
- Schoener, T. W. (1968). The *Anolis*Lizards of Bimini: Resource Partitioning in a Complex Fauna. *Ecology*, 49(4):704–726. ⁷³⁴
- Seehausen, O. (1997). Cichlid Fish Diversity Threatened by Eutrophication That Curbs Sexual Selection. *Science*, 277(5333):1808–1811. ⁷³⁵
- Sigmund, W. R. (1983). Female Preference for *Anolis carolinensis* Males as a Function of Dewlap Color and Background Coloration. *Journal of Herpetology*, 17(2):137–143. ⁷³⁶
- Stapley, J., Wordley, C., and Slate, J. (2011). No Evidence of Genetic Differentiation Between Anoles With Different Dewlap Color Patterns. *Journal of Heredity*, 102(1):118–124. ⁷³⁷
- 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. *Biological Journal of the Linnean Society*, 111(3):646–655. ⁷³⁸
- Steffen, J. E., Hill, G. E., and Guyer, C. (2010). Carotenoid Access, Nutritional Stress, and the Dewlap Color of Male Brown Anoles. *Copeia*, 2010(2):239–246. ⁷³⁹
- Steffen, J. E. and McGraw, K. J. (2007). Contributions of pterin and carotenoid pigments to dewlap coloration in two anole species. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 146(1):42–46. ⁷⁴⁰
- Steffen, J. E. and McGraw, K. J. (2009). How dewlap color reflects its carotenoid and pterin content in male and female brown anoles (*Norops sagrei*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 154(3):334–340. ⁷⁴¹
- Thorpe, R. S. (2002). Analysis of Color Spectra in Comparative Evolutionary Studies: Molecular Phylogeny and Habitat Adaptation in the St. Vincent Anole (*Anolis trinitatis*). *Systematic Biology*, 51(4):554–569. ⁷⁴²

- 769 Thorpe, R. S. and Stenson, A. G. (2002). Phylogeny, Paraphyly and Ecological Adaptation of 768
770 the Colour and Pattern in the Anolis Roquet Complex on Martinique: Interaction Between
771 Phylogeny and Adaptation. *Molecular Ecology*, 12(1):117–132.
- 772 Tokarz, R. R. (2002). An Experimental Test of the Importance of the Dewlap in Male Mating
773 Success in the Lizard *Anolis sagrei*. *Herpetologica*, 58(1):87–94.
- 774 Tokarz, R. R. (2006). Importance of Prior Physical Contact with Familiar Females in the Develop-
775 ment of a Male Courtship and Mating Preference for Unfamiliar Females in the Lizard *Anolis*
Sagrei. *Herpetologica*, 62(2):115–124.
- 776 Tokarz, R. R., Paterson, A. V., and McMann, S. (2005). Importance of Dewlap Display in
777 Male Mating Success in Free-Ranging Brown Anoles (*Anolis sagrei*). *Journal of Herpetology*,
778 39(1):174–177.
- 779 van de Schoot, M. (2016). *Within and between Island Radiation and Genetic Variation in Anolis*
780 *Sagrei*. PhD thesis, Wageningen University, Wageningen, The Netherlands.
- 781 Vanhooydonck, B., Herrel, A., Meyers, J. J., and Irschick, D. J. (2009). What determines dewlap
782 diversity in *Anolis* lizards? An among-island comparison. *Journal of Evolutionary Biology*,
783 22(2):293–305.
- 784 Willi, Y. and Hoffmann, A. A. (2012). Microgeographic adaptation linked to forest fragmentation
785 and habitat quality in the tropical fruit fly *Drosophila birchii*. *Oikos*, 121(10):1627–1637.
- 786 Williams, E. E. (1969). The Ecology of Colonization as Seen in the Zoogeography of Anoline
787 Lizards on Small Islands. *The Quarterly Review of Biology*, 44(4):345–389.
- 788 Williams, E. E. and Rand, A. S. (1977). Species Recognition, Dewlap Function and Faunal Size.
789 *American Zoologist*, 17(1):261–270.
- 790 Zuur, A. F., editor (2009). *Mixed Effects Models and Extensions in Ecology with R*. Statistics for
791 Biology and Health. Springer, New York, NY.

⁷⁹² Supplementary Figures

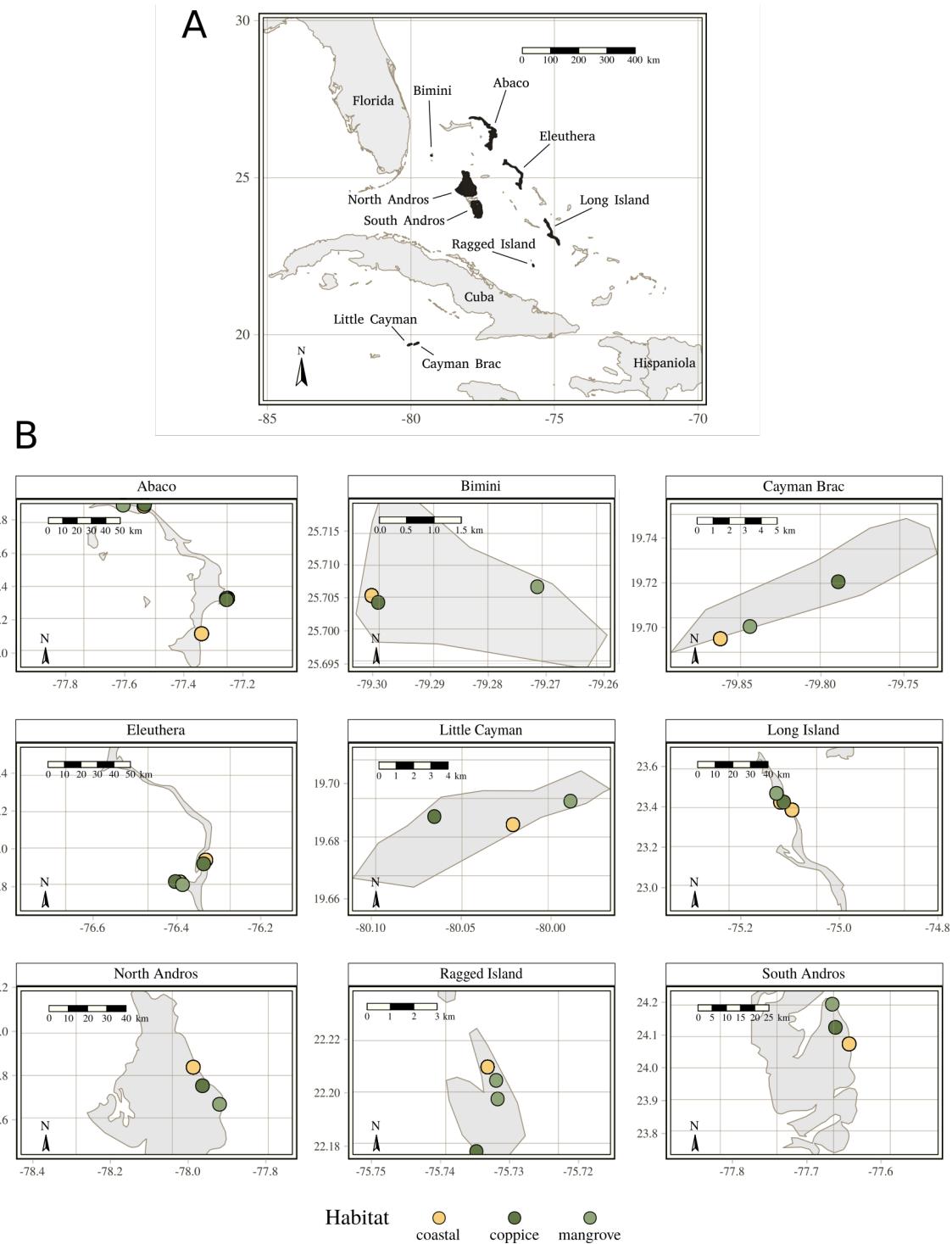


Figure S1: Maps of the islands. (A) Map of the West Indies with sampled islands highlighted in black. (B) Sampling sites within islands colored after their respective habitat-types.

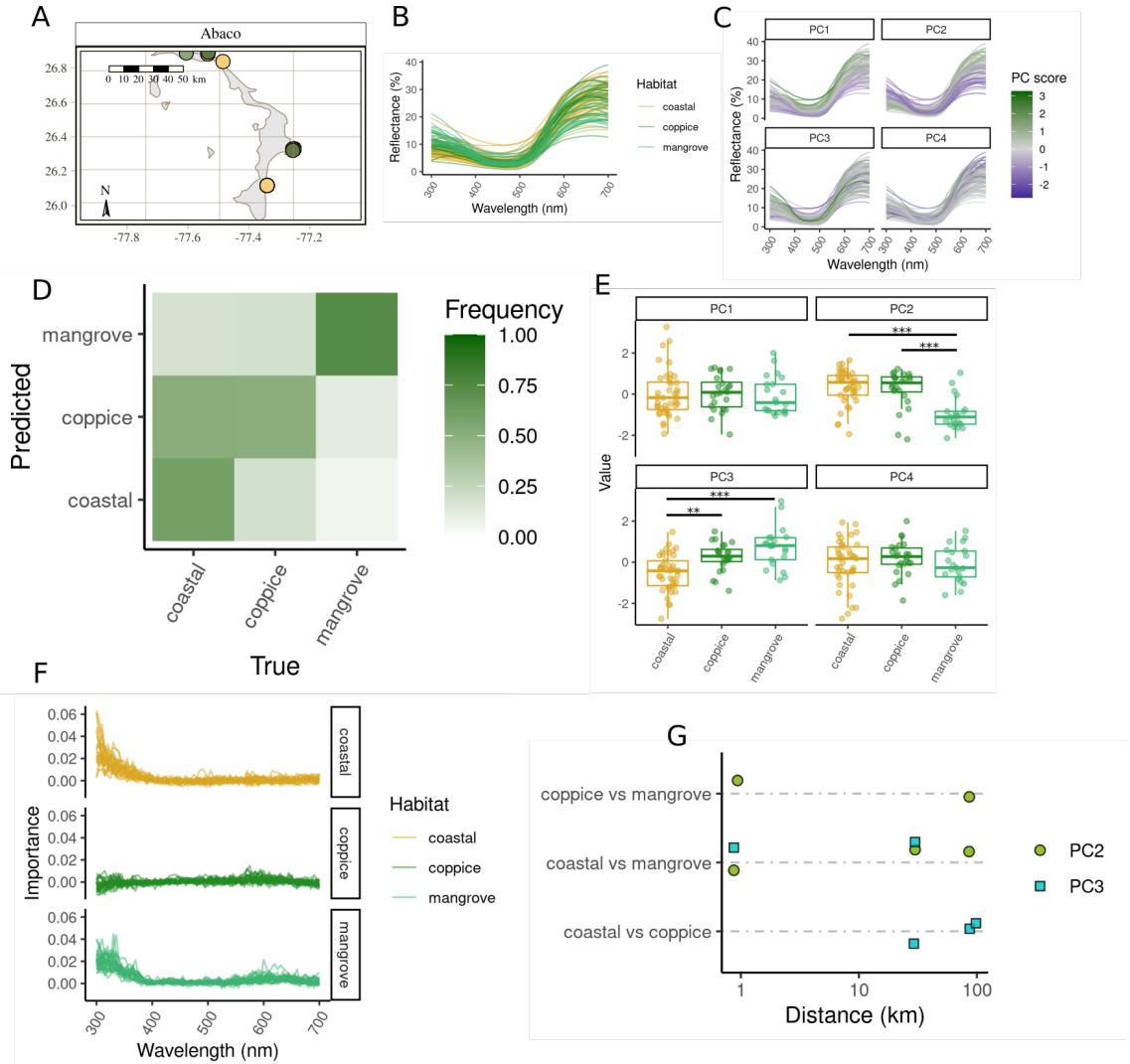


Figure S2: Comparison of dewlap coloration across habitats on Abaco, with extended results. (A–E) Legend as per Figure 2. (F) One-dimensional sensitivity analysis showing the relative importance (mean decrease in accuracy) of the various wavelengths in random forest classification of the whole spectrum. (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.

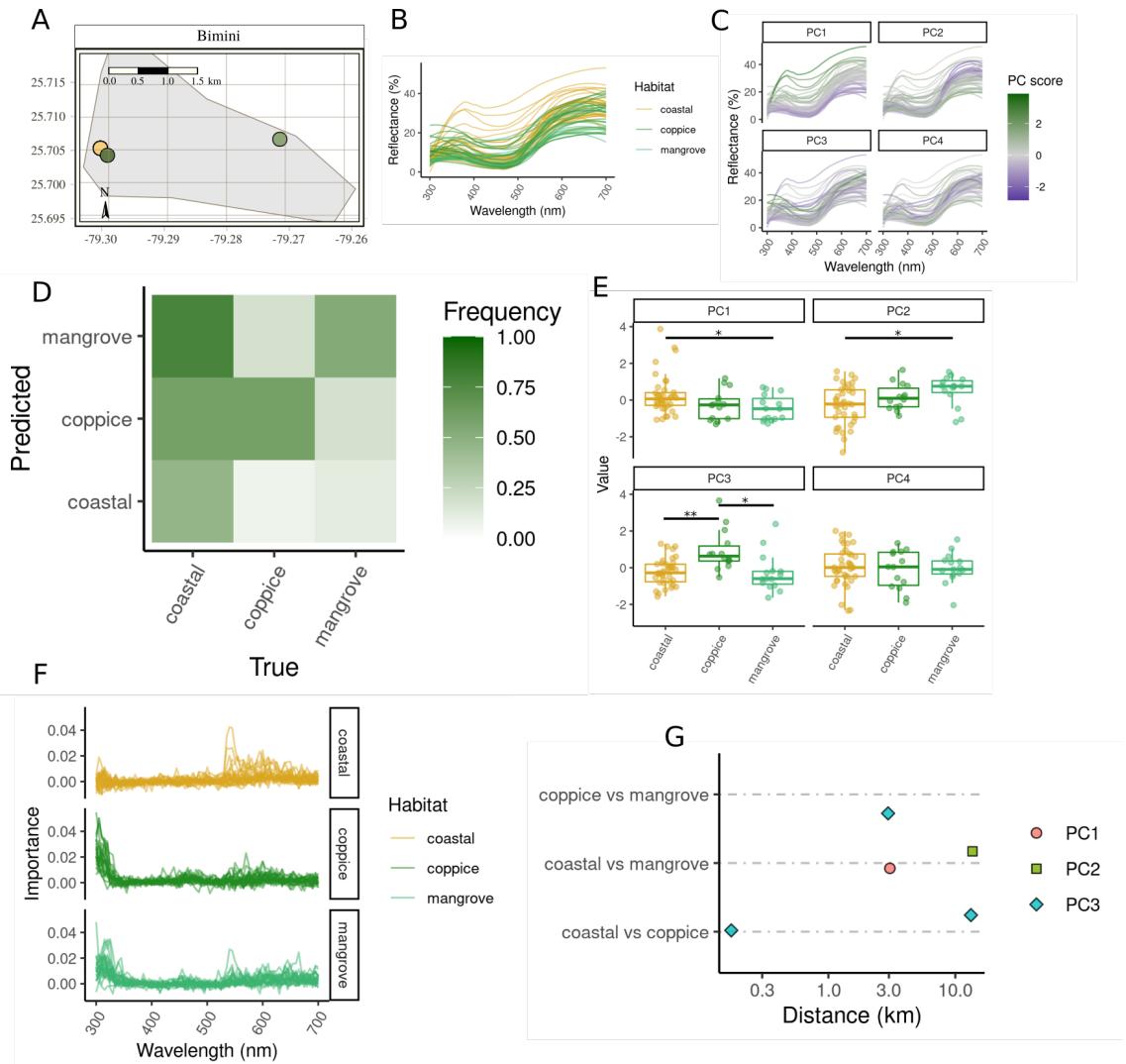


Figure S3: Comparison of dewlap coloration across habitats on Bimini. Legend is as per Figure S2.

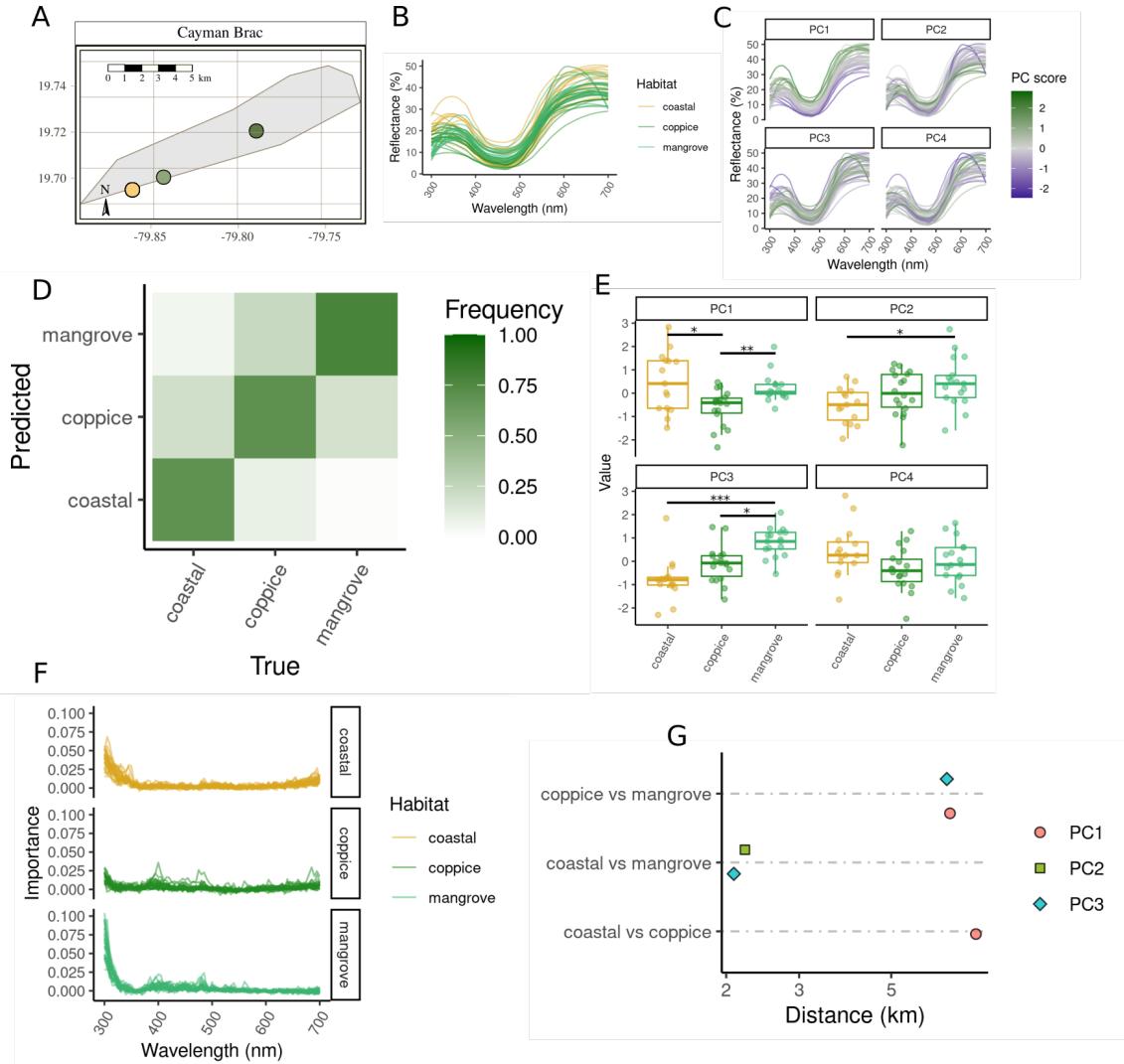


Figure S4: Comparison of dewlap coloration across habitats on Cayman Brac. Legend is as per Figure S2.

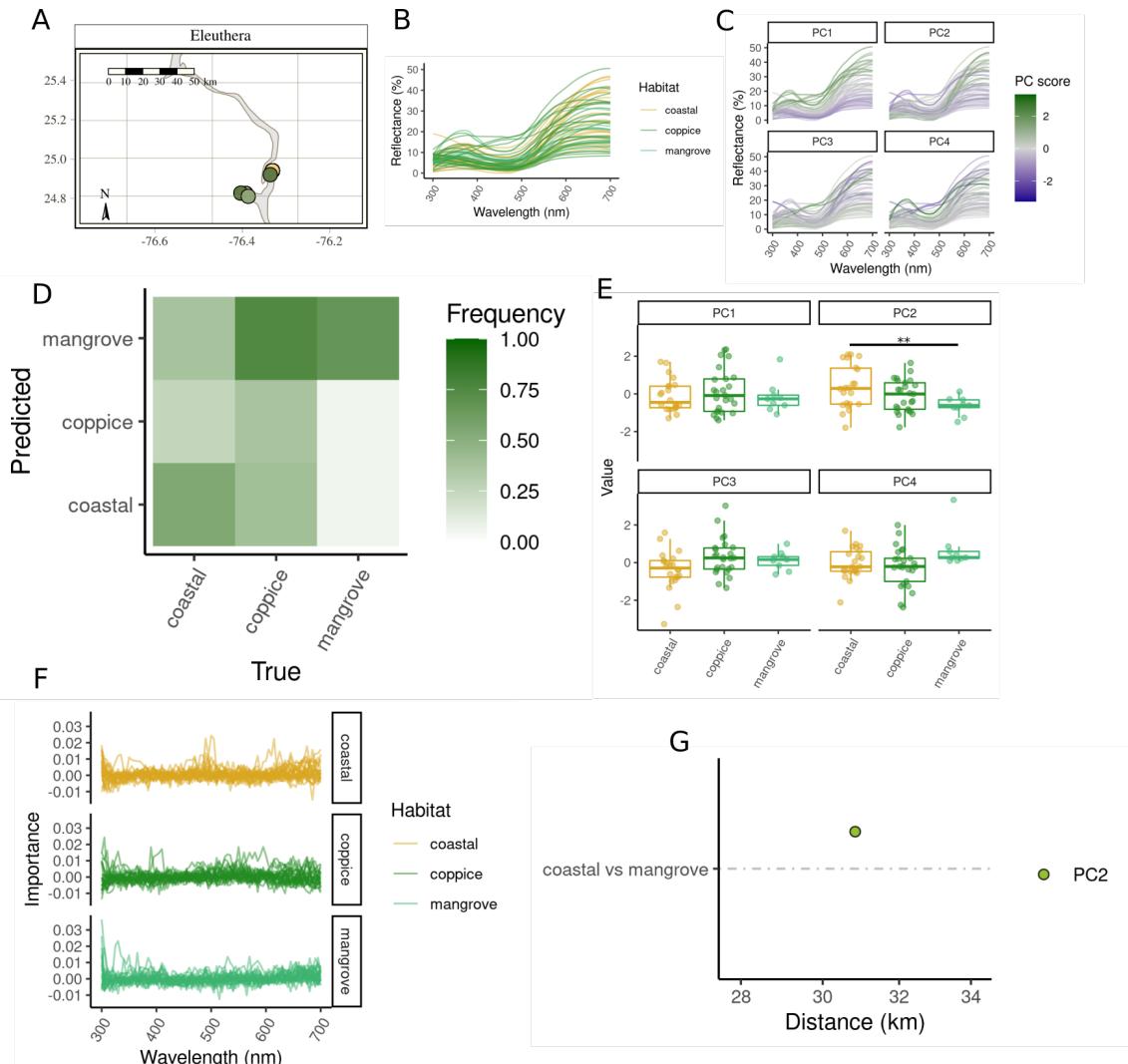


Figure S5: Comparison of dewlap coloration across habitats on Eleuthera. Legend is as per Figure S2.

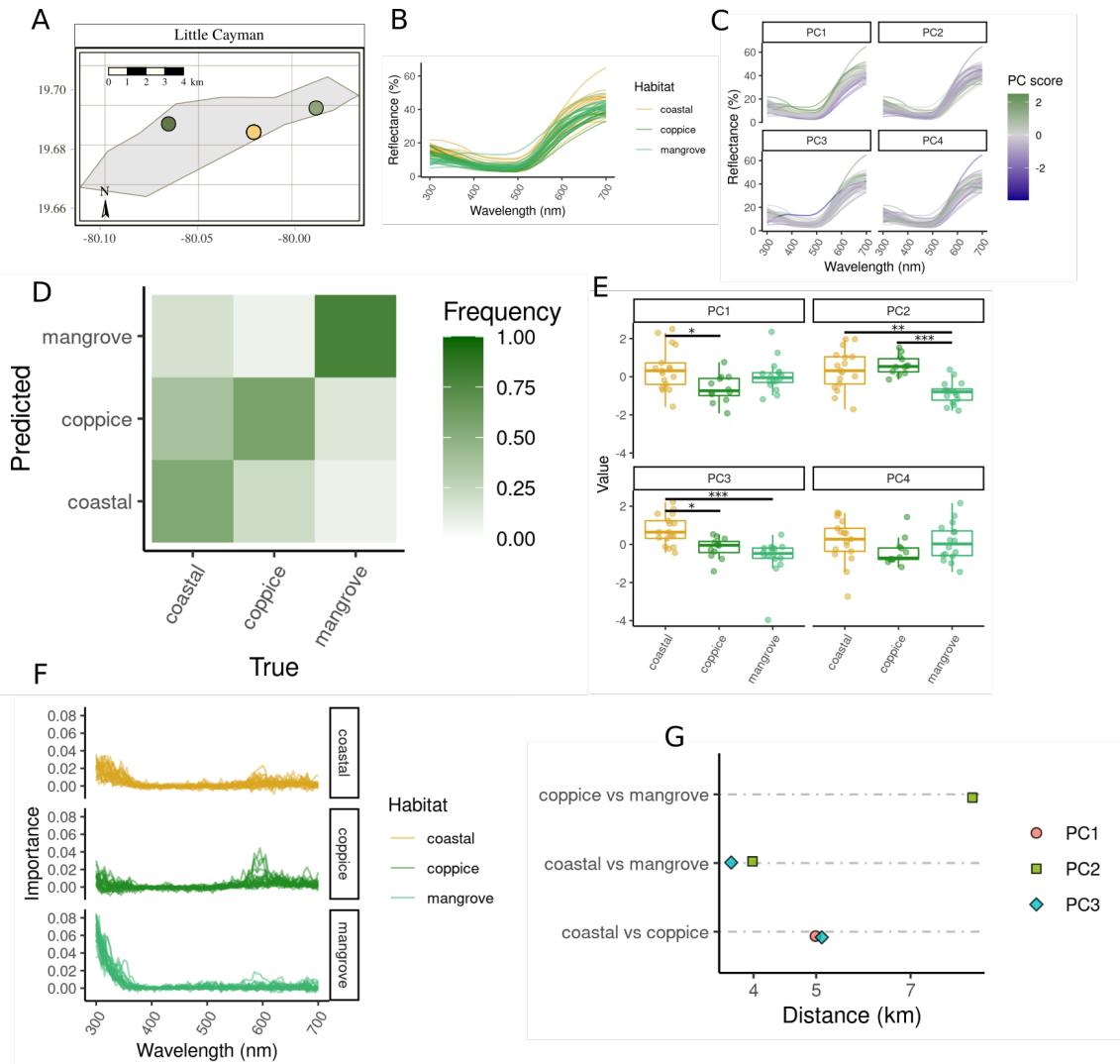


Figure S6: Comparison of dewlap coloration across habitats on Little Cayman. Legend is as per Figure S2.

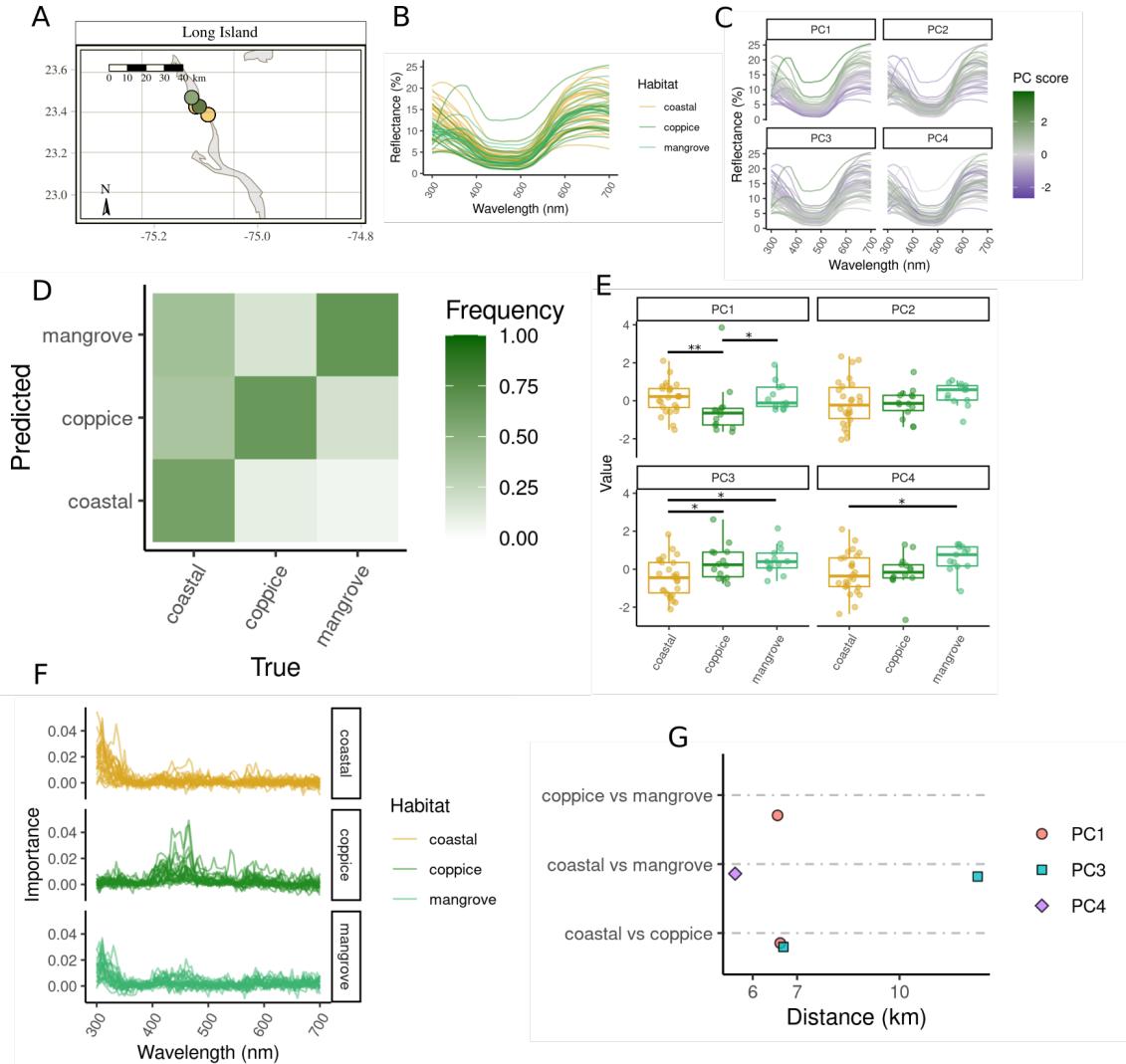


Figure S7: Comparison of dewlap coloration across habitats on Long Island. Legend is as per Figure S2.

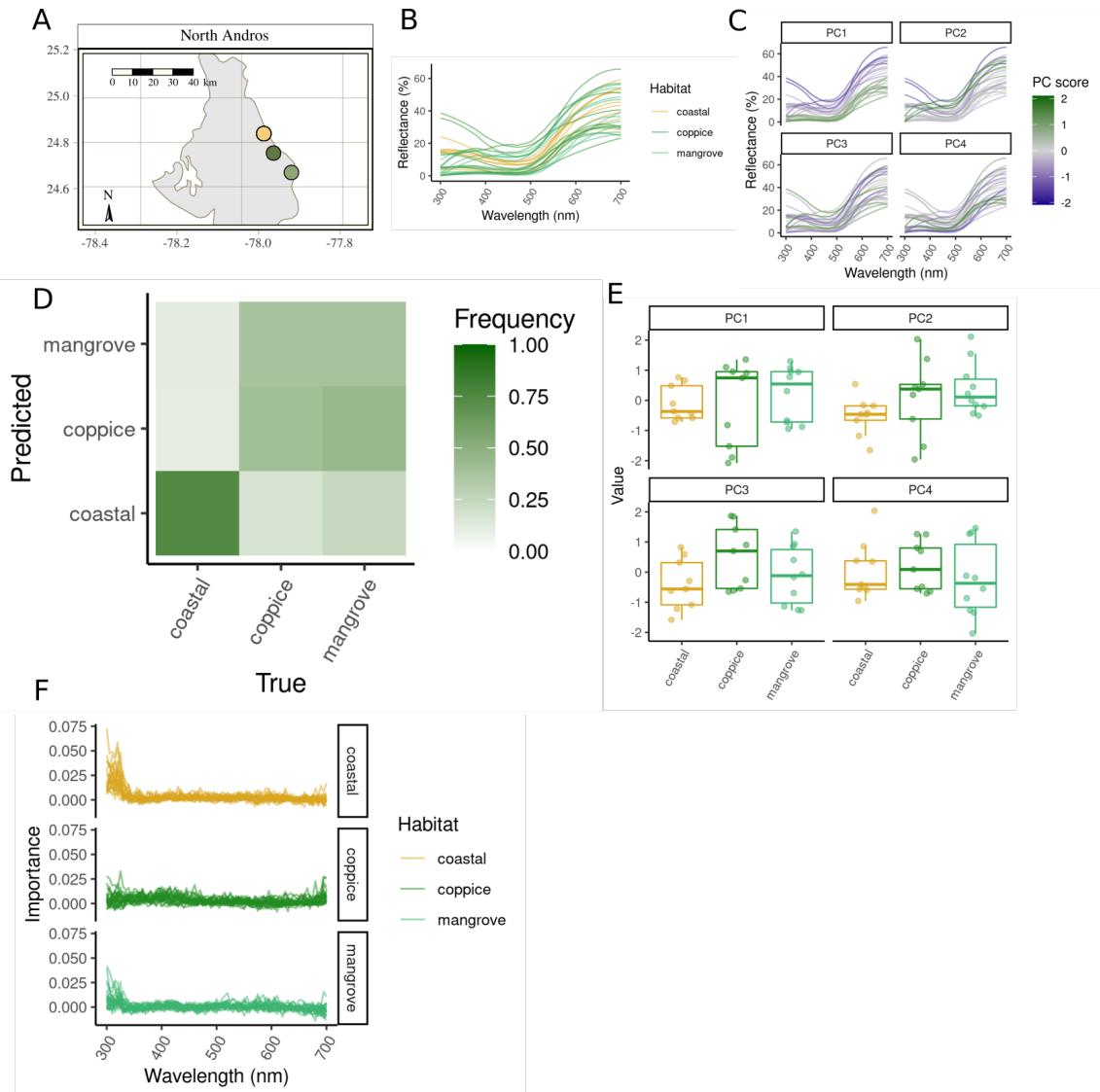


Figure S8: Comparison of dewlap coloration across habitats on North Andros. Legend is as per Figure S2, but without panel G.

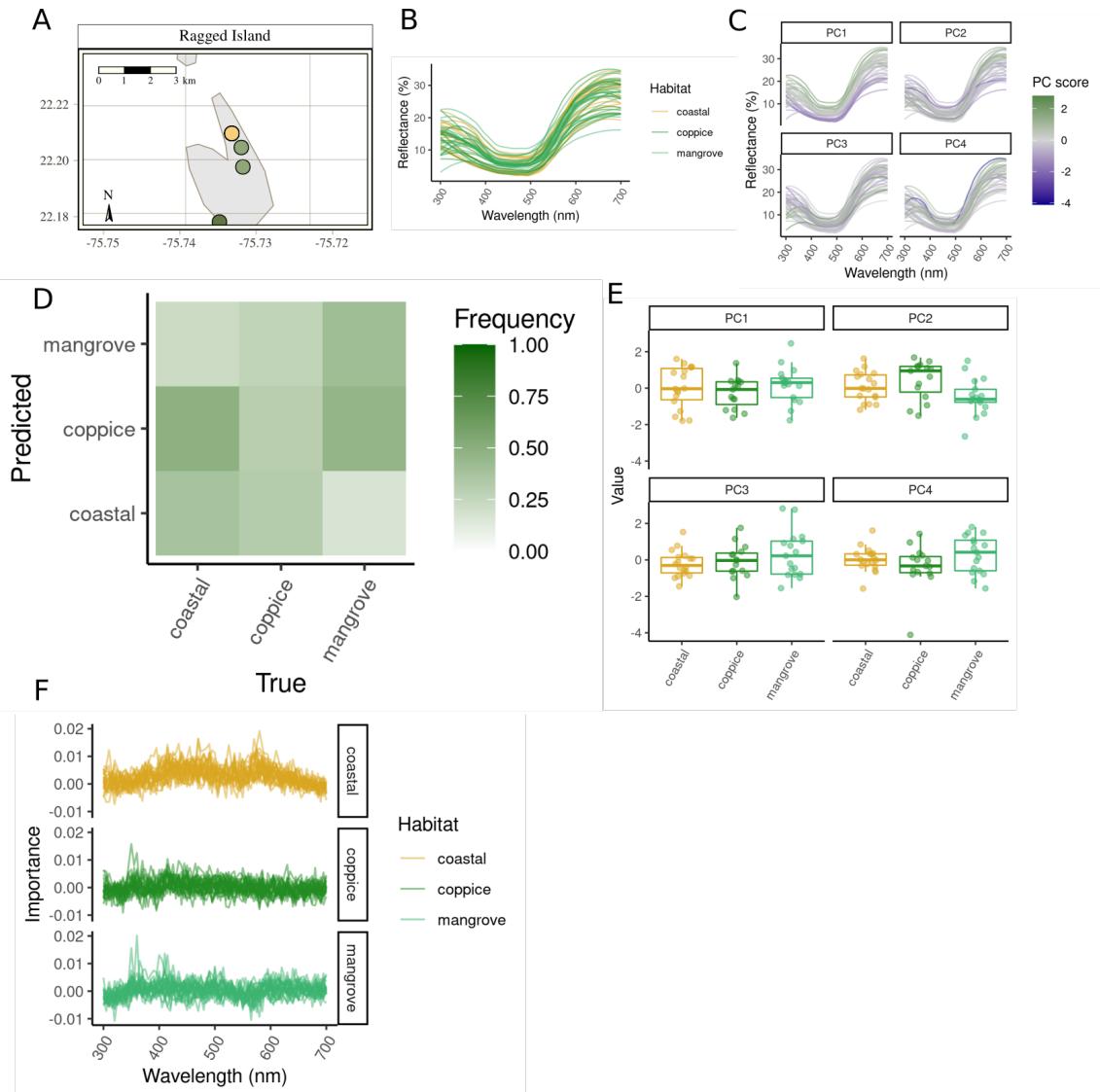


Figure S9: Comparison of dewlap coloration across habitats on Ragged Island. Legend is as per Figure S2, but without panel G.

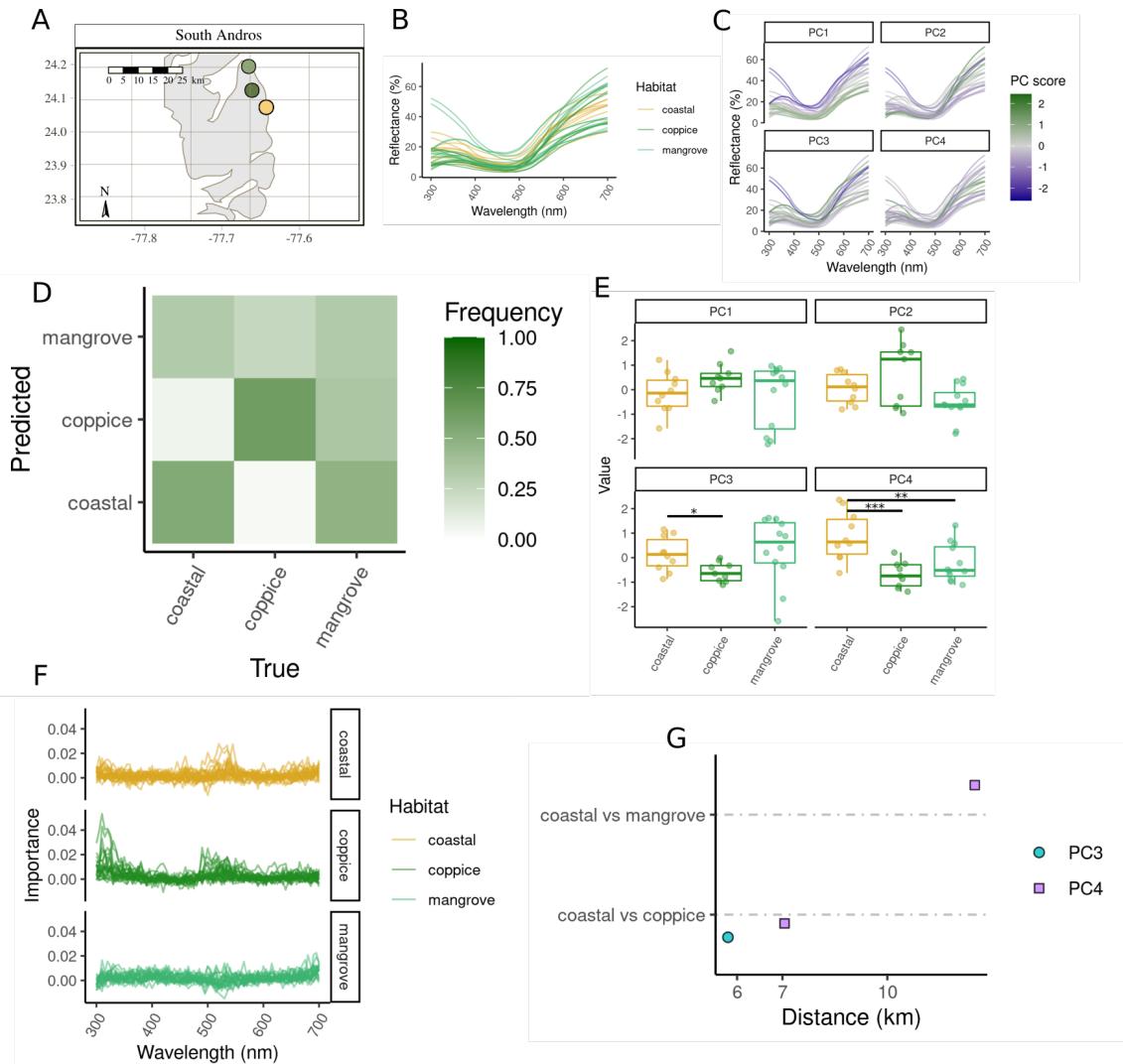


Figure S10: Comparison of dewlap coloration across habitats on South Andros. Legend is as per Figure S2.

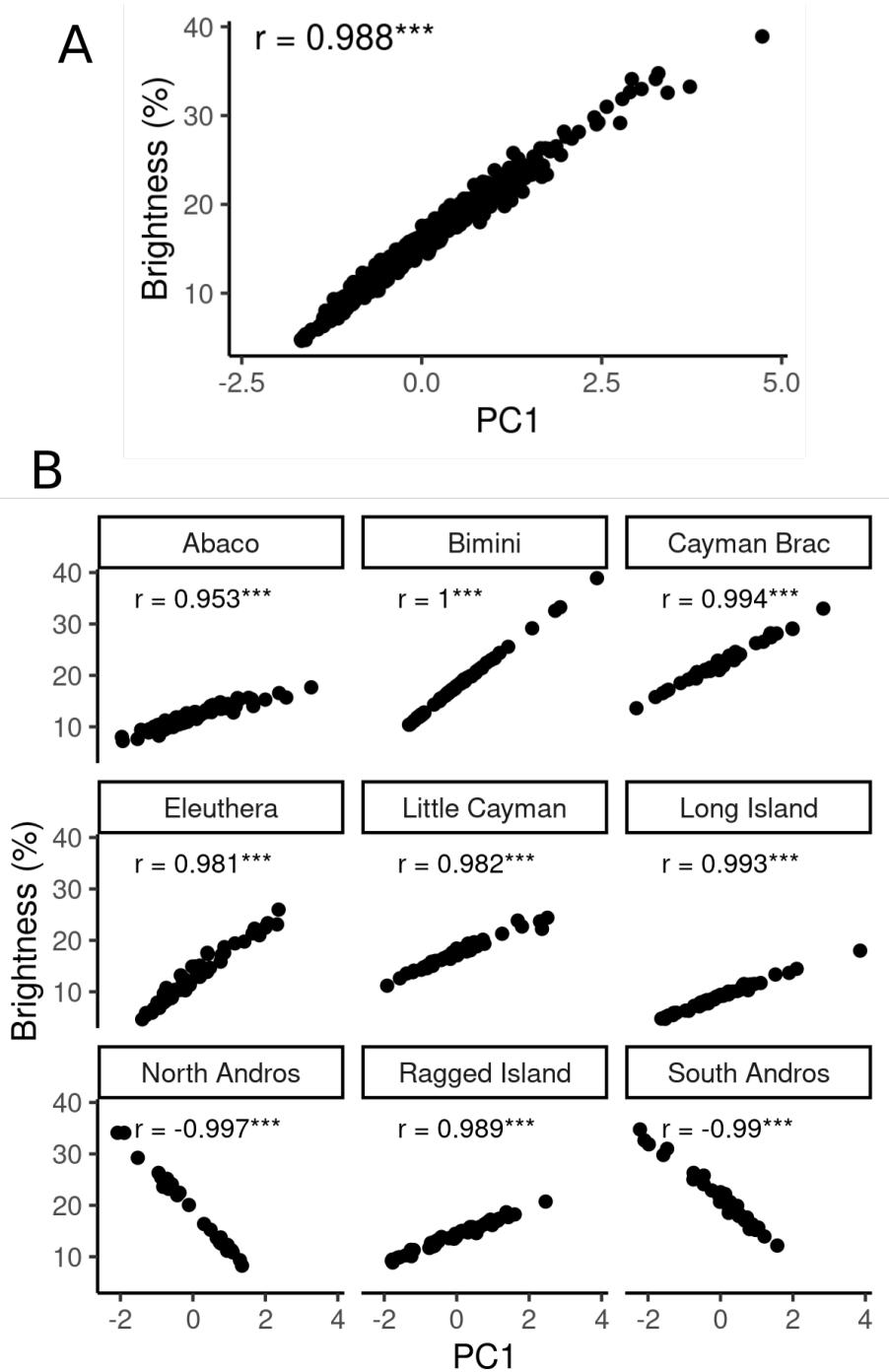


Figure S11: PC1 captures brightness across all islands. (A) Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across all islands. (B) Correlation between brightness and within-island PC1, for each island. Pearson's correlation coefficients are reported. ***, $P < 0.001$.

Supplementary Tables

793

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	χ^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	**