

# 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, 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 seem to 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 of dewlap color variation across islands.

**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

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

37 detection by unintended recipients such as predators (Endler, 1984, 1990, 1991; Halfwerk et al.,  
38 2014). This selective pressure may drive the local adaptation of communication signals.

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

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

84 *Anolis sagrei* is widespread across islands of the West Indies (Reynolds et al., 2020). It has  
85 been the subject of numerous studies concerning local adaptation (Losos et al., 1994, 1997, 2001;  
86 Kolbe et al., 2012), biological invasion (Kolbe et al., 2008), and sexual selection (Tokarz, 2002;  
87 Tokarz et al., 2005; Tokarz, 2006; Driessens et al., 2014; Steffen and Guyer, 2014; Driessens et al.,  
88 2015) among many other topics. Between-island variation in the mainly orange-red color of its  
89 dewlap was shown to be better explained by climatic variables such as annual precipitation and  
90 solar radiation (proposed to affect the average vegetation type on each island and among other

things, its ambient light environment, Driessens et al. 2017), than by proxies for biotic factors such as sexual selection or predation pressure (Vanhooijdonck et al., 2009; 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 species. 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 Shoot 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 8.45km. While this species has traditionally been considered territorial, a recent study revealed that they are polygynandrous and that gene flow is not impeded by territorial-like behaviors exhibited by some males (Kamath and Losos 2018). 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. 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 (Harvey and Pagel, 1991; Losos, 2011).

124 **Methods**

125 **Data collection**

126 We sampled 455 male *A. sagrei* from seven islands in the Bahamas Archipelago – Abaco, North  
127 Andros, South Andros, South Bimini, Eleuthera, Long Island, and Ragged Island – and two in the  
128 Cayman Islands – Cayman Brac and Little Cayman (Figure S1A). These islands were chosen to  
129 span the breadth of the West Indian range of *A. sagrei*, because they have highly similar habitat  
130 types, and because the *A. sagrei* on each island group are derived from ancient and distinct  
131 colonization events from Cuba (i.e. relatively evolutionarily independent, Reynolds et al. 2020).  
132 Three habitats were sampled on each island based on characterizations by Howard (1950) and  
133 Schoener (1968). Each habitat is clearly distinguishable by its dominant vegetation type — xeric  
134 beach scrub (open, relatively dry habitat consisting of low vegetation or isolated trees), primary  
135 coppice forest (closed-canopy forest) and mangrove forest (wet coastal habitat with trees growing  
136 in brackish water and high light penetration, although lizards were sampled in dry soil areas).  
137 Sample sizes are given in Table S1. Our sampling design enabled us to test for differences between  
138 habitats at a coarse and fine geographical scale. The median distance between two localities within  
139 an island was 8.5km (Figure S1B), and 79.3% of all pairwise distances within islands were less  
140 than 50km. Additionally, there are no major barriers to dispersal (such as mountains or grassland)  
141 on any of the islands that we sampled.

142 **Reflectance measurements**

143 We measured reflectance between 300nm and 700nm wavelength, a range from ultraviolet to red  
144 that encompasses the colors visible to most lizards and vertebrates in general (Lazareva et al.,  
145 2012). Measurements were taken with an Ocean Optics USB4000 spectrometer, a pulsed Xenon  
146 light source (PX-2, Ocean Optics, Largo, FL, USA) and a reflectance probe protected by a black  
147 anodized aluminum sheath. Measurements were taken with a 45-degree inclination to prevent  
148 specular reflection (Endler, 1990). The device was regularly standardized with a Spectralon white  
149 standard (Labsphere, North Sutton, NH, USA). Reflectance was measured at the center of the  
150 dewlap. Reflectance curves were smoothed using the R package pavo (Maia et al., 2013) as well  
151 as with custom R functions, down to one reflectance value at each nanometer in wavelength from  
152 300 to 700nm.

153 **Analysis**

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

172

173 All analyses in this study were performed in R 3.6.1 (R Core Team, 2019).

## Dimensionality reduction

174

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 S11), while the other PCs captured the chromatic variation (i.e. irrespective of brightness) in dewlap color.

## Among-island variance partitioning

183

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

191

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. These coordinates, or variables, are 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 tree being trained on a subset of observations sampled with replacement from the dataset, and each tree being 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 with 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 are 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 five 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 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.

## Spatial autocorrelation

281

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. For this, we performed Mantel's test (Legendre and Legendre 2012, R package vegan; Oksanen et al. 2019) on each island, using 999 permutations and geographical distances computed as geodesic distances from latitude and longitude data (R package geosphere, Hijmans 2019).

282

283

284

285

286

287

288

## Site differences

289

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 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 were detected with our analyses of variance. The P-values were adjusted using a Benjamini-Hochberg correction for multiple testing (Benjamini and Hochberg, 1995).

290

291

292

293

294

295

296 **Results**

297 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  
298 scrub, primary coppice and mangroves). We found that most of the variation in coloration was  
299 partitioned between islands (two-way PERMANOVA,  $F(df = 8) = 45.38$ ,  $P = 0.001$ , explained  
300 variance  $R^2 = 41.4\%$ ). Nonetheless, we did find evidence for differences in dewlap coloration  
301 between habitat types, and those were mostly island-specific (habitat-by-island interaction term,  
302  $F(16) = 4.78$ ,  $P = 0.001$ ,  $R^2 = 8.7\%$ ), with a significant portion of the variation explained by  
303 an habitat effect across all islands, but this effect was relatively small ( $F(2) = 4.46$ ,  $P = 0.001$ ,  
304  $R^2 = 1\%$ ).

305  
306 We subsequently tested for differences in dewlap coloration between habitat-populations within  
307 each island, using within-island principal component scores (to maximize the variation captured  
308 for each island, see Methods). Our within-island random forest classification analyses revealed  
309 detectable differences in dewlap coloration on seven out of the nine islands in our sample: Abaco,  
310 Bimini, Cayman Brac, Eleuthera, Little Cayman, Long Island, and North Andros. The accuracy of  
311 random forest classification exceeded random expectation more often than expected by chance for  
312 all these islands (Table 1). Accuracy was as high as 74.8% for Cayman Brac. We obtained similar  
313 results using other machine learning approaches such as support vector machines (Table S7) and  
314 linear discriminant analysis (Table S8). We describe in details the specific differences detected on  
315 each island in the Appendix, and focus here on the general patterns emerging from our data.

316  
317 Overall, we found significant differences in dewlap coloration between populations that were  
318 often in close geographical proximity. On Bimini, notably, we found a significant difference be-  
319 tween dewlaps from beach scrub and primary coppice forest, at a distance of a few hundred meters,  
320 making this contrast the smallest geographical scale at which differences in coloration were found  
321 in our study (Fig. S3). We also detected significant differences in dewlap coloration at distances  
322 below one kilometer on Abaco (Fig. S2G), and at distances between one and ten kilometers on  
323 Bimini (Fig. S3G), Cayman Brac (Fig. S4G), Little Cayman (Fig. S6G), Long Island (Fig. S7G)  
324 and North Andros (Fig. S8G).

325  
326 We found evidence of spatial autocorrelation in dewlap coloration between the sites within is-  
327 lands for Abaco (Table 2), suggesting that populations from closer sites tend to have more similar  
328 dewlaps on this island than expected by chance. Abaco was the island we sampled at the largest  
329 scale, with some sites nearly a hundred kilometers away from each other (Fig. 2A). That said, some  
330 sites were also in close proximity, and significant differences in coloration were detected between  
331 habitats sometimes less than a kilometer away (Fig. S2G), suggesting that differences in dewlap  
332 coloration between distant sites may be partly attributable to isolation-by-distance, but this may  
333 not necessarily be the case for sites in close proximity. We did not find evidence for spatial auto-  
334 correlation on other islands than Abaco (although Eleuthera was nearly significant, Table 2).

335  
336 A striking feature of our data was inconsistency in between-habitat differences among islands,  
337 in terms of which habitats differ from which, which dimensions of coloration were involved, and  
338 in which direction. For example, while on Cayman Brac the random forests could well distinguish  
339 between all three habitats (Fig. S4D), on Abaco dewlaps from beach scrub and primary coppice  
340 were often mistaken, and on Bimini beach scrub dewlaps were more often classified into primary  
341 coppice or mangrove than into beach scrub (Fig. S3D). In terms of variable importance, for multi-  
342 ple islands the random forests used information in the UV range to discriminate between at least  
343 some habitats, particularly on Abaco (Fig. S2F), Bimini (Fig. S3F), Cayman Brac (Fig. S4F),  
344 Little Cayman (Fig. S6F) and Long Island (Fig. S7F), but differences in UV reflectance involved  
345 different habitats and were in different directions among these islands.

346  
347

## Discussion

348

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.

349

350

351

352

353

354

355

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

403

404

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), and we would have expected gene flow to cause a more homogeneous distribution of color phenotypes within islands. While little is known about the cruising range of individuals from our study populations (but see Steinberg and Leal 2017; Kamath and Losos 2018b for other systems), *A. sagrei* are polygynandrous (both males and females mate with multiple mates, Kamath and Losos 2017, 2018a,b), thus offering opportunity for gene flow, especially given that lizards were distributed continuously and at high densities within the islands we sampled. Consistent with that, while populations from different islands were monophyletic, individuals within islands were not monophyletic with respect to habitat based on mitochondrial haplotypes (van de Schoot 2016 unpublished thesis).

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 found differences in dewlap coloration between habitats 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 populations from different habitats across islands, given the apparent environmental consistency each of the three habitat types across the islands we sampled. In particular, we would have expected divergence in line with increased detectability given local light conditions, such as the high contrasts with background vegetation found in the UV range in Leal and Fleishman (2002) and Leal and Fleishman (2004). We might also have expected mangrove and beach scrub lizards, both inhabiting areas with high light penetration, to have more similar dewlaps, and to differ significantly from lizards from the coppice habitat, where irradiance is low. Instead, we found inconsistencies in the way dewlap color differed 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. Overall, the observed heterogeneity of divergence patterns across islands provides no support to a sensory-drive explanation.

It is presently not known if the reported differences in coloration have a genetic basis. Yet, we find it unlikely that these differences arose through phenotypic plasticity, as although the carotenoids that partly make up the red and orange colors of anole dewlaps must be found in the diet (Goodwin, 1984; Hill et al., 2002; Hill and McGraw, 2006), 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 *A. distichus* (another species with a carotenoid-based dewlap), found little support for phenotypic and developmental plasticity in dewlap coloration. One exception is a study demonstrating that lizards heavily parasitized by skin mites had duller dewlaps

405 (Cook et al., 2013), but we found no sign of that in our study.

406

407 Genetic drift could contribute to some of the observed variation. Indeed, while only Abaco  
408 showed significant patterns consistent with isolation-by-distance (which may emerge under limited  
409 dispersal and drift, Wright 1943; Kimura and Weiss 1964; Slatkin 1987), there may have been  
410 too few sites on most islands to conclusively detect it, and even then, the absence of detectable  
411 isolation-by-distance may not necessarily constitute evidence for the absence of drift. Besides,  
412 spatial autocorrelation was the strongest on islands sampled at the largest scales (e.g. some sites  
413 on Abaco were nearly 100km apart, and Eleuthera – the second strongest signal, albeit nonsignif-  
414 icant – had sites more than 30km apart), such that it is possible that neutral processes and/or  
415 dispersal limitations might contribute to shaping variation over long distances. That said, many  
416 significant differences were found between habitats in close proximity, contrary to what would be  
417 expected under isolation-by-distance, including on islands where spatial autocorrelation was de-  
418 tected. Moreover, *A. sagrei* was distributed across the islands continuously, usually at relatively  
419 high population densities, rather than in small and isolated populations particularly prone to drift.  
420 Together with the fact that isolation-by-distance may not necessarily only emerge from drift (e.g.  
421 if there is a spatial environmental gradient), this indicates that genetic drift may have limited  
422 potential to explain the differences observed between habitats, at least at a local scale.

423

424 In this study, we found larger differences among than within islands, a pattern already reported  
425 and linked to climatic conditions (Driessens et al., 2017) and to densities of predators and of anole  
426 congeners (Vanhooydonck et al., 2009; Baeckens et al., 2018). Differences among habitats within  
427 islands, however, are still difficult to account for. Remaining hypotheses may include, for exam-  
428 ple, runaway sexual selection (i.e. arbitrary preferences of females for some colors over others,  
429 Andersson 1994) operating in different directions across islands, but no evidence so far suggests  
430 that dewlap is a target of mate choice in anoles (Tokarz, 2002; Tokarz et al., 2005; Lailvaux and  
431 Irschick, 2006; Nicholson et al., 2007). Another hypothesis is that the different genetic constitutu-  
432 tions of different islands, perhaps resulting from founder effects (the islands have been colonized  
433 independently, van de Schoot 2016 unpubl.; Driessens et al. 2017; Reynolds et al. 2020), may have  
434 predisposed populations to adapt differently to similarly selective circumstances. Either way, new  
435 data would be needed to test these hypotheses. Visual modeling, for instance, would be a valuable  
436 follow-up analysis as it would indicate whether the differences we detected may be detectable by  
437 the organisms themselves (as has been done in *Anolis* in Leal and Fleishman 2004; Fleishman  
438 et al. 2020), but such approach requires irradiance profiles from the habitats in the field, which we  
439 presently do not have.

440

441 Last, some patterns may arise owing to factors we did not measure. For one, signalling can be a  
442 multifaceted behavior, and while we focused on dewlap color here, other potentially important el-  
443 ements of behavioral interactions include dewlap size, dewlap patterning, display activity as well as  
444 behaviors associated with dewlap extensions such as headbobs and pushups (Vanhooydonck et al.,  
445 2005; Driessens et al., 2014, 2015; Lailvaux et al., 2015). These have been linked in various ways  
446 (and not always consistently across studies) not only with environmental variables but also with  
447 proxies for sexual selection, predation and species recognition (Vanhooydonck et al., 2005; Lailvaux  
448 and Irschick, 2007; Vanhooydonck et al., 2009; Driessens et al., 2017; Baeckens et al., 2018), which  
449 do vary among islands but may also vary within islands, possibly in interaction with dewlap color.  
450 Besides, while the three habitats were clearly recognizable and consistent across islands to the hu-  
451 man eye (Fig. 1), we did not precisely quantify irradiance within each habitat and did not consider  
452 any more subtle environmental differences there might have been for a given habitat among islands.

453

454 Altogether, our results show that dewlap color of *A. sagrei* commonly varies between habitat  
455 types, even in close geographical proximity, within islands of the West Indies. However, coloration  
456 differs in different ways across similar habitats from one island to another. We discussed several  
457 non-mutually exclusive mechanisms that could explain these observations. Nevertheless, heteroge-  
458 neous patterns of divergence across islands do not support an adaptive sensory-drive scenario, and  
459 we propose that within-island dewlap color variation may be underlain by a more subtle mosaic of  
460 factors.

## Acknowledgements

461

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, 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 Templeton 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).

462

463

464

465

466

467

468

469

470

471

472

473

474 **Figures**

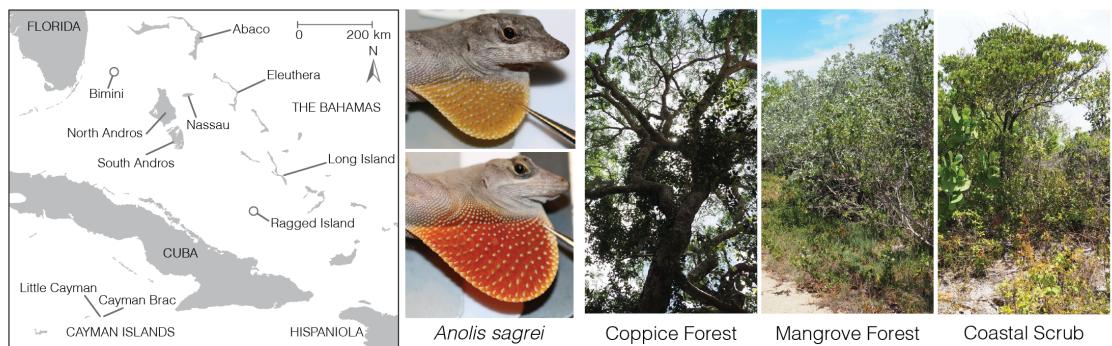


Figure 1: Overview of our study design, including a map of the Bahamas and the Cayman Islands, on which are indicated the nine islands we sampled, two representatives of our study species *Anolis sagrei* with their dewlaps deployed, and the three types of habitats we considered on each island.

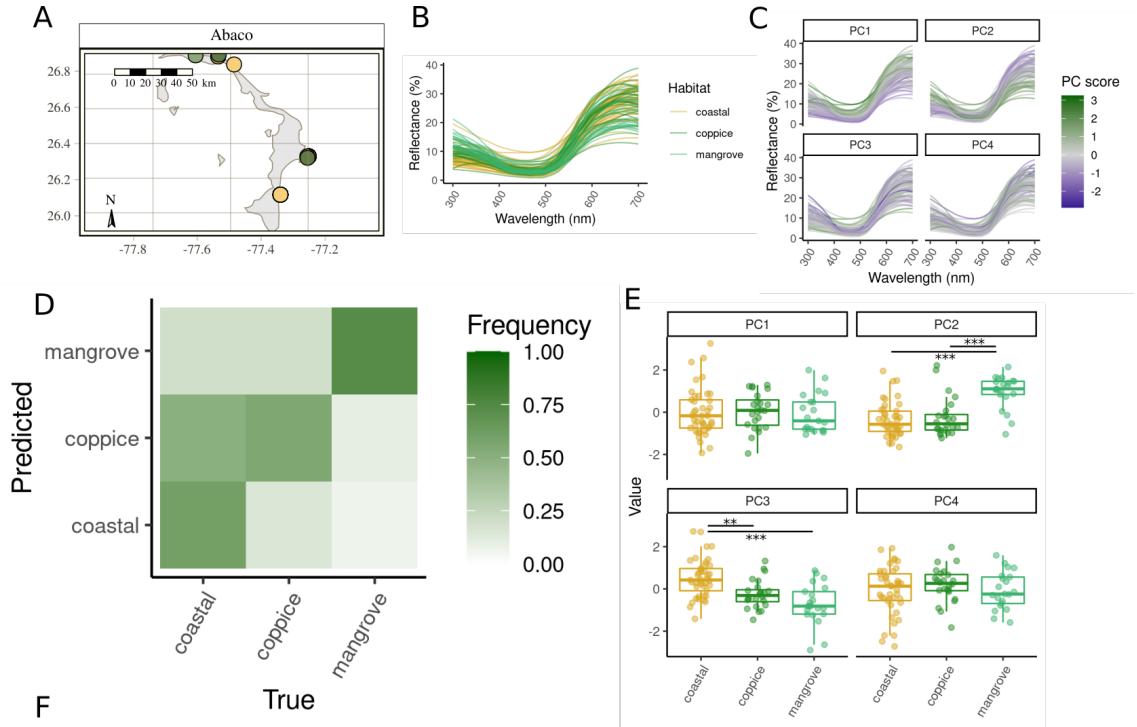


Figure 2: Comparison of dewlap coloration across habitats on Abaco. (A) Map of the island with the sampling sites colored by habitat. (B) Reflectance profiles of all the dewlaps on the island. (C) How reflectance profiles map onto the within-island principal components. (D) Confusion matrix showing the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

<sup>475</sup> **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	0.623	< 0.0001 ***
Bimini	57	0.460	0.0194 *
Cayman Brac	50	0.748	< 0.0001 ***
Eleuthera	55	0.520	0.0023 **
Little Cayman	45	0.676	< 0.0001 ***
Long Island	53	0.611	< 0.0001 ***
North Andros	28	0.693	< 0.0001 ***
Ragged Island	50	0.412	0.1259
South Andros	31	0.419	0.1152

Table 2: Mantel's 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 population means were randomly permuted among sites (999 permutations). \*,  $P < 0.05$ .

Island	$\rho$	$P$	
Abaco	0.439	0.032	*
Bimini	-0.725	1.000	
Cayman Brac	-0.737	0.833	
Eleuthera	0.827	0.058	
Little Cayman	-0.042	0.667	
Long Island	-0.077	0.583	
North Andros	-0.968	1.000	
Ragged Island	-0.363	0.708	
South Andros	0.963	0.167	

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	$\Delta\text{AICc}$	AICw	Model	Log-lik.	$\chi^2$	df	P
Abaco	PC1	255.81	2.06	0.737	OLS	-121.46	0.14	2	0.9318
Abaco	PC2	225.29	3.98	0.880	OLS	-105.64	31.77	2	< 0.0001 ***
Abaco	PC3	229.85	1.44	0.673	OLS	-108.01	27.04	2	< 0.0001 ***
Abaco	PC4	254.59	0.72	0.589	OLS	-120.82	1.41	2	0.4945
Bimini	PC1	162.92	-0.32	0.540	GLS	-72.43	10.03	2	0.0066 **
Bimini	PC2	165.36	3.08	0.824	OLS	-76.52	7.70	2	0.0212 *
Bimini	PC3	163.58	3.13	0.827	OLS	-75.58	9.59	2	0.0083 **
Bimini	PC4	172.47	2.43	0.771	OLS	-80.27	0.20	2	0.9035
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	166.33	2.26	0.756	OLS	-77.29	0.49	2	0.7827
Eleuthera	PC2	155.78	-2.38	0.767	GLS	-68.74	12.80	2	0.0017 ***
Eleuthera	PC3	160.47	-0.22	0.527	GLS	-71.18	5.59	2	0.0613
Eleuthera	PC4	160.61	3.85	0.873	OLS	-74.27	6.54	2	0.0380 *
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	145.51	3.73	0.866	OLS	-66.41	16.58	2	0.0003 ***
Long Island	PC2	158.82	-1.29	0.656	GLS	-70.56	1.35	2	0.5103
Long Island	PC3	154.36	3.02	0.819	OLS	-71.10	7.19	2	0.0274 *
Long Island	PC4	155.59	0.47	0.558	OLS	-71.75	5.89	2	0.0525
North Andros	PC1	89.00	2.87	0.808	OLS	-39.05	0.35	2	0.8406
North Andros	PC2	74.74	-0.37	0.547	GLS	-27.50	17.24	2	0.0002 ***
North Andros	PC3	87.62	0.25	0.531	OLS	-38.28	1.89	2	0.3893
North Andros	PC4	73.56	5.39	0.937	OLS	-30.40	17.64	2	0.0001 ***

## References

- Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26(1):32–46. 476  
477  
478
- Andersson, M. B. (1994). *Sexual Selection*. Monographs in Behavior and Ecology. Princeton University Press, Princeton, N.J. 479  
480
- Arnold, S. J. (1983). Morphology, Performance and Fitness. *American Zoologist*, 23(2):347–361. 481
- 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. 482  
483  
484
- Bartoń, K. (2019). MuMIn: Multi-Model Inference. 485
- 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. 486  
487  
488
- Bradbury, J. W. and Vehrenkamp, S. L. (2011). *Principles of Animal Communication*. Sinauer Associates, Sunderland, Mass, 2nd ed edition. 489  
490
- Breiman, L. (2001). Random forests. *Machine Learning*, 45(1):5–32. 491
- 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. 492  
493
- Cortez, P. (2020). Rminer: Data Mining Classification and Regression Methods. 494
- 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. 495  
496  
497
- Cristianini, N. and Shawe-Taylor, J. (2000). *An Introduction to Support Vector Machines and Other Kernel-based Learning Methods*. Cambridge University Press, first edition. 498  
499
- 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. 500  
501  
502
- Dieckmann, U. and Doebeli, M. (1999). On the origin of species by sympatric speciation. *Nature*, 400(6742):354–357. 503  
504
- 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. 505  
506  
507
- 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. 508  
509  
510
- 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. 511  
512  
513
- Endler, J. (1998). Sensory ecology, receiver biases and sexual selection. *Trends in Ecology & Evolution*, 13(10):415–420. 514  
515
- 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. 516  
517  
518
- 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. 519  
520

- 521 Endler, J. A. (1991). Variation in the appearance of guppy color patterns to guppies and their  
522 predators under different visual conditions. *Vision Research*, 31(3):587–608.
- 523 Endler, J. A. (1992). Signals, Signal Conditions, and the Direction of Evolution. *The American  
524 Naturalist*, 139:S125–S153.
- 525 Endler, J. A. (1993a). The Color of Light in Forests and Its Implications. *Ecological Monographs*,  
526 63(1):1–27.
- 527 Endler, J. A. (1993b). Some general comments on the evolution and design of animal communi-  
528 cation systems. *Philosophical Transactions of the Royal Society of London. Series B: Biological  
529 Sciences*, 340(1292):215–225.
- 530 Endler, J. A. and McLellan, T. (1988). The Processes of Evolution: Toward a Newer Synthesis.  
531 *Annual Review of Ecology and Systematics*, 19(1):395–421.
- 532 Felsenstein, J. (1976). The Theoretical Population Genetics of Variable Selection and Migration.  
533 *Annual Review of Genetics*, 10(1):253–280.
- 534 Fleishman, L. J. (2000). *Signal Function, Signal Efficiency and the Evolution of Anoline Lizard  
535 Dewlap Color*, pages 209–236. Tapir Academic, Trondheim.
- 536 Fleishman, L. J., Leal, M., and Persons, M. H. (2009). Habitat light and dewlap color diversity in  
537 four species of Puerto Rican anoline lizards. *Journal of Comparative Physiology A*, 195(11):1043–  
538 1060.
- 539 Fleishman, L. J. and Persons, M. (2001). The influence of stimulus and background colour on  
540 signal visibility in the lizard *Anolis cristatellus*. *The Journal of Experimental Biology*, 204(Pt  
541 9):1559–1575.
- 542 Fleishman, L. J., Wadman, C. S., and Maximov, K. J. (2020). The interacting effects of total  
543 light intensity and chromatic contrast on visual signal visibility in an *Anolis* lizard. *Animal  
544 Behaviour*, page S0003347220302037.
- 545 García-Ramos, G. and Kirkpatrick, M. (1997). Genetic Models of Adaptation and Gene Flow in  
546 Peripheral Populations. *Evolution*, 51(1):21–28.
- 547 Geneva, A. J., Hilton, J., Noll, S., and Glor, R. E. (2015). Multilocus phylogenetic analyses of  
548 Hispaniolan and Bahamian trunk anoles (*distichus* species group). *Molecular Phylogenetics and  
549 Evolution*, 87:105–117.
- 550 Goodwin, T. W. (1984). *The Biochemistry of the Carotenoids*. Springer Netherlands, Dordrecht.
- 551 Halfwerk, W., Jones, P. L., Taylor, R. C., Ryan, M. J., and Page, R. A. (2014). Risky Ripples Allow  
552 Bats and Frogs to Eavesdrop on a Multisensory Sexual Display. *Science*, 343(6169):413–416.
- 553 Harvey, P. H. and Pagel, M. D. (1991). *The Comparative Method in Evolutionary Biology*. Oxford  
554 Series in Ecology and Evolution. Oxford University Press, Oxford ; New York.
- 555 Hendry, A. P., Day, T., and Taylor, E. B. (2007a). Population Mixing and the Adaptive Divergence  
556 of Quantitative Traits in Discrete Populations: A Theoretical Framework for Empirical Tests.  
557 *Evolution*, 55(3):459–466.
- 558 Hendry, A. P., Taylor, E. B., and McPhail, J. D. (2007b). Adaptive Divergence and the Balance  
559 Between Selection and Gene Flow: Lake and Stream Stickleback in the Misty System. *Evolution*,  
560 56(6):1199–1216.
- 561 Henze, N. and Zirkler, B. (1990). A class of invariant consistent tests for multivariate normality.  
562 *Communications in Statistics - Theory and Methods*, 19(10):3595–3617.
- 563 Hijmans, R. J. (2019). Geosphere: Spherical Trigonometry.
- 564 Hill, G. E., Inouye, C. Y., and Montgomerie, R. (2002). Dietary carotenoids predict plumage  
565 coloration in wild house finches. *Proceedings of the Royal Society of London. Series B: Biological  
566 Sciences*, 269(1496):1119–1124.

Hill, G. E. and McGraw, K. J., editors (2006). <i>Bird Coloration</i> . Harvard University Press, Cambridge, Mass.	567 568
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.	569 570 571
Howard, R. A. (1950). Vegetation of the Bimini Island Group: Bahamas, B. W. I. <i>Ecological Monographs</i> , 20(4):317–349.	572 573
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.	574 575
Kamath, A. and Losos, J. (2017). The erratic and contingent progression of research on territoriality: A case study. <i>Behavioral Ecology and Sociobiology</i> , 71(6):89.	576 577
Kamath, A. and Losos, J. (2018a). Reconsidering territoriality is necessary for understanding <i>Anolis</i> mating systems. <i>Behavioral Ecology and Sociobiology</i> , 72(7):106.	578 579
Kamath, A. and Losos, J. B. (2018b). 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.	580 581 582
Kimura, M. and Weiss, G. H. (1964). The Stepping Stone Model of Population Structure and the Decrease of Genetic Correlation with Distance. <i>Genetics</i> , 49(4):561–576.	583 584
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.	585 586 587
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.	588 589 590
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.	591 592
Lailvaux, S. P. and Irschick, D. J. (2006). No Evidence for Female Association with High-Performance males in the Green Anole Lizard, <i>Anolis carolinensis</i> . <i>Ethology</i> , 112(7):707–715.	593 594
Lailvaux, S. P. and Irschick, D. J. (2007). The Evolution of Performance-Based Male Fighting Ability in Caribbean Anolis Lizards. <i>The American Naturalist</i> , 170(4):573–586.	595 596
Lailvaux, S. P., Leifer, J., Kircher, B. K., and Johnson, M. A. (2015). The incredible shrinking dewlap: Signal size, skin elasticity, and mechanical design in the green anole lizard ( <i>Anolis carolinensis</i> ). <i>Ecology and Evolution</i> , 5(19):4400–4409.	597 598 599
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.	600 601 602
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.	603 604
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.	605 606 607
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.	608 609
Leal, M. and Rodríguez-Robles, J. A. (1995). Antipredator Responses of <i>Anolis cristatellus</i> (Sauria: Polychrotidae). <i>Copeia</i> , 1995(1):155–161.	610 611

- 612 Leal, M. and Rodriguez-Robles, J. A. (1997). Antipredator Responses of the Puerto Rican Giant  
613 Anole, *Anolis cuvieri* (Squamata: Polychrotidae). *Biotropica*, 29(3):372–375.
- 614 Leal, M. and Rodríguez-Robles, J. A. (1997). Signalling displays during predator–prey interactions  
615 in a Puerto Rican anole, *Anolis cristatellus*. *Animal Behaviour*, 54(5):1147–1154.
- 616 Legendre, P. and Legendre, L. (2012). *Numerical Ecology*. Number 24 in Developments in Envi-  
617 ronmental Modelling. Elsevier, Amsterdam, third english edition edition.
- 618 Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*,  
619 17(4):183–189.
- 620 Liaw, A. and Wiener, M. (2002). Classification and Regression by randomForest. *R News*, 2(3):18–  
621 22.
- 622 Losos, J. B. (1985). An Experimental Demonstration of the Species-Recognition Role of *Anolis*  
623 Dewlap Color. *Copeia*, 1985(4):905–910.
- 624 Losos, J. B. (2009). *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*.  
625 University of California Press.
- 626 Losos, J. B. (2011). Convergence, Adaptation, and Constraint. *Evolution*, 65(7):1827–1840.
- 627 Losos, J. B., Irschick, D. J., and Schoener, T. W. (1994). Adaptation and Constraint in the  
628 Evolution of Specialization of Bahamian *Anolis* Lizards. *Evolution*, 48(6):1786–1798.
- 629 Losos, J. B., Schoener, T. W., Warheit, K. I., and Creer, D. (2001). Experimental studies of  
630 adaptive differentiation in Bahamian *Anolis* lizards. *Genetica*, 112–113:399–415.
- 631 Losos, J. B., Warheit, K. I., and Schoener, T. W. (1997). Adaptive differentiation following  
632 experimental island colonization in *Anolis* lizards. *Nature*, 387(6628):70–73.
- 633 Macedonia, J. M. (2001). Habitat light, colour variation, and ultraviolet reflectance in the Grand  
634 Cayman anole, *Anolis conspersus*. *Biological Journal of the Linnean Society*, 73(3):299–320.
- 635 Macedonia, J. M., Clark, D. L., Riley, R. G., and Kemp, D. J. (2013). Species recognition of color  
636 and motion signals in *Anolis grahami*: Evidence from responses to lizard robots. *Behavioral  
637 Ecology*, 24(4):846–852.
- 638 Macedonia, J. M., Clark, D. L., and Tamasi, A. L. (2014). Does Selection Favor Dewlap Colors that  
639 Maximize Detectability? A Test with Five Species of Jamaican *Anolis* Lizards. *Herpetologica*,  
640 70(2):157–170.
- 641 Macedonia, J. M. and Stamps, J. A. (1994). Species Recognition in *Anolis grahami* (Sauria,  
642 Iguanidae): Evidence from Responses to Video Playbacks of Conspecific and Heterospecific  
643 Displays. *Ethology*, 98(3-4):246–264.
- 644 Maia, R., Eliason, C. M., Bitton, P.-P., Doucet, S. M., and Shawkey, M. D. (2013). Pavo: An  
645 R package for the analysis, visualization and organization of spectral data. *Methods in Ecology  
646 and Evolution*, pages n/a–n/a.
- 647 Ng, J., Geneva, A. J., Noll, S., and Glor, R. E. (2017). Signals and Speciation: *Anolis* Dewlap  
648 Color as a Reproductive Barrier. *Journal of Herpetology*, 51(3):437–447.
- 649 Ng, J. and Glor, R. E. (2011). Genetic differentiation among populations of a Hispaniolan trunk  
650 anole that exhibit geographical variation in dewlap colour. *Molecular Ecology*, 20(20):4302–4317.
- 651 Ng, J., Kelly, A. L., MacGuigan, D. J., and Glor, R. E. (2013). The Role of Heritable and Dietary  
652 Factors in the Sexual Signal of a Hispaniolan *Anolis* Lizard, *Anolis distichus*. *Journal of Heredity*,  
653 104(6):862–873.
- 654 Ng, J., Landeen, E. L., Logsdon, R. M., and Glor, R. E. (2012). Correlation Between *Anolis*  
655 Lizard Dewlap Phenotype and Environmental Variation Indicates Adaptive Divergence of a  
656 Signal Important to Sexual Selection and Species Recognition. *Evolution*, 67(2):573–582.

Ng, J., Ossip-Klein, A. G., and Glor, R. E. (2016). Adaptive signal coloration maintained in the face of gene flow in a Hispaniolan <i>Anolis</i> Lizard. <i>BMC Evolutionary Biology</i> , 16(1):193.	657 658
Nicholson, K. E., Harmon, L. J., and Losos, J. B. (2007). Evolution of <i>Anolis</i> Lizard Dewlap Diversity. <i>PLoS ONE</i> , 2(3):e274.	659 660
Nosil, P. and Crespi, B. J. (2004). Does Gene Flow Constrain Adaptive Divergence or Vice Versa? A Test Using Ecomorphology and Sexual Isolation in <i>Timema cristinae</i> Walking-Sticks. <i>Evolution</i> , 58(1):102–112.	661 662 663
Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H. (2019). Vegan: Community Ecology Package. R package version 2.5-6.	664 665 666
Pinheiro, J. and Bates, D. (2000). <i>Mixed-Effects Models in S and S-PLUS</i> . Statistics and Computing. Springer-Verlag, New York.	667 668
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and Team, R. C. (2020). Nlme: Linear and Nonlinear Mixed Effects Models.	669 670
Pohlert, T. (2020). PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended. R package version 1.4.4.	671 672
R Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.	673 674
Rand, A. S. and Williams, E. E. (1970). An Estimation of Redundancy and Information Content of Anole Dewlaps. <i>The American Naturalist</i> , 104(935):99–103.	675 676
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.	677 678 679 680
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.	681 682
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.	683 684
Schoener, T. W. (1968). The <i>Anolis</i> Lizards of Bimini: Resource Partitioning in a Complex Fauna. <i>Ecology</i> , 49(4):704–726.	685 686
Seehausen, O. (1997). Cichlid Fish Diversity Threatened by Eutrophication That Curbs Sexual Selection. <i>Science</i> , 277(5333):1808–1811.	687 688
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.	689 690
Slatkin, M. (1987). Gene Flow and the Geographic Structure of Natural Populations. <i>Science</i> , 236(4803):787–792.	691 692
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.	693 694
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.	695 696 697
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.	698 699
Steinberg, D. S. and Leal, M. (2017). An Unexpected Case of Homing in a Territorial Lizard. <i>Integrative and Comparative Biology</i> , 57:E418.	700 701

- 702 Thorpe, R. S. (2002). Analysis of Color Spectra in Comparative Evolutionary Studies: Molecular  
703 Phylogeny and Habitat Adaptation in the St. Vincent Anole (*Anolis trinitatis*). *Systematic*  
704 *Biology*, 51(4):554–569.
- 705 Thorpe, R. S. and Stenson, A. G. (2002). Phylogeny, Paraphyly and Ecological Adaptation of  
706 the Colour and Pattern in the Anolis Roquet Complex on Martinique: Interaction Between  
707 Phylogeny and Adaptation. *Molecular Ecology*, 12(1):117–132.
- 708 Tokarz, R. R. (2002). An Experimental Test of the Importance of the Dewlap in Male Mating  
709 Success in the Lizard *Anolis sagrei*. *Herpetologica*, 58(1):87–94.
- 710 Tokarz, R. R. (2006). Importance of Prior Physical Contact with Familiar Females in the Devel-  
711 opment of a Male Courtship and Mating Preference for Unfamiliar Females in the Lizard *Anolis*  
712 *Sagrei*. *Herpetologica*, 62(2):115–124.
- 713 Tokarz, R. R., Paterson, A. V., and McMann, S. (2005). Importance of Dewlap Display in  
714 Male Mating Success in Free-Ranging Brown Anoles (*Anolis sagrei*). *Journal of Herpetology*,  
715 39(1):174–177.
- 716 van de Schoot, M. (2016). *Within and between Island Radiation and Genetic Variation in Anolis*  
717 *Sagrei*. PhD thesis, Wageningen University, Wageningen, The Netherlands.
- 718 Vanhooydonck, B., Herrel, A., Meyers, J. J., and Irschick, D. J. (2009). What determines dewlap  
719 diversity in Anolis lizards? An among-island comparison. *Journal of Evolutionary Biology*,  
720 22(2):293–305.
- 721 Vanhooydonck, B., Herrel, A. Y., Van Damme, R., and Irschick, D. J. (2005). Does dewlap size  
722 predict male bite performance in Jamaican Anolis lizards? *Functional Ecology*, 19(1):38–42.
- 723 Willi, Y. and Hoffmann, A. A. (2012). Microgeographic adaptation linked to forest fragmentation  
724 and habitat quality in the tropical fruit fly *Drosophila birchii*. *Oikos*, 121(10):1627–1637.
- 725 Williams, E. E. (1969). The Ecology of Colonization as Seen in the Zoogeography of Anoline  
726 Lizards on Small Islands. *The Quarterly Review of Biology*, 44(4):345–389.
- 727 Williams, E. E. and Rand, A. S. (1977). Species Recognition, Dewlap Function and Faunal Size.  
728 *American Zoologist*, 17(1):261–270.
- 729 Wright, S. (1943). Isolation by Distance. *Genetics*, 28(2):114–138.
- 730 Zuur, A. F., editor (2009). *Mixed Effects Models and Extensions in Ecology with R*. Statistics for  
731 Biology and Health. Springer, New York, NY.

## Appendix

732

Here we describe more precisely the patterns identified on each island.

733

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 higher 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 higher 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). Abaco 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.

734

735

736

737

738

739

740

741

742

743

744

745

746

747

748

749

750

751

752

753

754

755

756

757

758

759

760

761

762

763

764

765

766

767

768

769

770

771

772

773

774

775

776

777

778

779

780

781

782

783

784

785

786

787

788

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). Coppice dewlaps had significantly higher PC2 scores than mangrove dewlaps (Fig. S3E), suggesting a higher curvature (higher UV and red reflectance than more intermediate wavelengths, Fig. S3C). For these two habitats the random forests were most sensitive to UV reflectance (Fig. S3F). Beach scrub dewlaps had higher PC3 scores than coppice dewlaps but it was not clear what properties of spectral shape this principal component mapped onto (Fig. S3C). 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. S4F). Consistent with this, coppice dewlaps were significantly different from all other dewlaps along PC1 (Fig. S4E, Table 3). At a distance between 2 and 3km (Fig. S4G), dewlaps in the beach scrub habitat reflected more red light (as represented by PC2, Fig. S4C, E) and more UV (as represented by PC3, along which coppice dewlaps were intermediate, Fig. S4C, E) than in the mangrove habitat.

On Eleuthera, 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. On Eleuthera, beach scrub and mangrove dewlaps were guessed relatively correctly, but coppice dewlaps were more often mistaken for mangrove dewlaps (Fig. S5D). Mangrove dewlaps had lower PC2 scores than beach scrub dewlaps (Fig. S5E, indicating higher UV relative to red, Fig. S5C), and higher PC4 scores than coppice dewlaps (Fig. S5E, suggesting more reflectance profiles with more curvature, Fig. S5C). Random forests did not seem to consistently capture the wavelengths responsible for these differences (Fig. S5F).

Little Cayman was characterized by a better discrimination of mangrove lizards from the rest than between beach scrub and coppice lizards even though all habitats were relatively well discriminated (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

789 represented by higher PC3 scores), than lizards from the other two habitats (Fig. S6C, E, Table 3).  
790

791 On Long Island the three habitats were relatively well discriminated (Fig. S7D). Importance  
792 profiles indicated that short wavelengths were used to discriminate between beach scrub and man-  
793 grove lizards (Fig. S7F). Beach scrub lizards had more curved reflectance profiles than in the  
794 mangrove, with higher levels of UV and red reflectance relative to intermediate wavelengths (PC3,  
795 Fig. S7C, E, Table 3). Coppice lizards were significantly darker than mangrove and beach scrub  
796 lizards (PC1, Fig. S7C, E, Table 3).

797 On North Andros beach scrub and coppice dewlaps could be discriminated better against each  
798 other than with mangrove dewlaps (Fig. S8D), with importance profiles supporting UV-reflectance  
799 as a predictor of coppice lizards (Fig. S8F). Coppice lizards had less curved reflectance profiles  
800 than beach scrub and mangrove lizards (PC2), and beach scrub dewlaps had the lowest scores on  
801 PC4, which was difficult to interpret (Fig. S8C, E, Table 3).

802  
803 Classification success was not significantly better than expected by chance on Ragged Island  
804 and South Andros (Table 1, S8, S7) where nearly no habitat could be differentiated from any other  
805 based on reflectance.  
806

## Supplementary Figures

807

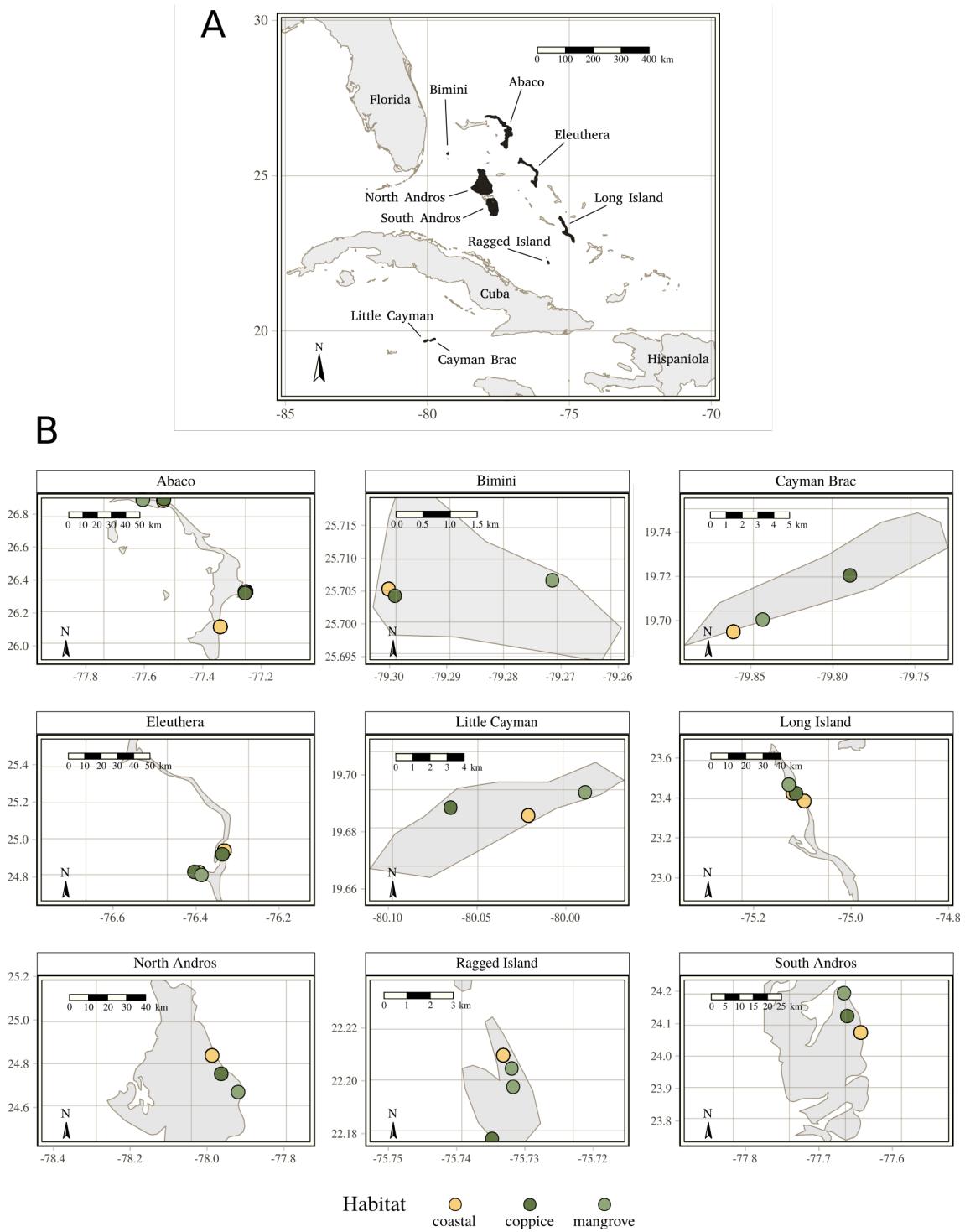


Figure S1: Maps of the islands. (A) Map of the West Indies with sampled islands highlighted in black. (B) Sampling sites within islands colored after their respective habitat types.

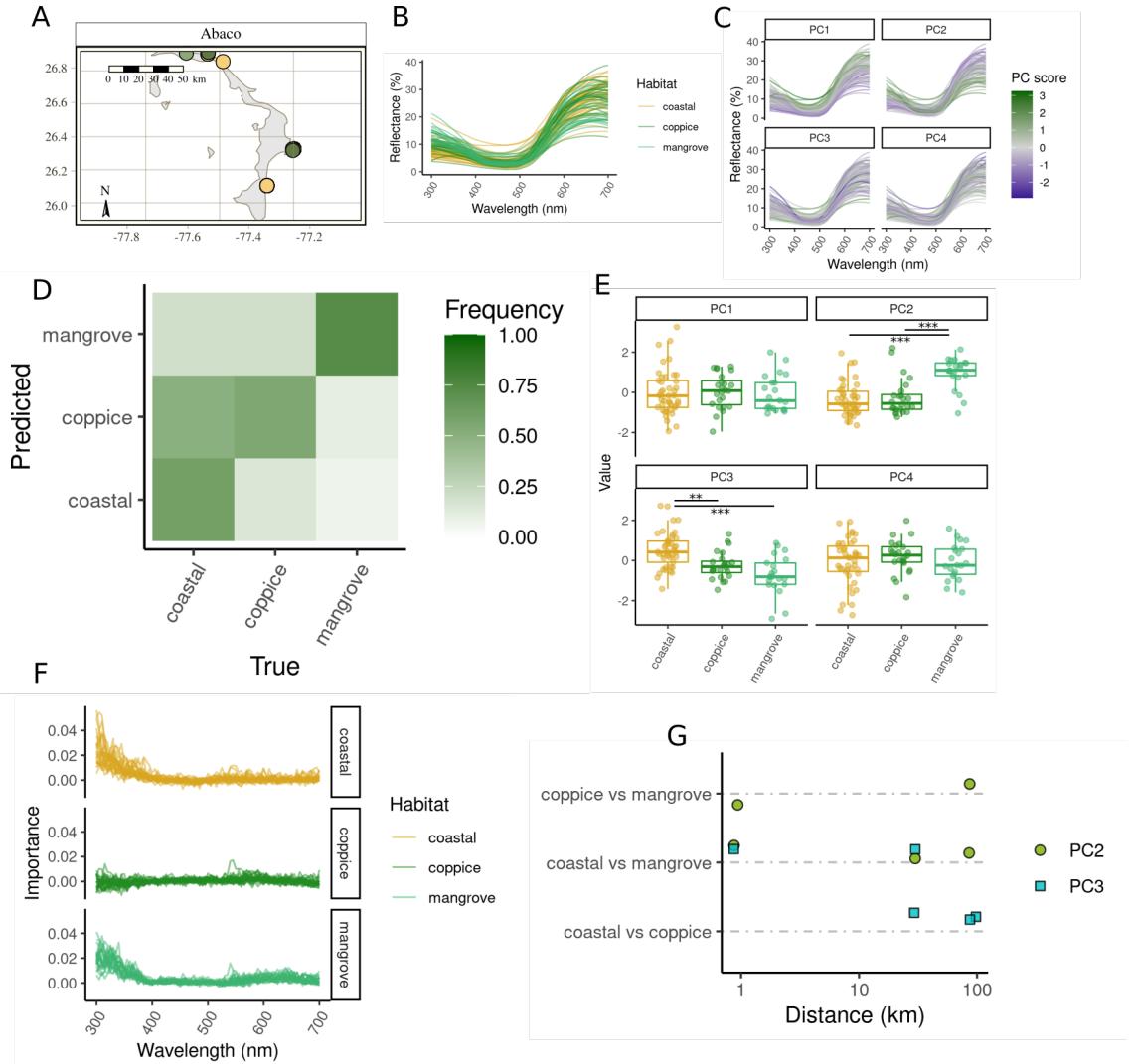


Figure S2: Comparison of dewlap coloration across habitats on Abaco, with extended results. (A–E) Legend as per Figure 2. (F) One-dimensional sensitivity analysis showing the relative importance (mean decrease in accuracy) of the various wavelengths in random forest classification of the whole spectrum.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

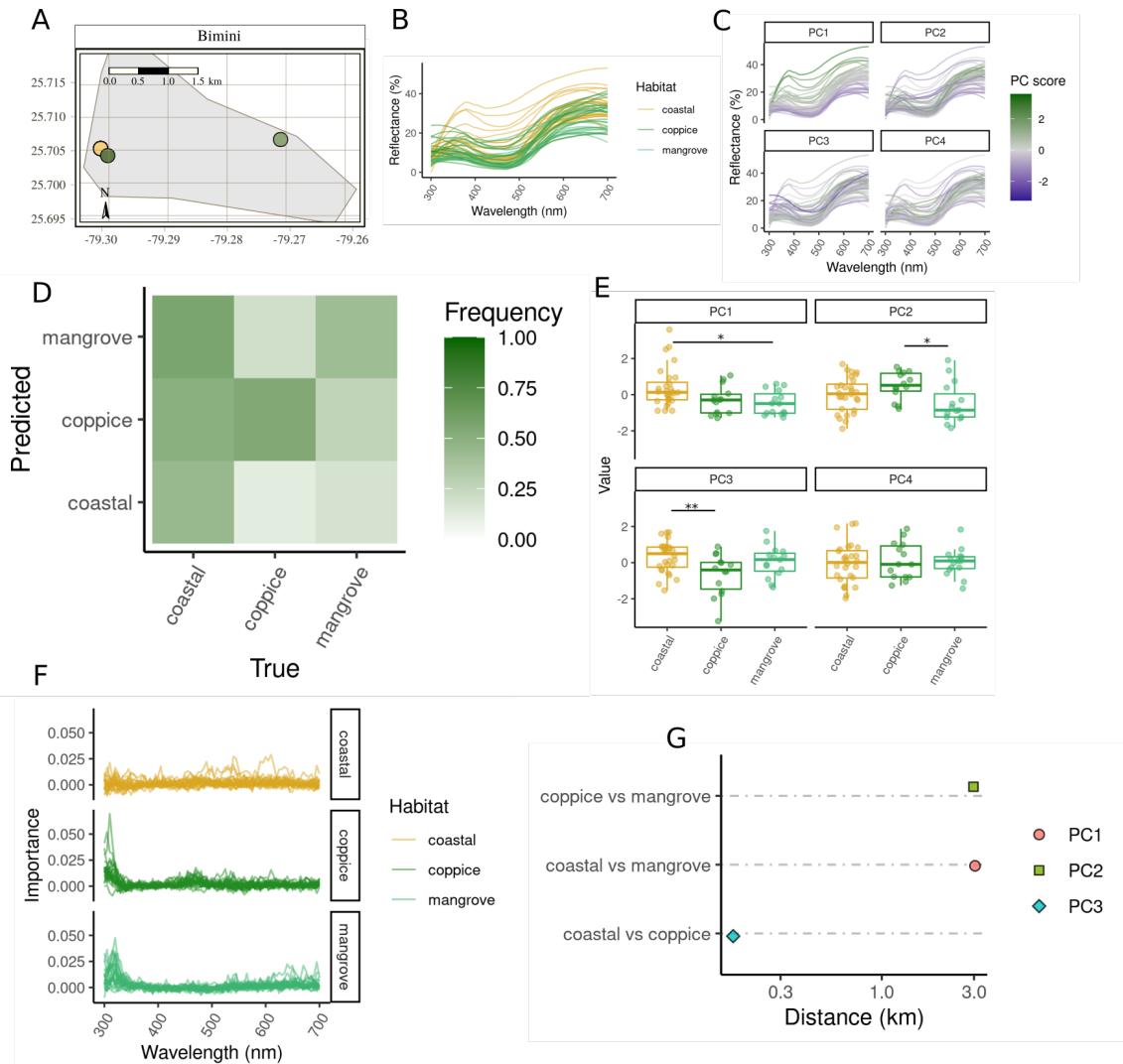


Figure S3: Comparison of dewlap coloration across habitats on Bimini. Legend is as per Figure S2.

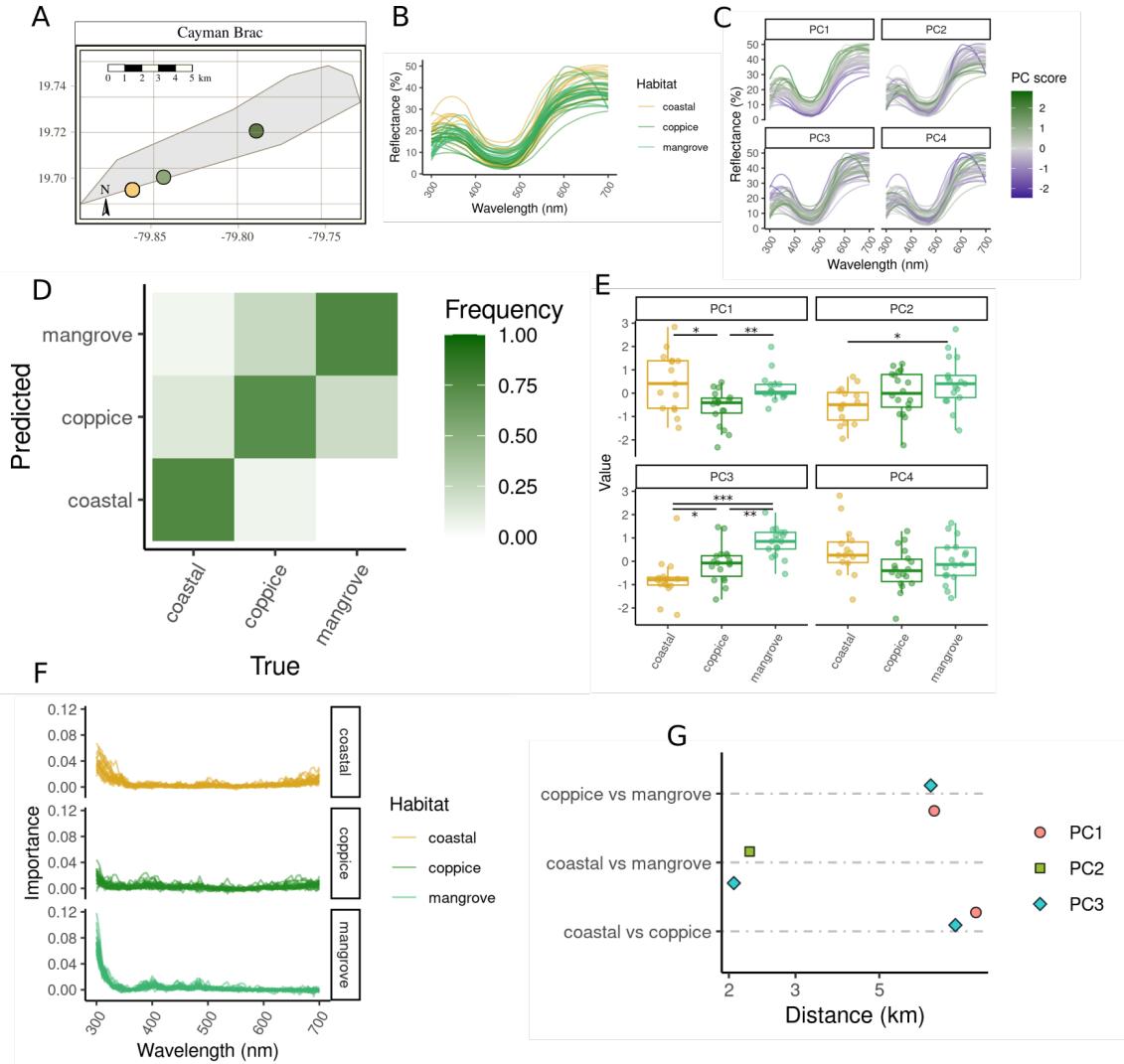


Figure S4: Comparison of dewlap coloration across habitats on Cayman Brac. Legend is as per Figure S2.

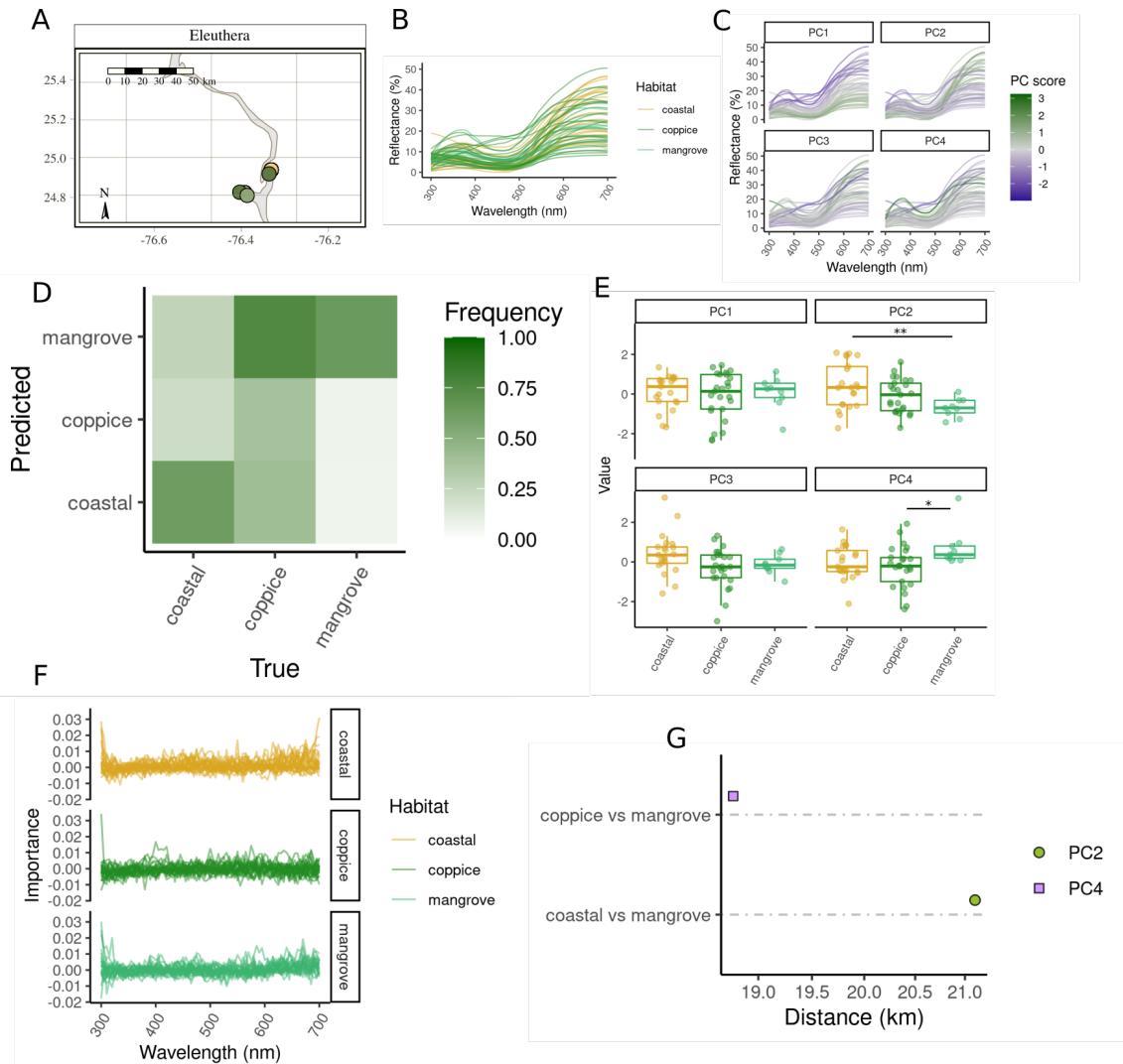


Figure S5: Comparison of dewlap coloration across habitats on Eleuthera. Legend is as per Figure S2.

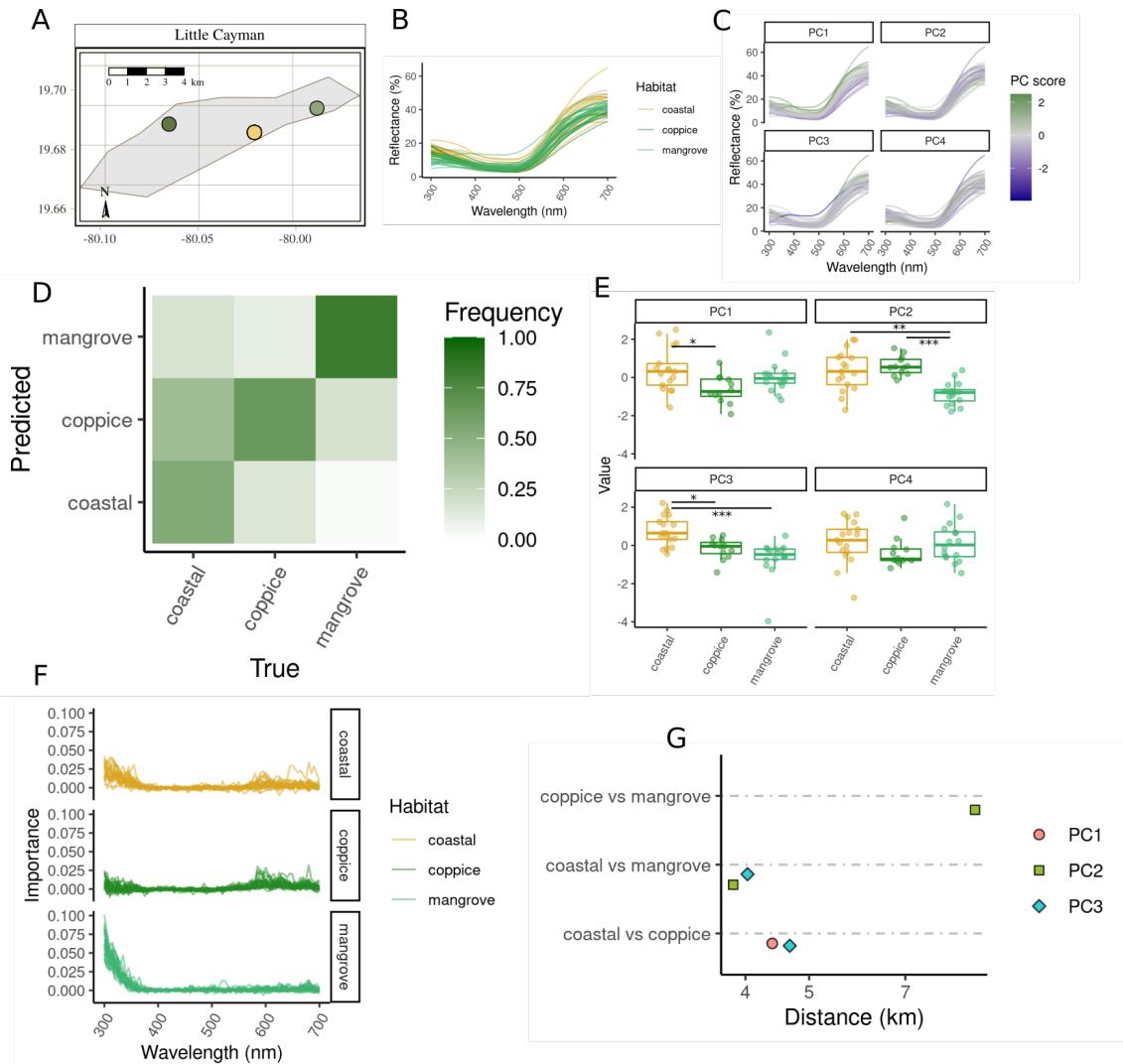


Figure S6: Comparison of dewlap coloration across habitats on Little Cayman. Legend is as per Figure S2.

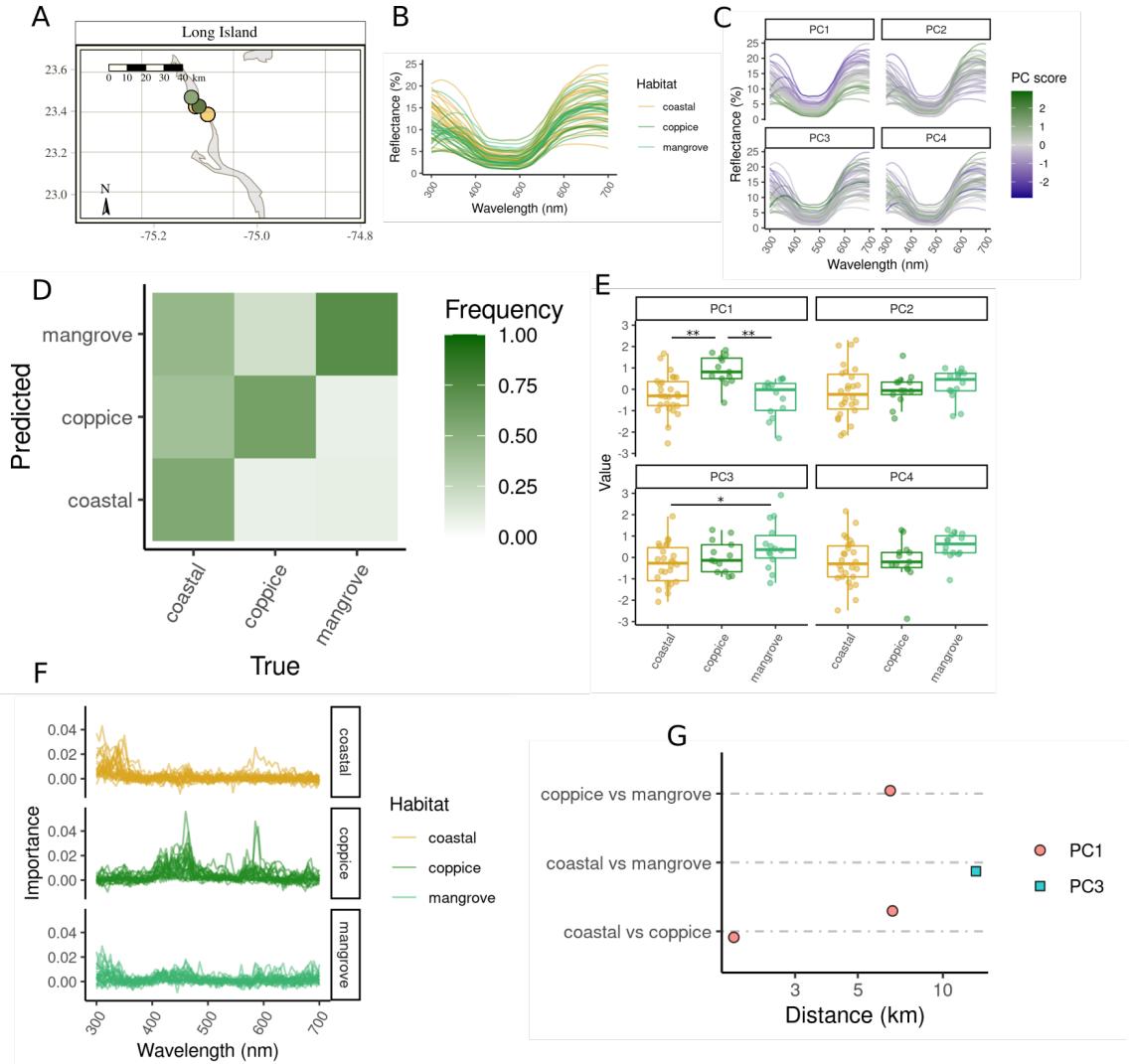


Figure S7: Comparison of dewlap coloration across habitats on Long Island. Legend is as per Figure S2.

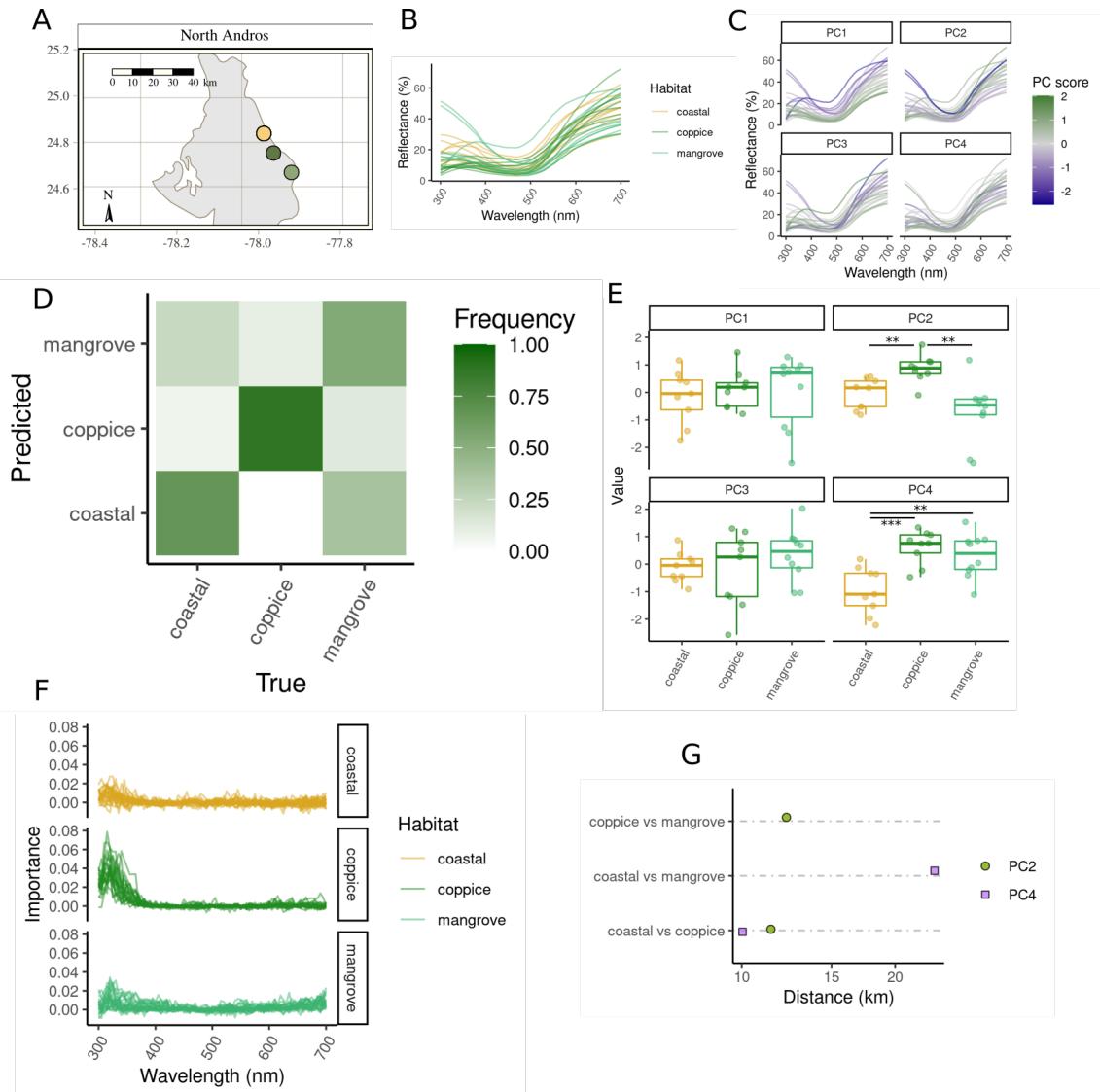


Figure S8: Comparison of dewlap coloration across habitats on North Andros. Legend is as per Figure S2.

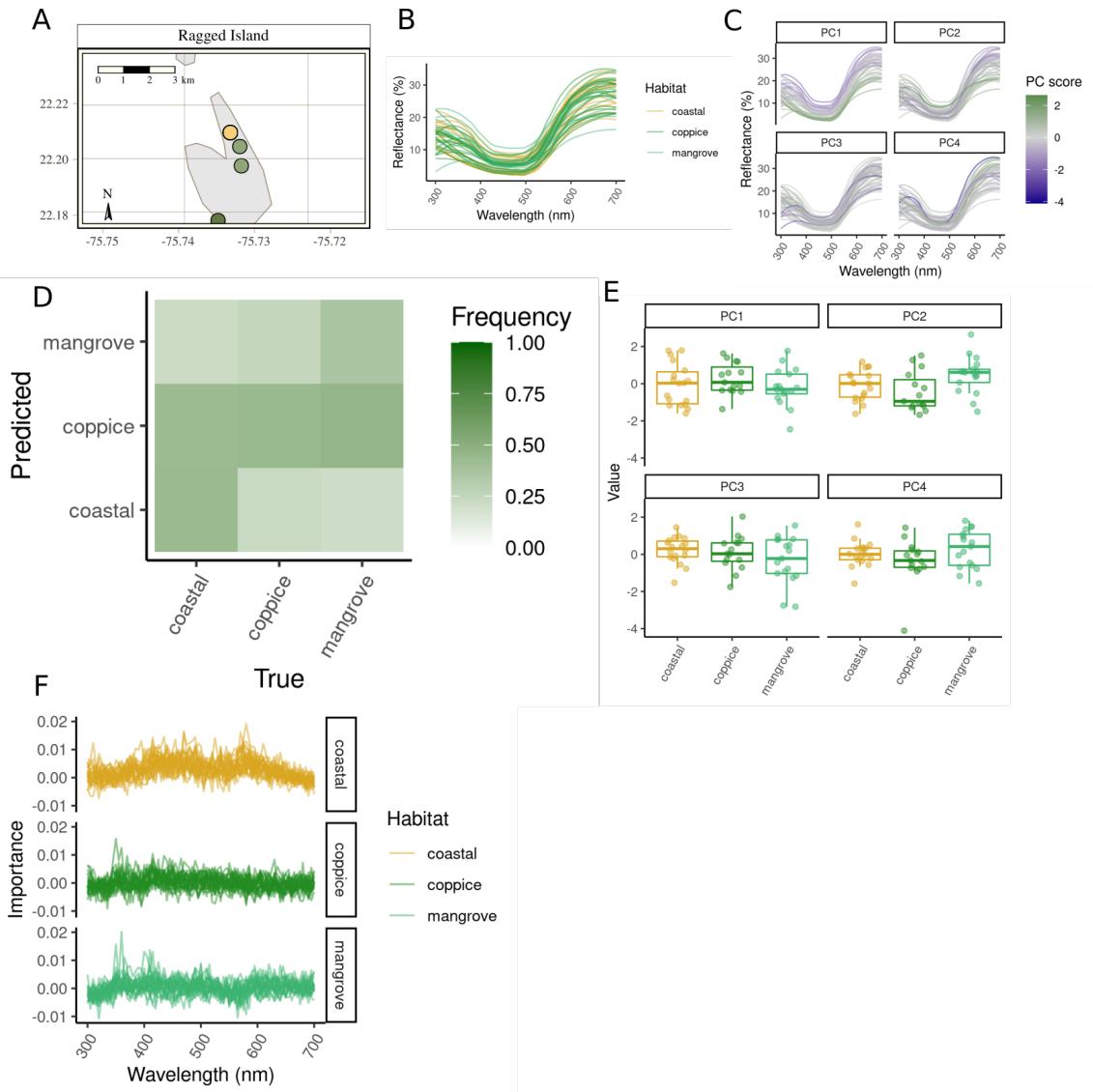


Figure S9: Comparison of dewlap coloration across habitats on Ragged Island. Legend is as per Figure S2, but without panel G.

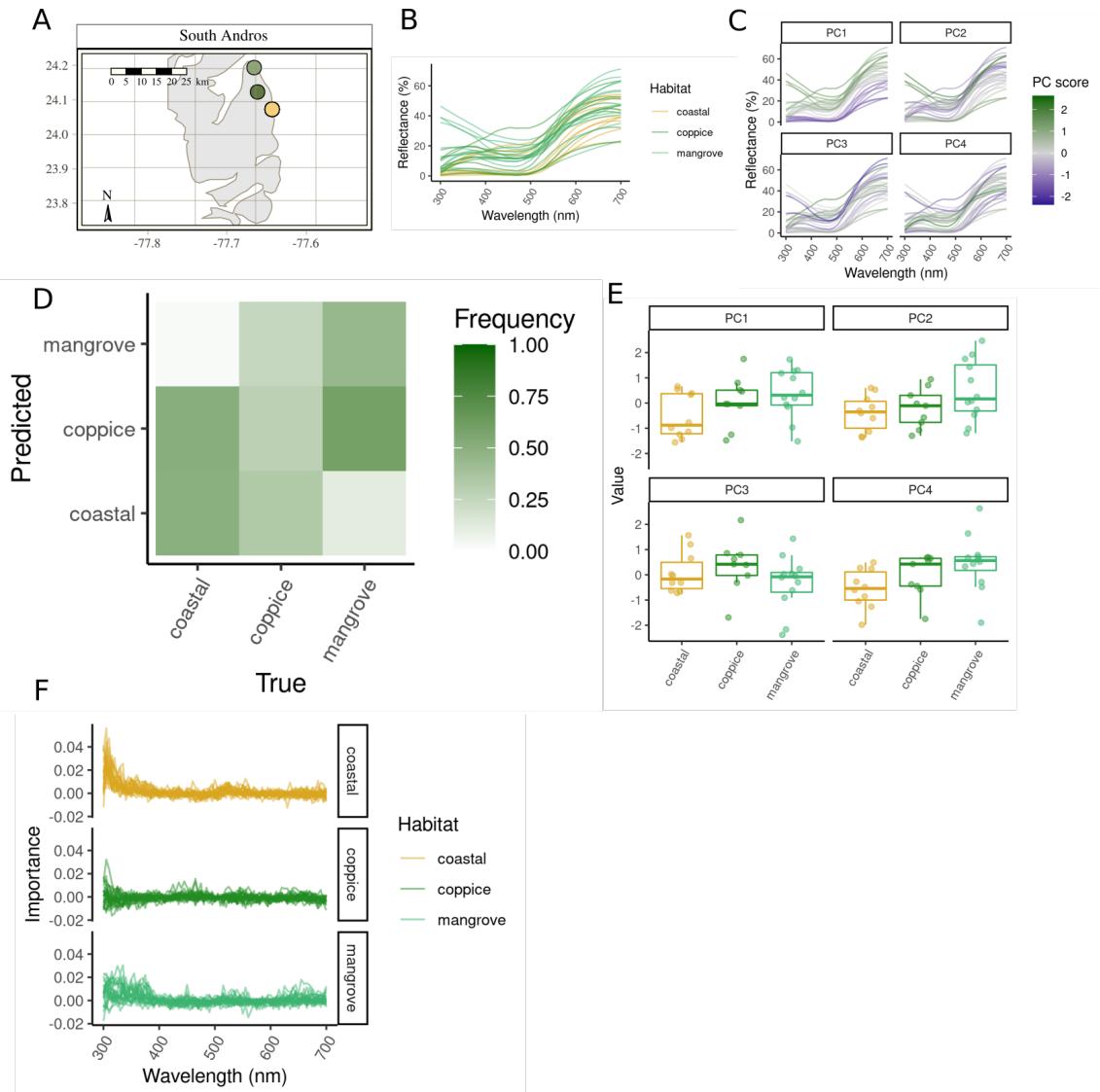


Figure S10: Comparison of dewlap coloration across habitats on South Andros. Legend is as per Figure S2, but without panel G.

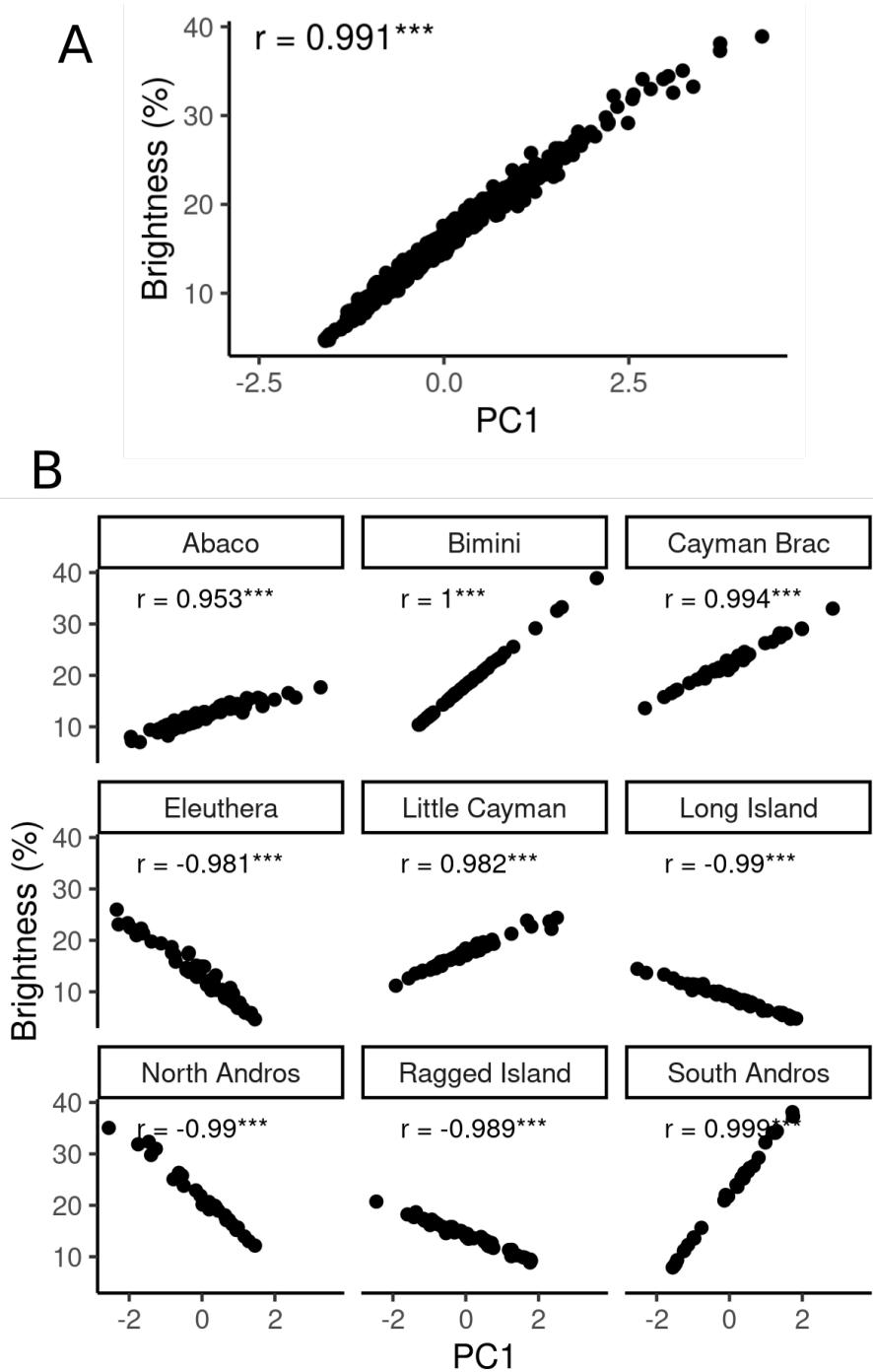


Figure S11: PC1 captures brightness across all islands. (A) Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across all islands. (B) Correlation between brightness and within-island PC1, for each island. Pearson's correlation coefficients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

**808 Supplementary Tables**

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

	coastal	coppice	mangrove
Abaco	41	24	21
Bimini	28	14	15
Cayman Brac	15	18	17
Eleuthera	21	25	9
Little Cayman	17	12	16
Long Island	26	13	14
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	0.277	0.149	0.079
Bimini	0.919	0.530	0.171	0.166	0.052
Cayman Brac	0.888	0.438	0.190	0.155	0.105
Eleuthera	0.926	0.486	0.235	0.138	0.066
Little Cayman	0.907	0.441	0.212	0.176	0.078
Long Island	0.916	0.501	0.215	0.152	0.048
North Andros	0.952	0.485	0.237	0.169	0.062
Ragged Island	0.907	0.483	0.226	0.127	0.072
South Andros	0.937	0.559	0.172	0.151	0.056
All islands	0.915	0.482	0.189	0.167	0.077

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	1.097	0.0028 **
Abaco	coppice	0	1.091	0.0016 **
Abaco	mangrove	0	1.052	0.0028 **
Bimini	coastal	0	0.965	0.0199 *
Bimini	coppice	0	0.828	0.0667
Bimini	mangrove	0	1.211	< 0.0001 ***
Cayman Brac	coastal	0	0.648	0.5261
Cayman Brac	coppice	0	0.691	0.4287
Cayman Brac	mangrove	0	0.671	0.4838
Eleuthera	coastal	0	1.446	< 0.0001 ***
Eleuthera	coppice	0	1.479	< 0.0001 ***
Eleuthera	mangrove	0	0.592	0.5556
Little Cayman	coastal	0	0.626	0.6547
Little Cayman	coppice	0	0.637	0.4895
Little Cayman	mangrove	0	0.867	0.0454 *
Long Island	coastal	0	0.834	0.1291
Long Island	coppice	0	1.001	0.0034 **
Long Island	mangrove	0	0.907	0.0197 *
North Andros	coastal	0	0.747	0.1123
North Andros	coppice	0	0.644	0.3623
North Andros	mangrove	0	0.839	0.0345 *
Ragged Island	coastal	0	0.757	0.2233
Ragged Island	coppice	0	0.810	0.0944
Ragged Island	mangrove	0	0.524	0.9341
South Andros	coastal	0	0.722	0.1765
South Andros	coppice	0	0.628	0.4212
South Andros	mangrove	0	0.684	0.3250

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	0.962	0.0128	0.0599
Abaco	PC2	0.960	0.0093	0.0518
Abaco	PC3	0.982	0.2963	0.6455
Abaco	PC4	0.983	0.3168	0.6455
Bimini	PC1	0.918	0.0009	0.0125 *
Bimini	PC2	0.985	0.7104	0.7956
Bimini	PC3	0.976	0.2997	0.6455
Bimini	PC4	0.977	0.3529	0.6455
Cayman Brac	PC1	0.986	0.8236	0.8869
Cayman Brac	PC2	0.989	0.9299	0.9299
Cayman Brac	PC3	0.934	0.0079	0.0518
Cayman Brac	PC4	0.981	0.5926	0.7214
Eleuthera	PC1	0.936	0.0058	0.0518
Eleuthera	PC2	0.972	0.2199	0.6455
Eleuthera	PC3	0.981	0.5224	0.6965
Eleuthera	PC4	0.977	0.3689	0.6455
Little Cayman	PC1	0.955	0.0821	0.3094
Little Cayman	PC2	0.982	0.6854	0.7956
Little Cayman	PC3	0.891	0.0005	0.0125 *
Little Cayman	PC4	0.977	0.4858	0.6965
Long Island	PC1	0.978	0.4223	0.6956
Long Island	PC2	0.980	0.5161	0.6965
Long Island	PC3	0.981	0.5605	0.7134
Long Island	PC4	0.976	0.3580	0.6455
North Andros	PC1	0.936	0.0884	0.3094
North Andros	PC2	0.966	0.4735	0.6965
North Andros	PC3	0.982	0.8977	0.9299
North Andros	PC4	0.959	0.3348	0.6455

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	1.005	-0.116	0.063	-1.255
Abaco	-77.6	26.9	coastal	0.157	-0.531	-0.086	-2.034
Abaco	-77.6	26.9	coppice	-0.081	-0.034	-0.631	-0.633
Abaco	-77.2	26.1	coastal	0.296	0.091	0.987	-0.158
Abaco	-77.0	26.3	mangrove	-0.312	1.224	-0.898	0.208
Abaco	-77.0	26.3	coppice	0.094	-0.314	-0.120	0.482
Abaco	-77.0	26.3	coastal	-0.334	-0.748	0.201	0.534
Bimini	-79.3	25.7	coastal	0.399	0.000	0.304	-0.049
Bimini	-79.3	25.7	coppice	-0.299	0.511	-0.657	0.097
Bimini	-79.3	25.7	mangrove	-0.466	-0.477	0.046	0.000
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	0.170	-0.855	-0.042	0.086
Eleuthera	-76.3	24.8	coastal	0.503	-0.653	0.564	-0.587
Eleuthera	-76.3	24.8	mangrove	0.067	-0.694	-0.119	0.718
Eleuthera	-76.2	24.9	coppice	-0.353	0.595	-0.544	-0.535
Eleuthera	-76.1	24.9	coastal	-0.215	1.220	0.297	0.396
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	-0.390	0.217	0.541	0.535
Long Island	-75.2	23.4	coastal	0.038	-0.807	0.015	-0.326
Long Island	-75.2	23.4	coppice	0.894	0.018	0.035	-0.161
Long Island	-75.1	23.4	coastal	-0.408	0.299	-0.512	-0.134
North Andros	-77.9	24.8	coastal	-0.152	-0.074	-0.104	-0.957
North Andros	-77.8	24.8	coppice	0.112	0.860	-0.258	0.621
North Andros	-77.8	24.7	mangrove	0.036	-0.707	0.326	0.303
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	0.384	0.492	-0.333	0.472
South Andros	-77.6	24.1	coppice	0.067	-0.211	0.358	-0.018
South Andros	-77.5	24.1	coastal	-0.520	-0.401	0.078	-0.550

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	$P$	
Bimini	PC1	9.31	2	0.0095	**
Little Cayman	PC3	19.95	2	< 0.0001	***

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

Island	<i>N</i>	Score	<i>P</i>	
Abaco	86	0.614	< 0.0001	***
Bimini	57	0.474	0.0098	**
Cayman Brac	50	0.732	< 0.0001	***
Eleuthera	55	0.476	0.0112	*
Little Cayman	45	0.724	< 0.0001	***
Long Island	53	0.623	< 0.0001	***
North Andros	28	0.721	< 0.0001	***
Ragged Island	50	0.368	0.2874	
South Andros	31	0.445	0.1152	

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

Island	<i>N</i>	Score	<i>P</i>
Abaco	86	0.670	< 0.0001 ***
Bimini	57	0.491	0.0046 **
Cayman Brac	50	0.736	< 0.0001 ***
Eleuthera	55	0.480	0.0112 *
Little Cayman	45	0.747	< 0.0001 ***
Long Island	53	0.562	0.0004 ***
North Andros	28	0.636	0.0008 ***
Ragged Island	50	0.388	0.1964
South Andros	31	0.458	0.0589