

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

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Abstract

Animal signals evolve in an ecological context. Locally adapting animal sexual signals can be especially important for initiating or reinforcing reproductive isolation during the early stages of speciation. Previous studies have demonstrated that dewlap color in *Anolis* lizards can be highly variable between populations in relation to both biotic and abiotic adaptive drivers at relatively large geographical scales. Here, we investigated differentiation of dewlap coloration among habitat types at a small spatial scale, within multiple islands of the West Indies, 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

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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 11.2km. While this species has traditionally been considered territorial, recent study reveals 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 466 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 ~ 11km (Figure S1B), and 80.3% 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.

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

Dimensionality reduction

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

Among-island variance partitioning

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

Within-island machine learning

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We performed a machine learning classification analysis on the first four principal components within each island separately, using random forests (Breiman, 2001). Random forests are a versatile, intuitive, and powerful algorithm commonly used in machine learning, using decision trees to predict the labels of particular observations based on their multivariate coordinates. 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

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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).

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Site differences

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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).

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296 **Results**

297 We tested for variation in *A. sagrei* dewlap coloration between populations living in three charac-
298 teristic habitat types across nine islands that span the West Indian range of the brown anole (beach
299 scrub, primary coppice and mangroves). We found that most of the variation in coloration was
300 partitioned between islands (two-way PERMANOVA, $F(df = 8) = 43.7$, $P = 0.0001$, explained
301 variance $R^2 = 40.9\%$). Nonetheless, we did find evidence for differences in dewlap coloration
302 between habitat types, and those were mostly island-specific (habitat-by-island interaction term,
303 $F(16) = 3.53$, $P = 0.0001$, $R^2 = 6.6\%$), with a significant portion of the variation explained by
304 an habitat effect across all islands, but this effect was relatively small ($F(2) = 4.7$, $P = 0.0001$,
305 $R^2 = 1.1\%$).

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 eight out of the nine islands in our sample: Abaco,
310 Bimini, Cayman Brac, Eleuthera, Little Cayman, Long Island, North Andros and South Andros.
311 The accuracy of random forest classification exceeded random expectation more often than ex-
312 pected by chance for all these islands (Table 1). Accuracy was as high as 73% for Cayman Brac.
313 We obtained similar results using other machine learning approaches such as support vector ma-
314 chines (Table S7) and linear discriminant analysis (Table S8), except that these methods did not
315 detect significant differences on Eleuthera and North Andros. We describe in details the specific
316 differences detected on each island in the Appendix, and focus here on the general patterns emerg-
317 ing from our data.

318 Overall, we found significant differences in dewlap coloration between populations that were
319 often in close geographical proximity. On Bimini, notably, we found a significant difference be-
320 tween dewlaps from beach scrub and primary coppice forest, at a distance of a few hundred meters,
321 making this contrast the smallest geographical scale at which differences in coloration were found
322 in our study (Fig. S3). We also detected significant differences in dewlap coloration at distances
323 below one kilometer on Abaco (Fig. S2G), and at distances between one and ten kilometers on
324 Bimini (Fig. S3G), Cayman Brac (Fig. S4G), Little Cayman (Fig. S6G), Long Island (Fig. S7G)
325 and South Andros (Fig. S10G).

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

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Discussion

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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.

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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 unpublished thesis), and (3) *A. sagrei* is highly mobile (Kamath and Losos, 2018b), we would have expected more homogeneous distributions of color phenotypes within islands due to gene flow, with fewer differences between populations, especially those in close proximity., 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

407 the diet (Goodwin, 1984; Hill et al., 2002; Hill and McGraw, 2006), studies testing the effect of
408 carotenoid deprivation (Steffen et al., 2010; Ng et al., 2013) and heritability (Cox et al., 2017) of
409 dewlap coloration in *A. sagrei* and *A. distichus* (another species with a carotenoid-based dewlap),
410 found little support for phenotypic and developmental plasticity in dewlap coloration. One excep-
411 tion is a study demonstrating that lizards heavily parasitized by skin mites had duller dewlaps
412 (Cook et al., 2013), but we found no sign of that in our study.

413

414 We found no evidence for a role of genetic drift in explaining the observed patterns either.
415 First, *A. sagrei* was distributed across the islands continuously, usually at relatively high popula-
416 tion densities, rather than in small and isolated populations where drift might be expected to have
417 a strong effect. Second, we found no evidence of isolation-by-distance except on Abaco, which was
418 sampled at the largest geographical scale, with sites nearly a hundred kilometers apart from each
419 other. Hence, while isolation-by-distance may explain long-range differences on this island, most of
420 the differences among habitats across the rest of the sampling region are unlikely to be explained
421 by genetic drift, as habitats were often in close proximity (less than 10km).

422

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

439

440 In this study, we found larger differences among than within islands, a pattern already reported
441 and linked to climatic conditions (Driessens et al., 2017) and to densities of predators and of anole
442 congeners (Vanhooijdonck et al., 2009; Baeckens et al., 2018). Differences among habitats within
443 islands, however, are still difficult to account for. Remaining hypotheses may include, for example,
444 runaway sexual selection (i.e. arbitrary preferences of females for some colors over others, Anders-
445 son 1994) arbitrarily operating in different directions across islands, but no evidence so far suggests
446 that dewlap is a target of mate choice in anoles (Tokarz, 2002; Tokarz et al., 2005; Lailvaux and
447 Irschick, 2006; Nicholson et al., 2007). Another hypothesis is that the different genetic constitu-
448 tions of different islands, perhaps resulting from founder effects (the islands have been colonized
449 independently, van de Schoot 2016 unpubl.; Driessens et al. 2017; Reynolds et al. 2020), may have
450 predisposed populations to adapt differently to similarly selective circumstances. Either way, new
451 data would be needed to test these hypotheses. Visual modeling, for instance, would be a valuable
452 follow-up analysis as it would indicate whether the differences we detected may be detectable by
453 the organisms themselves (as has been done in *Anolis* in Leal and Fleishman 2004; Fleishman
454 et al. 2020), but such approach requires irradiance profiles from the habitats in the field, which we
455 presently do not have.

456

457 Last, some patterns may arise owing to factors we did not measure. For one, signalling can be a
458 multifaceted behavior, and while we focused on dewlap color here, other potentially important el-
459 ements of behavioral interactions include dewlap size, dewlap patterning, display activity as well as
460 behaviors associated with dewlap extensions such as headbobs and pushups (Vanhooijdonck et al.,
461 2005; Driessens et al., 2014, 2015; Lailvaux et al., 2015). These have been linked in various ways
462 (and not always consistently across studies) not only with environmental variables but also with
463 proxies for sexual selection, predation and species recognition (Vanhooijdonck et al., 2005; Lailvaux
464 and Irschick, 2007; Vanhooydonck et al., 2009; Driessens et al., 2017; Baeckens et al., 2018), which

do vary among islands but may also vary within islands, possibly in interaction with dewlap color.
Besides, while the three habitats were clearly recognizable and consistent across islands to the hu-
man eye (Fig. 1), we did not precisely quantify irradiance within each habitat and did not consider
any more subtle environmental differences there might have been for a given habitat among 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. However, coloration
differs in different ways across similar habitats from one island to another. We discussed several
non-mutually exclusive mechanisms that could explain these observations. Nevertheless, heteroge-
neous patterns of divergence across islands do not support an adaptive sensory-drive scenario, and
⁴⁶⁵ we propose that within-island dewlap color variation may be underlain by a more subtle mosaic of
⁴⁶⁶ factors.
⁴⁶⁷

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490 **Figures**

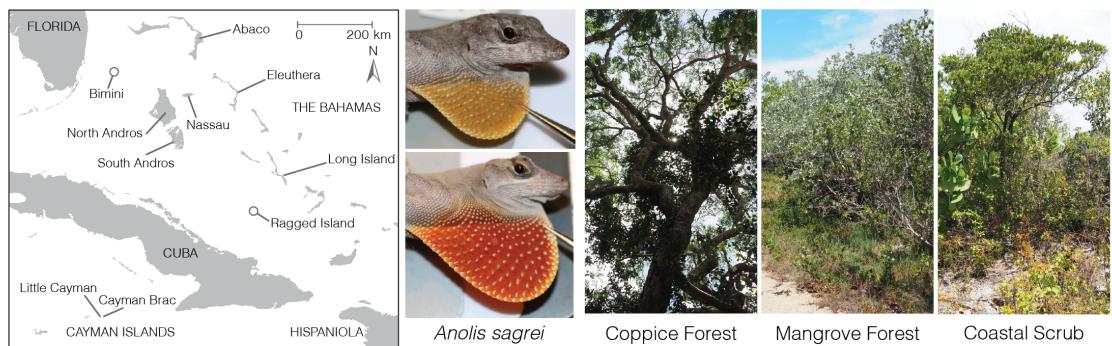


Figure 1: Overview of our study design, including a map of the Bahamas and the Cayman Islands, on which are indicated the nine islands we sampled, two representatives of our study species *Anolis sagrei* with their dewlaps deployed, and the three types of habitats we considered on each island.

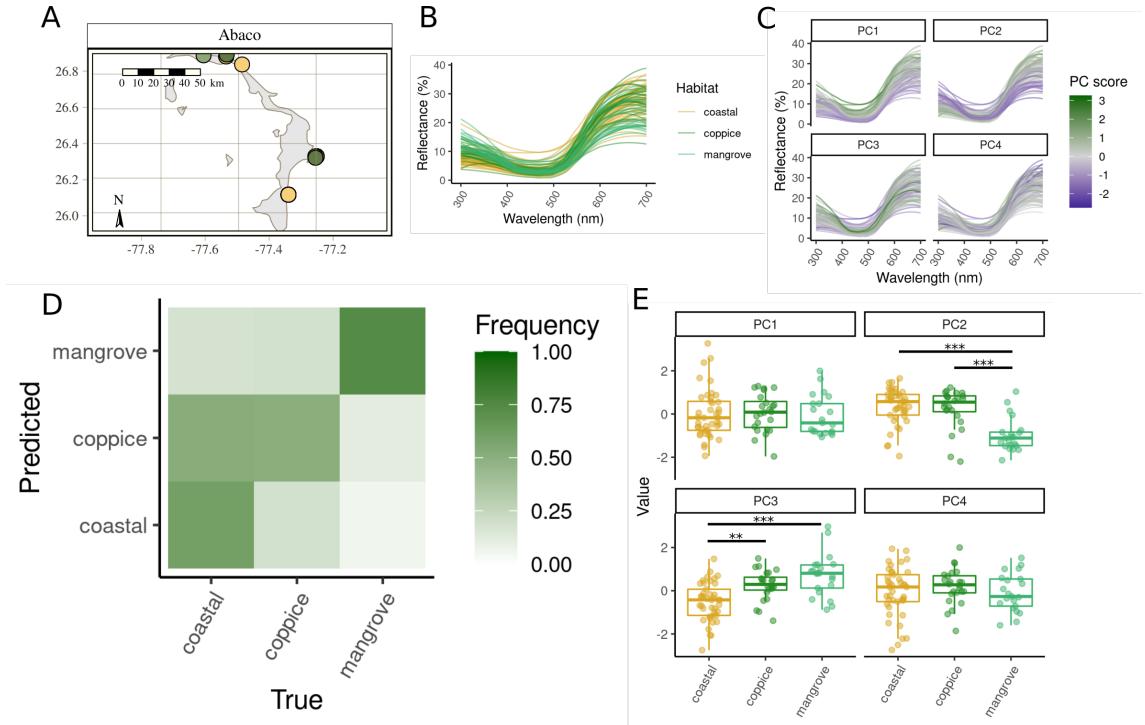


Figure 2: Comparison of dewlap coloration across habitats on Abaco. (A) Map of the island with the sampling sites colored by habitat. (B) Reflectance profiles of all the dewlaps on the island. (C) How reflectance profiles map onto the within-island principal components. (D) Confusion matrix showing the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

491 **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: Mantel’s 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 population means were randomly permuted among sites (999 permutations). *, $P < 0.05$.

Island	ρ	P	
Abaco	0.448	0.033	*
Bimini	0.810	0.250	
Cayman Brac	-0.737	0.833	
Eleuthera	0.844	0.058	
Little Cayman	-0.042	0.667	
Long Island	0.367	0.333	
North Andros	0.051	0.667	
Ragged Island	-0.363	0.708	
South Andros	-0.979	1.000	

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 ***

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748 Appendix

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

750 On Abaco, dewlaps from the mangrove habitat were the best discriminated, while dewlaps from
751 the beach scrub habitat were often mistaken for dewlaps from the coppice habitat (Fig. 2D). Im-
752 portance analysis revealed that beach scrub and mangrove lizards mostly differed in reflectance in
753 the ultraviolet (UV) end of the spectrum (below 400nm, Fig. S2F), where mangrove dewlaps had
754 higher UV reflectance relative to beach scrub lizards, and coppice lizards had an intermediate UV
755 reflectance between the two other habitats (Fig. 2B). Consistent with this, our analyses of variance
756 detected significantly lower PC2 scores in mangrove lizards than in the two other habitats (Fig.
757 2E, Table 3), representing a higher UV-reflectance relative to red (Fig. 2C). Beach scrub lizards
758 also scored lower on PC3 (Fig. 2E, Table 3), indicating less curvature of the reflectance profile
759 and relatively higher reflectance at intermediate wavelengths (blue-to-yellow) than at the ends of
760 the range (Fig. 2C). Differences were detected between sites both at large ($\sim 100\text{km}$) and short
761 ($< 1\text{km}$) distances (Fig. S2G). Abaco was the only island where we detected significant spatial
762 autocorrelation (Table 2), that is, sites that were closer geographically tended to have populations
763 of lizards with more similar dewlap colors.

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

781 On Cayman Brac, all three habitats could be well discriminated against each other (Fig. S4D),
782 with UV reflectance appearing to be an important variable differentiating beach scrub and man-
783 grove dewlaps (Fig. S4F). In contrast, coppice dewlaps had a relatively flat importance profile,
784 suggesting that brightness made them more distinct rather than any particular wavelength (Fig.
785 S4F). Consistent with this, coppice dewlaps were significantly different from all other dewlaps
786 along PC1 (Fig. S4E, Table 3). At a distance between 2 and 3km (Fig. S4G), dewlaps in the
787 beach scrub habitat reflected more red light (as represented by PC2, Fig. S4C, E) and more UV
788 (as represented by PC3, Fig. S4C, E) than in the mangrove habitat. Coppice lizards were also
789 characterized by a higher UV reflectance than mangrove dewlaps (PC3, Fig. S4C, E, Table 3).

791 On Eleuthera, although random forests detected between-habitat differences in dewlap color,
792 other approaches did not (Tables S8 and S7), suggesting that the differences may be small. Consis-
793 tent with this, the only significant univariate difference detected was for PC2 between beach scrub
794 and mangrove lizards, where beach scrub lizards had higher levels of red reflectance and mangrove
795 lizards higher levels of UV reflectance (Fig. S5C, E, Table 3).

797 Little Cayman was characterized by a better discrimination of mangrove lizards from the rest
798 than between beach scrub and coppice lizards (Fig. S6D). Mangrove dewlaps were most distinct
799 with respect to their reflectance in short wavelengths (Fig. S6F), with significantly lower UV re-
800 flectance (as represented by PC2, Fig. S6C, E, Table 3). Beach scrub lizards were characterized
801 by brighter dewlaps than coppice lizards (PC1), and also more convex curves, i.e. slightly higher
802 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.

Supplementary Figures

836

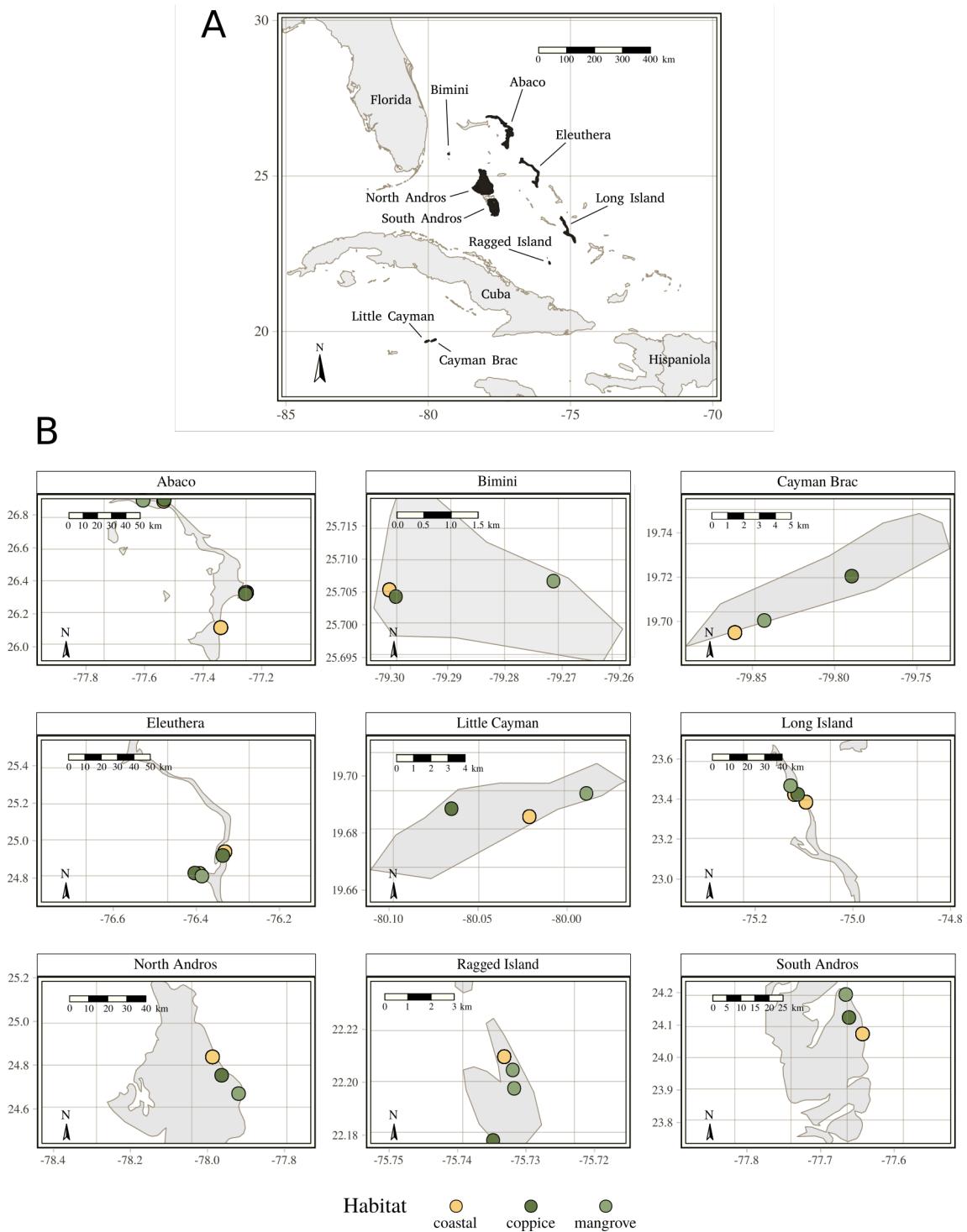


Figure S1: Maps of the islands. (A) Map of the West Indies with sampled islands highlighted in black. (B) Sampling sites within islands colored after their respective habitat types.

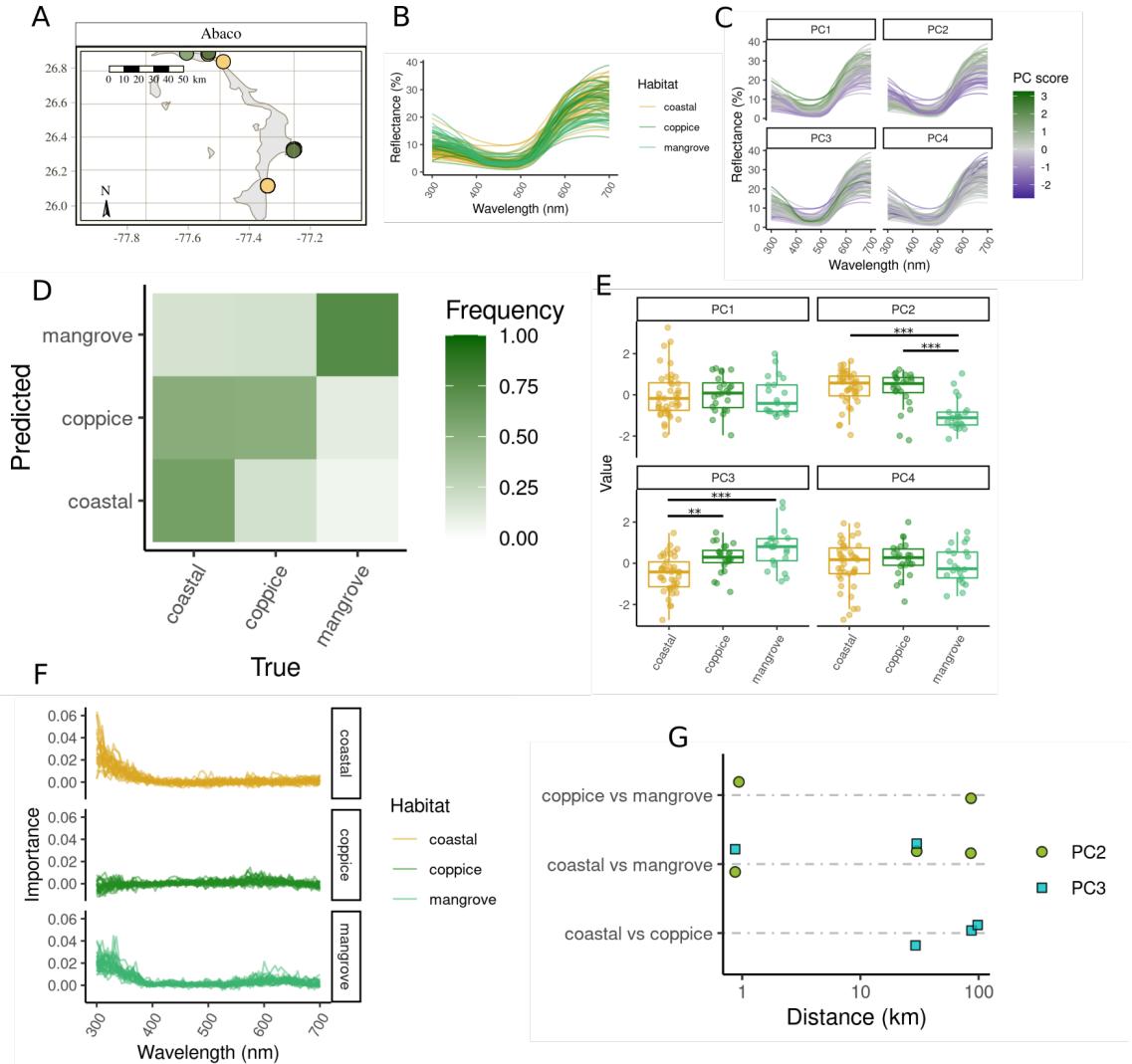


Figure S2: Comparison of dewlap coloration across habitats on Abaco, with extended results. (A–E) Legend as per Figure 2. (F) One-dimensional sensitivity analysis showing the relative importance (mean decrease in accuracy) of the various wavelengths in random forest classification of the whole spectrum.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

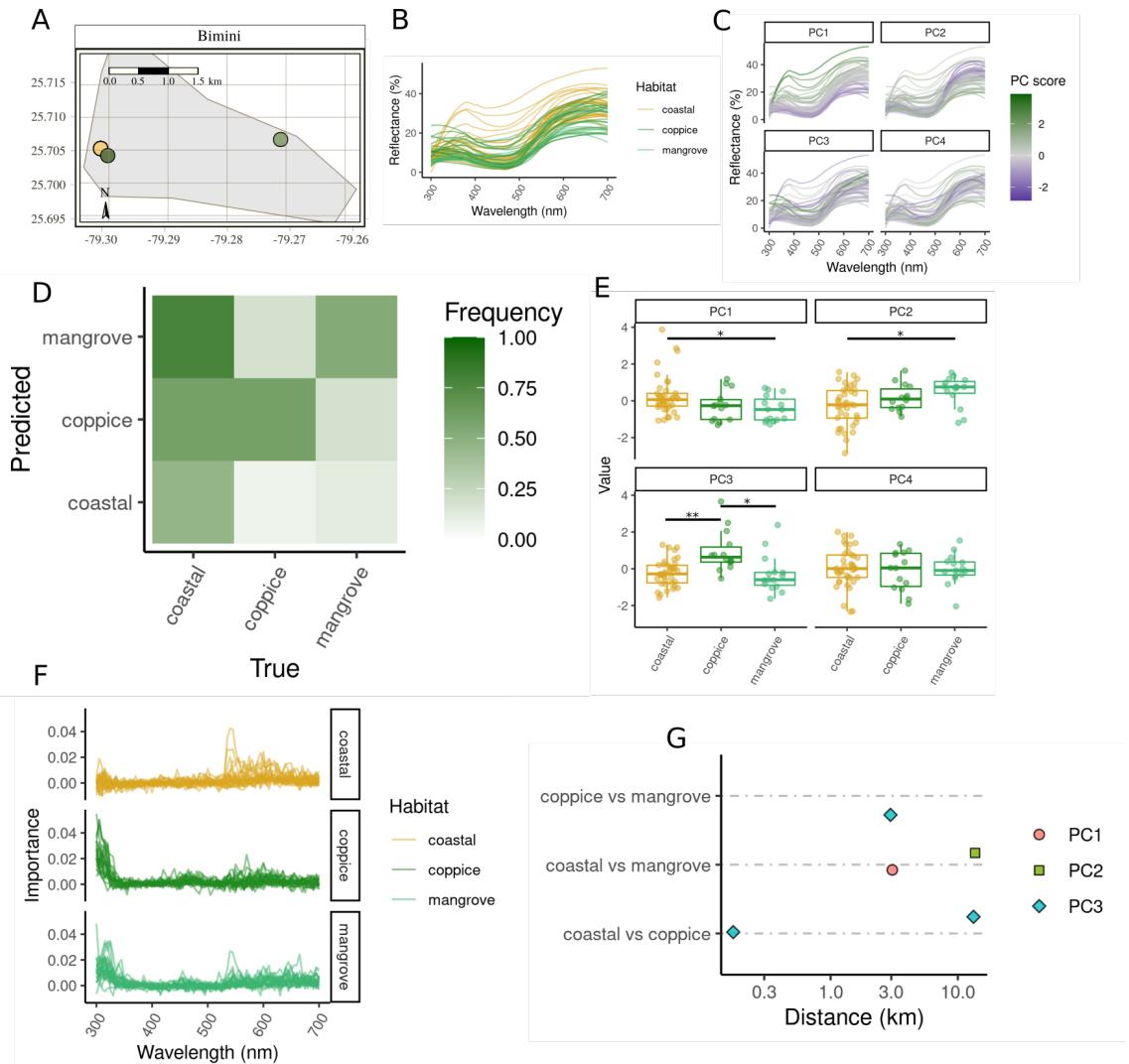


Figure S3: Comparison of dewlap coloration across habitats on Bimini. Legend is as per Figure S2.

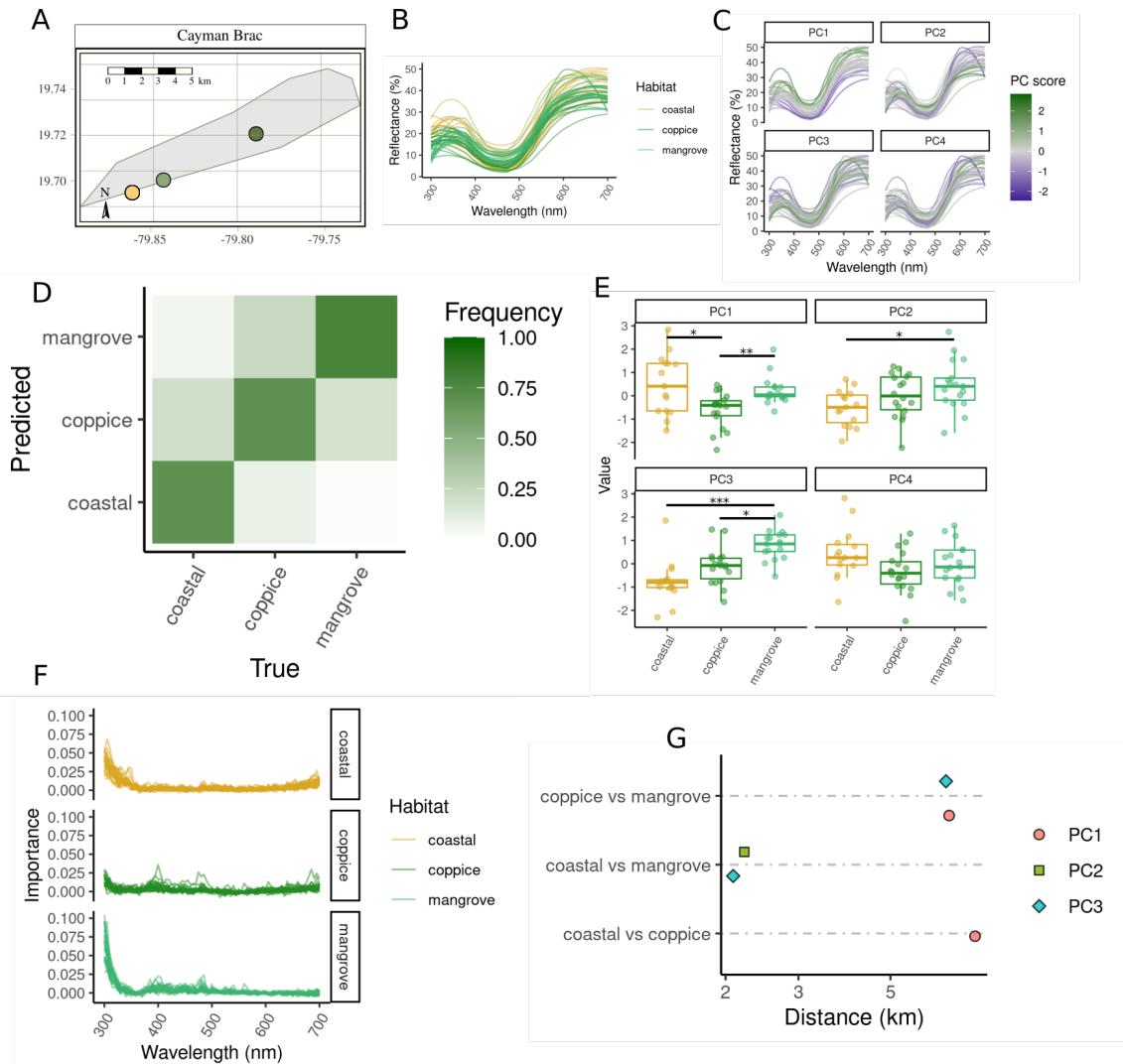


Figure S4: Comparison of dewlap coloration across habitats on Cayman Brac. Legend is as per Figure S2.

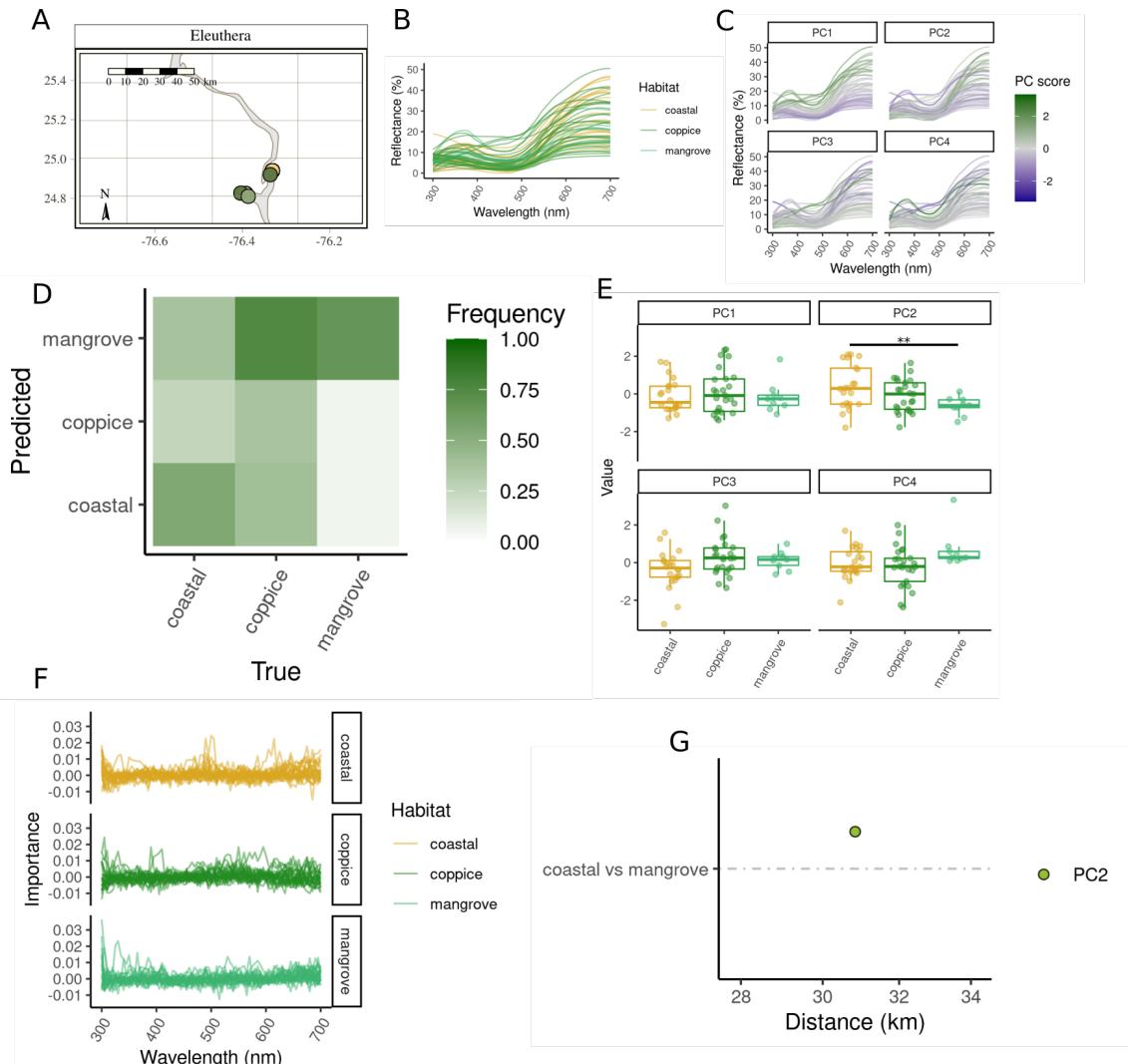


Figure S5: Comparison of dewlap coloration across habitats on Eleuthera. Legend is as per Figure S2.

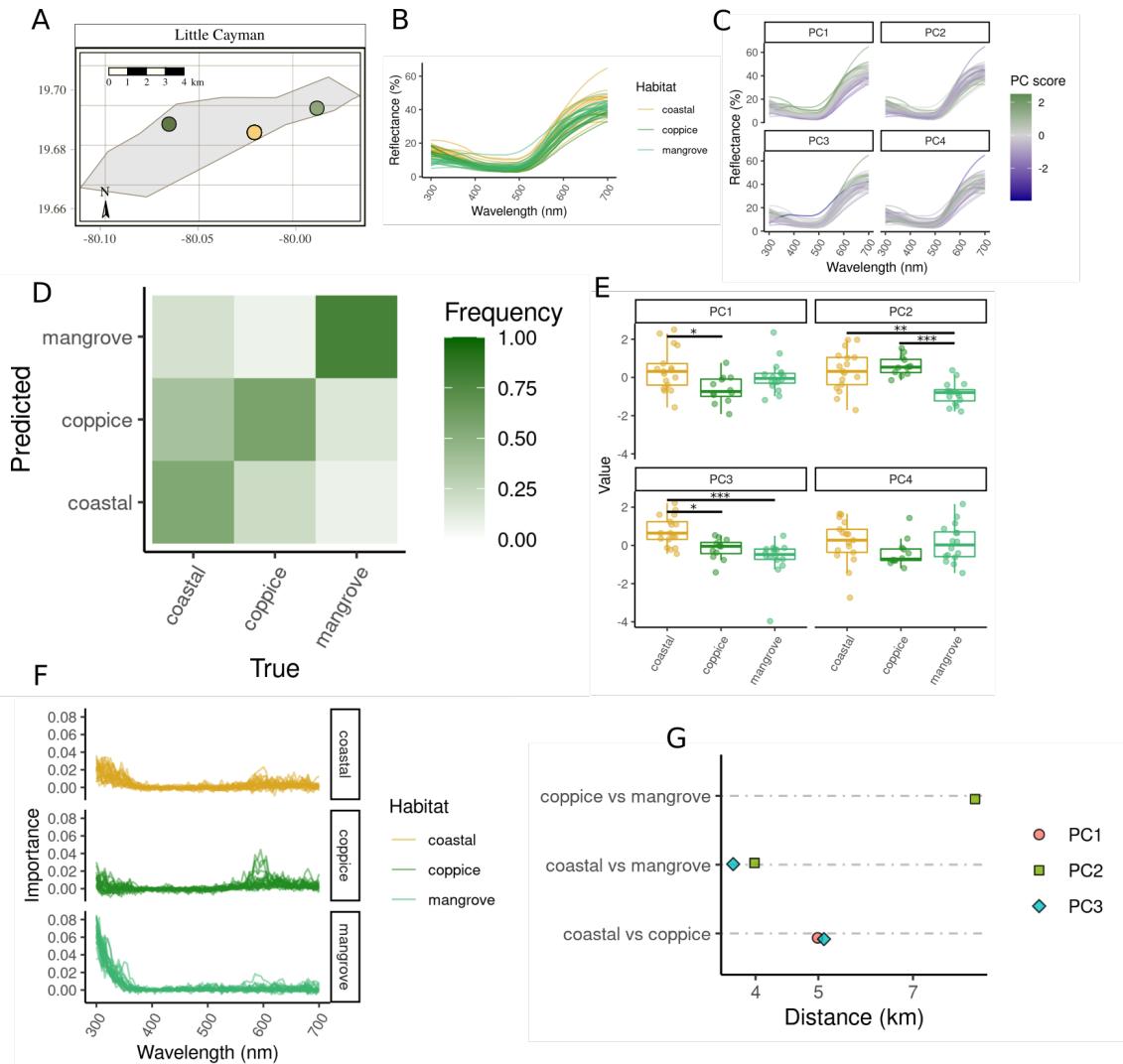


Figure S6: Comparison of dewlap coloration across habitats on Little Cayman. Legend is as per Figure S2.

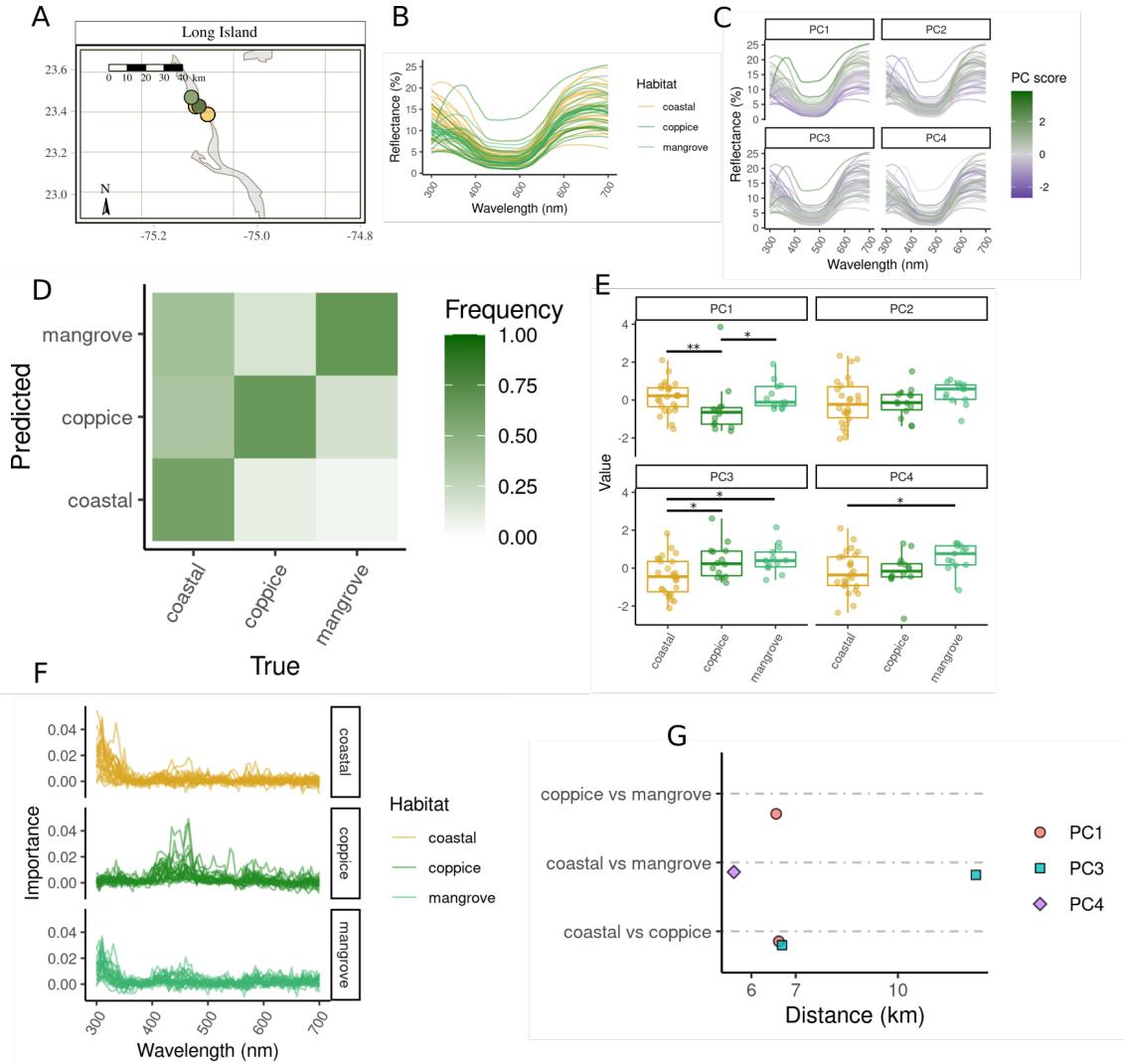


Figure S7: Comparison of dewlap coloration across habitats on Long Island. Legend is as per Figure S2.

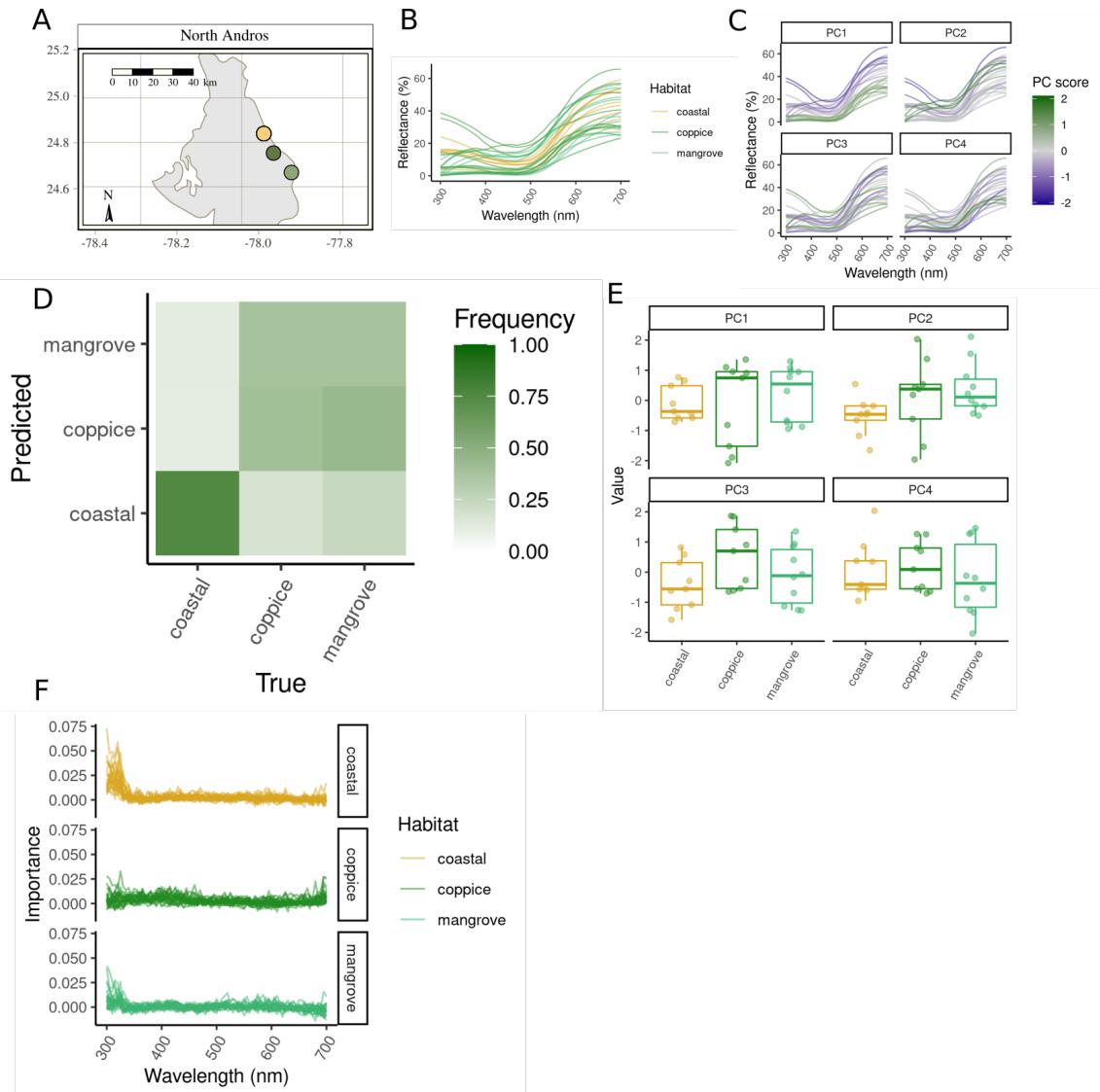


Figure S8: Comparison of dewlap coloration across habitats on North Andros. Legend is as per Figure S2, but without panel G.

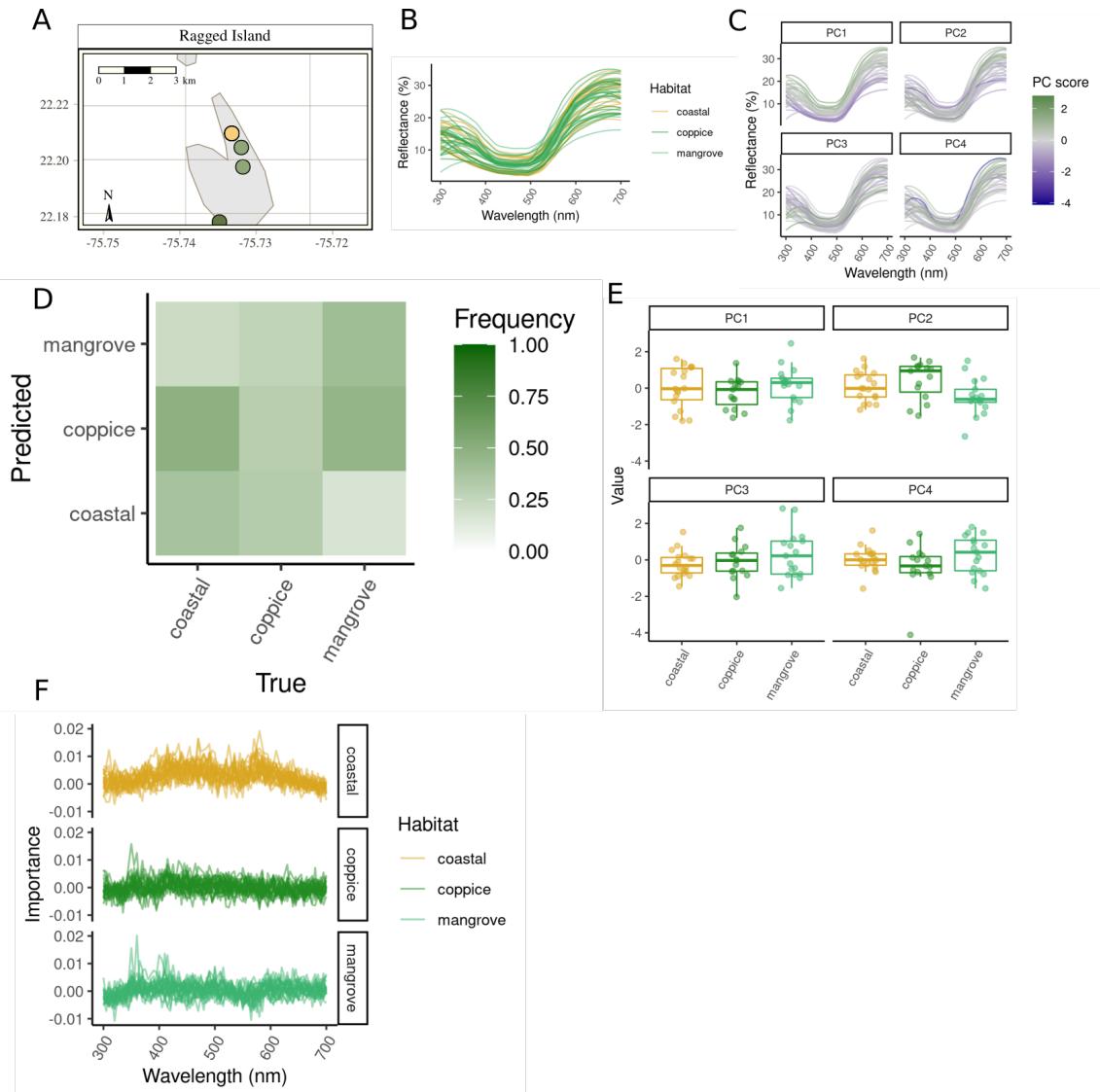


Figure S9: Comparison of dewlap coloration across habitats on Ragged Island. Legend is as per Figure S2, but without panel G.

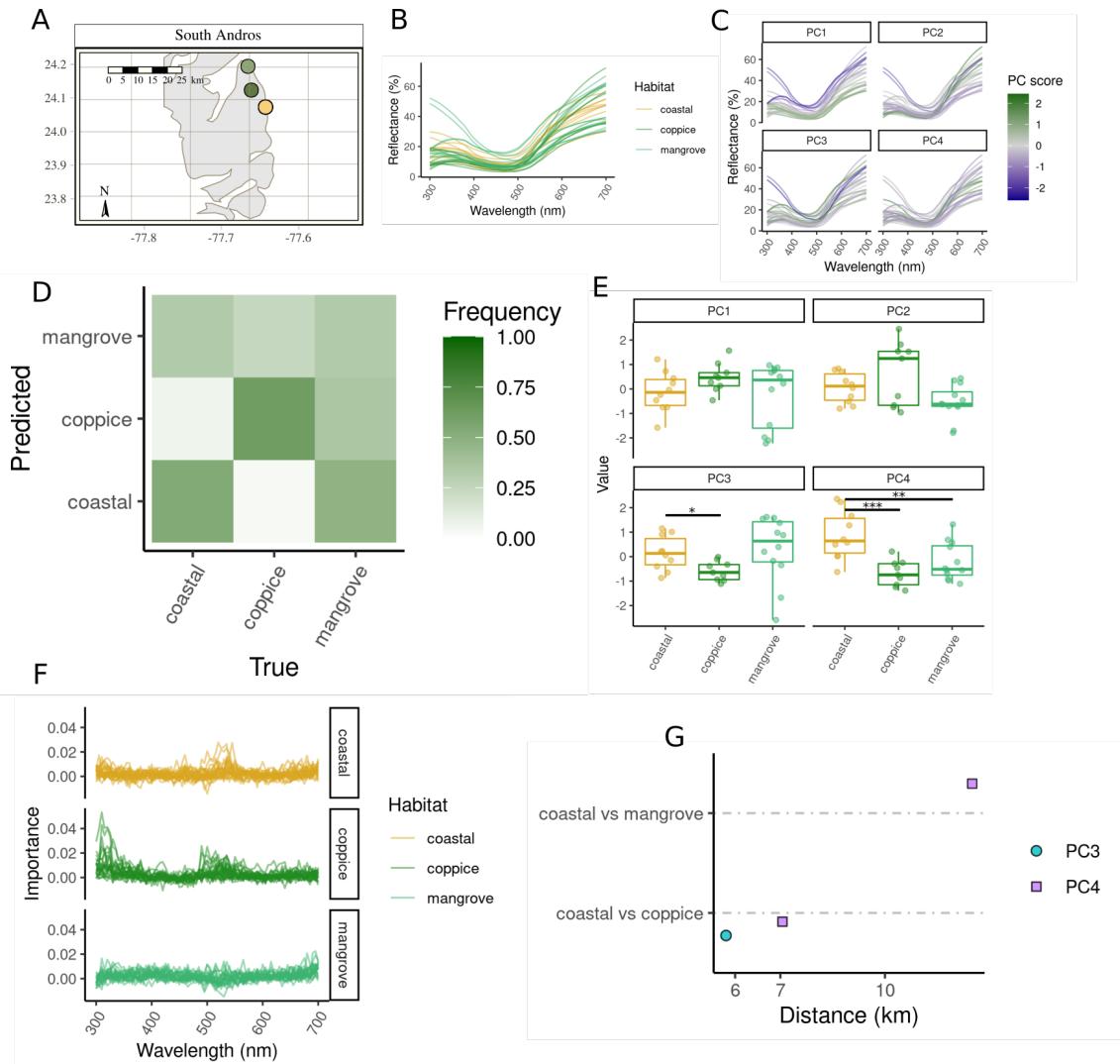


Figure S10: Comparison of dewlap coloration across habitats on South Andros. Legend is as per Figure S2.

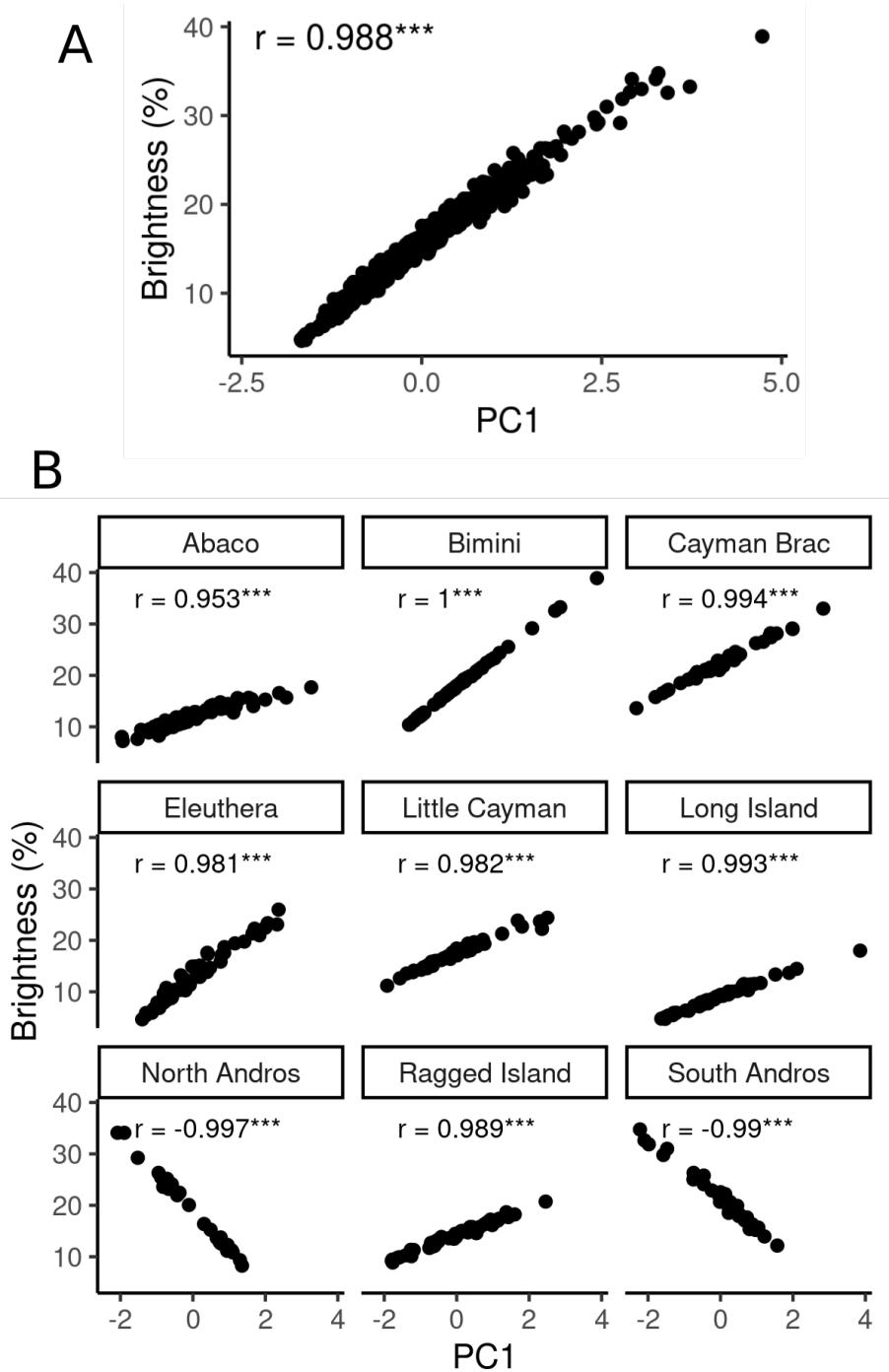


Figure S11: PC1 captures brightness across all islands. (A) Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across all islands. (B) Correlation between brightness and within-island PC1, for each island. Pearson's correlation coefficients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

Supplementary Tables

837

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	**