

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

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Abstract

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

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

Introduction

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

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37 signals.

38
39 One potential barrier to the maintenance of localized signal divergence is the homogenizing effect
40 of gene flow. Population genetics theory suggests that gene flow may counteract local adaptation
41 between localities and prevent divergence altogether, especially at small spatial scales, because
42 of the inflow of maladapted alleles or because of the breaking of linkage between coevolving loci
43 (Felsenstein, 1976; García-Ramos and Kirkpatrick, 1997; Dieckmann and Doebeli, 1999; Lenormand,
44 2002; Hendry et al., 2007a). This genetic homogenization has been confirmed empirically
45 in systems such as stick-insects (Nosil and Crespi, 2004) and sticklebacks (Hendry et al., 2007b).
46 Yet, examples of microgeographic adaptation, i.e. adaptation at smaller scales than the range of
47 dispersal, exist, highlighting the potential of some organisms to respond to selection in the face of
48 gene flow (see Richardson et al. 2014 and references therein). Examples include small scale adap-
49 tation in fragmented areas in Australian fruit flies (Willi and Hoffmann, 2012), or local adaptation
50 to predation pressure in North American salamanders (Richardson and Urban, 2013). Therefore,
51 despite evidence that local adaptation may be particularly difficult at small spatial scales where
52 gene flow tends to cause adjoining populations to remain genetically homogeneous, the potential
53 adaptive response of species traits, in particular communication signals, to localized differences in
54 habitats remains relatively unknown (Richardson et al., 2014).

55
56 Lizards of the neotropical genus *Anolis* are an excellent group for studying the eco-evolutionary
57 dynamics of local adaptation and natural selection (Losos, 2009). A particularly conspicuous trait
58 of anoles is their dewlap; an extensible flap of skin that is typically sexually dimorphic and used as
59 a communication signal in courtship (Sigmund, 1983; Driessens et al., 2014, 2015) and territorial
60 displays (Losos, 1985; Macedonia and Stamps, 1994; Macedonia et al., 2013) as well as in predator
61 deterrence (Leal and Rodríguez-Robles, 1995, 1997; Leal and Rodriguez-Robles, 1997). Dewlap
62 characteristics vary widely among the approximately 400 species of the genus (Nicholson et al.,
63 2007). Interspecific variation in dewlap coloration is implicated in species recognition (Rand and
64 Williams, 1970; Williams, 1969; Williams and Rand, 1977; Losos, 1985; Macedonia and Stamps,
65 1994; Fleishman, 2000; Macedonia et al., 2013), and this function could have had a role in initiating
66 and/or reinforcing reproductive isolation during speciation (Lambert et al., 2013; Geneva et al.,
67 2015; Ng et al., 2017).

68
69 Within species, studies have shown a link between variation in dewlap coloration and differ-
70 ences in habitats or climatic conditions (Macedonia, 2001; Leal and Fleishman, 2002; Thorpe and
71 Stenson, 2002; Thorpe, 2002; Leal and Fleishman, 2004; Vanhooydonck et al., 2009; Ng et al., 2012,
72 2013, 2016; Vanhooydonck et al., 2009; Driessens et al., 2017). Some studies suggest that those
73 differences may be adaptive, and that dewlaps may have evolved to maximize detectability given
74 local light conditions (Fleishman and Persons, 2001; Leal and Fleishman, 2002, 2004). Although
75 this claim is further supported by recent findings that dewlap colors are perceived differently un-
76 der different levels of shading (Fleishman et al., 2020), other studies found conflicting patterns
77 of between-habitat variation that did not support the sensory drive hypothesis (Fleishman et al.,
78 2009; Ng et al., 2012; Macedonia et al., 2014).

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

87
88 The species *Anolis sagrei* is widespread across islands of the West Indies (Reynolds et al.,
89 2020). It has been the subject of numerous studies concerning local adaptation (Losos et al., 1994,
90 1997, 2001; Kolbe et al., 2012), biological invasion (Kolbe et al., 2008), sexual selection (Tokarz,
91 2002; Tokarz et al., 2005; Tokarz, 2006; Driessens et al., 2014; Steffen and Guyer, 2014; Driessens
92 et al., 2015) and many other topics. Between-island variation in the mainly orange-red color of
93 its dewlap was shown to be better explained by climatic variables (Driessens et al., 2017) than by
94 proxies for biotic factors such as sexual selection or predation pressure (Vanhooydonck 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 promiscuous species (Kamath and Losos, 2018). These islands all share three characteristic native West Indian habitat-types – beach scrub bush, closed-canopy primary coppice forest, and mangrove forest – that are often spatially intermingled. These habitats contrast in environmental parameters including vegetation community, light irradiance, humidity and temperature (Howard, 1950; Schoener, 1968). The Cayman Islands and the Bahamas have been colonized independently by *A. sagrei* from Cuba (Reynolds et al. 2020, van de Schoot et al. unpubl.), such that these archipelagos constitute an ideal suite of natural replicates to explore within-island dewlap diversity across multiple islands.

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

Methods

Data collection

We sampled 466 male *Anolis sagrei* from seven islands in the Bahamas Archipelago – Abaco, North Andros, South Andros, South Bimini, Eleuthera, Long Island, Ragged Island – and two in the Cayman Islands – Cayman Brac and Little Cayman (Figure 1). These islands were chosen to span the breadth of the West Indian range of *A. sagrei*, because they have highly similar habitat types, and because the *A. sagrei* on each island group are derived from ancient and distinct colonization events from Cuba (i.e. relatively evolutionarily independent, Reynolds et al. 2020). Three habitats were sampled on each island based on characterizations by Howard (1950) and Schoener (1968). Each habitat is clearly distinguishable by its dominant vegetation type — xeric coastal scrub (open, relatively dry habitat consisting of low vegetation or isolated trees), primary coppice forest (closed-canopy forest) and mangrove forest (wet coastal habitat with trees growing in brackish water and high light penetration). Sample sizes are given in Table S1. Our sampling design enabled us to test for differences between habitats at a coarse and fine geographical scale. The median distance between two localities within an island was ~ 11km, with some islands being sampled at smaller or larger scales (Figure S1, Table S2), and 80.3% of all pairwise distances within islands were less than 50km. Additionally, there are no major barriers to dispersal (such as mountains or grassland) on any of the islands that we sampled.

Reflectance measurements

We measured reflectance between 300 and 700nm wavelength, a range that encompasses the colors visible to most lizards and vertebrates in general (Lazareva et al., 2012). Measurements were taken with an Ocean Optics USB4000 spectrometer, a pulsed Xenon light source (PX-2, Ocean Optics, Largo, FL, USA) and a reflectance probe protected by a black anodized aluminum sheath. Measurements were taken with a 45-degree inclination to prevent specular reflection (Endler, 1990). The device was regularly standardized with a Spectralon white standard (Labsphere, North Sutton, NH, USA). Reflectance was measured at the center of the dewlap.

147 **Analysis**

148 All analyses in this study were performed in R 3.6.1 ([R Core Team, 2019](#)) and the source code can
149 be found at <https://github.com/rscherrer/dewlap>, presently private. We used our newly developed
150 R package nmgc (Nested Multivariate Group Comparisons; <https://github.com/rscherrer/nmgc>),
151 to perform all analyses. This package provides a streamlined and powerful interface to perform
152 group comparison analyses in hierarchical and/or nested by combining both classical statistical
153 tools (e.g. MANOVA, ANOVAs, Kruskal-Wallis) and machine learning tools (e.g. LDA, SVMs)
154 into complete analysis pipelines. The package also facilitates PCA-transformations, assumption
155 testing and automatic choice of suitable posthoc tests, along with full cross-validation, binomial
156 testing of accuracy scores and independent replicate trials for machine learning procedures.

157 **Dimensionality reduction**

158 Reflectance curves were smoothed using the R package pavo ([Maia et al., 2013](#)) as well as with
159 custom R functions, down to one reflectance value at each nanometer in wavelength from 300
160 to 700nm. Because neighboring wavelengths are highly collinear in reflectance, we reduced the
161 dimensionality of the data using principal component analysis (PCA), as per [Cuthill et al. \(1999\)](#)
162 and [Leal and Fleishman \(2002\)](#). We performed PCA on each island separately and systematically
163 retained the first four principal components (PC), which together always explained more than
164 88.8% of the variance across islands (Table S3). PC1 explained between 40 and 56% of the variance
165 across islands; PC2 explained 17.4–27.9%; PC3 12.7–17.6% and PC4 4.3–10.5%. The first four PCs
166 explained similar proportions of variance when calculated for all islands together (Table S3). PCs
167 need not represent the same wavelengths across islands because they are fitted on different datasets.
168 Nevertheless, PC1 was very collinear with brightness for all islands (Figure S2, Table S4). PC2
169 correlated highly with the red and ultraviolet ends of the spectrum, which were inversely correlated
170 with each other (Fig. 3A). Higher PCs corresponded to various combinations of wavelengths.
171 Because PC1 correlated uniformly with all wavelengths across the spectrum we considered PC2
172 onwards to capture the chromatic dimensions of color space, i.e. the relative contributions of the
173 wavelengths regardless of brightness.

174 **Pooled analyses**

175 In addition to within-island PCA, we performed a PCA on pooled data from all islands. The
176 first four principal components explained 91.3% of the variance (Table S3). Again PC1 strongly
177 correlated with brightness (Fig. S3, Table S4). PC2 was positively correlated to short wavelengths
178 (ultraviolet to blue) and negatively correlated to long wavelengths (green to red, Fig. S4B). PC3
179 was strongly negatively correlated with UV reflectance and positively correlated with blue-green.
180 PC4 was made of a mosaic of wavelengths, correlating positively with blue and red but negatively
181 with ultraviolet and yellow.

182

183 We used this dataset to partition the variance in dewlap coloration among islands, habitats
184 and habitats within islands, using a two-way multivariate analysis of variance (MANOVA) with
185 an interaction term. However, because the assumptions of parametric MANOVA were violated
186 for all islands but Ragged Island (multivariate normality, Henze-Zirkler's test, [Henze and Zirkler
1990](#), R package MVN, [Korkmaz et al. 2014](#), Table S5; and homogeneity of covariance matrices,
187 Box's M-test, [Box 1949](#); [Morrison 1988](#), R package heplots, [Fox et al. 2018](#), Table S6), we used
188 a semi-parametric MANOVA instead (R package MANOVA.RM, [Friedrich et al. 2018](#)), with P-
189 values calculated from a bootstrap procedure with 1,000 iterations. We calculated the proportion
190 of variance explained by islands, habitats and the habitat-by-island interaction using partial effect
191 sizes η^2 on a MANOVA-approximation of the analysis (R package heplots, [Fox et al. 2018](#)).

193 **Machine learning**

194 Because of the aforementioned violations of MANOVA assumptions, and to reduce the chances
195 of false discovery, we conducted multivariate group comparisons using support vector machines
196 (SVMs), a model-free, powerful nonparametric supervised machine learning technique.

197

198 Machine learning for group comparison has become more common in ecology and evolution
199 in recent years (e.g. [Pigot et al. 2020](#)). In particular, SVMs are designed to find the best pos-

sible nonlinear boundaries between labelled groups of points in multidimensional spaces, without assumptions about the distribution of the data (Cortes and Vapnik, 1995; Cristianini and Shawe-Taylor, 2000; Kim and von Oertzen, 2018). This makes them well suited to field biological data, which often violate the assumptions of classical linear modeling (Kim and von Oertzen, 2018) and can be, as in the case of coloration, inherently highly multivariate (Cuthill et al., 1999). First, a machine is trained to recognize differences between groups within a subset of the data called the training set. Significance of differences is then assessed by testing the accuracy of that fitted machine in predicting the group-labels of data points that were not included in the training, called a testing set, based solely on their multivariate coordinates. This cross-validation procedure results in a proportion of correctly classified points, or generalization accuracy score, which can be compared to that expected under random guessing using a binomial test.

In this study, we performed SVM classifications on each island separately. We used a standard five-fold cross-validation procedure, where the data were randomly split into five bins of approximately equal sizes. Each bin was in turn taken as the testing set while the rest was used as a training set, thus resulting in five trained machines per cross-validation. We replicated this procedure 100 times for each island to account for stochastic outcomes. We performed binomial tests to evaluate the significance of deviations in observed mean generalization accuracy per island to null expectations under random guessing. Each training data set was downsampled to the size of its least represented habitat to ensure balanced training samples. We ensured that each habitat was represented by at least five data points in the training set.

All classification analyses were repeated using the more classical linear discriminant analysis (LDA), a supervised machine learning technique finding linear boundaries that maximize the differences between groups, albeit assuming multivariate normality and homogeneity of covariance matrices (Ripley, 1996). We used the R package rminer (Cortez, 2010, 2016) for SVMs, and MASS (Venables and Ripley, 2002) for LDAs. We used rminer's default heuristic search option to automatically tune the Gaussian kernel parameter σ and the complexity parameter C for the SVMs.

The same procedure was repeated on principal components from the whole archipelago (see Pooled analyses) to evaluate the significance of archipelago-wide differences in dewlap coloration across habitats.

All machine learning classifications performed on principal components were also repeated on the original reflectance datasets reduced to 50-nm spaced wavelengths from 300 to 700nm.

We conducted one-dimensional sensitivity analyses using rminer (Cortez and Embrechts, 2013) to determine the relative importance of the different input variables during classification where significant differences were detected, both on machines trained on principal components and machines trained on non-transformed reflectance at various wavelengths. In parallel, we conducted univariate analyses of variance to independently test the importance of different variables in between-habitat variation, on islands where the machines detected significant differences based on binomial tests (next section).

Univariate analyses

For each island where significant differences in multivariate dewlap coloration were detected between habitats, we used multiple univariate analyses of variance (ANOVA) to identify which variables were responsible for 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, Barton 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

256 including a habitat-term and a null model lacking the habitat-term.

257
258 We tested the assumptions of the parametric ANOVA for each island included in the univariate
259 analyses. For all islands where deviations from multivariate normality were detected in at least one
260 habitat (Table S5), we assessed univariate normality for each principal component (Shapiro-Wilk's
261 test, Table S7). For skewed PCs that deviated significantly from normality, we repeated the anal-
262 ysis using a nonparametric Kruskal-Wallis test (Hollander et al., 2013). We found no multivariate
263 outliers based on the Mahalanobis distance (package MVN, Korkmaz et al. 2014). We used the
264 cases of better fit of the GLS model relative to the OLS model as evidence for heterogeneity of
265 variances, which were then accounted for by the GLS approach (Table 1).

266
267 Significant *post hoc* contrasts were assessed using Tukey's Honest Significant Difference (HSD)
268 test whenever the assumptions of normality and homogeneity of variances was met (Tukey, 1949),
269 Dunnett's T3 method when only homogeneity of variances was violated but not normality (Dun-
270 nett, 1980), and Nemenyi's test when normality was violated (Nemenyi, 1963). All *post hoc* tests
271 were performed with the R package PMCMRplus (Pohlert, 2020).

272
273 We used the same procedure to investigate which variables, if any, were involved in archipelago-
274 wide multivariate differences between habitats detected in our two-way MANOVA design (see
275 Pooled analyses). However, in the first step of our model comparison procedure, we added mixed-
276 effect equivalents of our OLS and GLS models, this time with island as a random effect. The
277 resulting four models were compared and the best fitting variance structure was retained as ex-
278 plained above.

279 Spatial autocorrelation

280 We tested for within-island spatial autocorrelation between the geographical distances among sam-
281 pling sites and their Euclidean distances in multivariate color space (mean PC1 to PC4 per site,
282 Table S2), regardless of habitat-type. Because often only a few sites were sampled per island, we
283 could not get meaningful results from tests that use sites as units of observation, such as Moran's
284 I test (Gittleman and Kot, 1990). Instead, we designed a permutation test where we randomly
285 reshuffled individual lizards across sites within islands 1,000 times each, and systematically re-
286 calculated Pearson's correlation coefficient between geographic distances (computed as geodesic
287 distances in the R package geosphere; Hijmans 2019) and phenotypic distances. We used the re-
288 sulting null distributions of correlation coefficients to assess the significance of the observed spatial
289 autocorrelation for each island.

290 Site differences

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

297 Results

298 We tested for variation in *A. sagrei* dewlap coloration between populations living in three charac-
299 teristic habitat types across nine islands that span the West Indian range of the brown anole (Fig
300 1, S1). We found that most of the variation in coloration is partitioned between islands (two-way
301 semi-parametric MANOVA, modified ANOVA-type statistic (MATS) = 2009.6, $P < 0.001$, Fig.
302 S5, explained variance $\eta^2 = 44.3\%$, MANOVA approximation). Nonetheless, we did find evidence
303 for differences in dewlap coloration between habitat-types, and those were mostly island-specific
304 (habitat-by-island interaction term, MATS = 384.4, $P < 0.001$, explained variance $\eta^2 = 11.4\%$),
305 with a small but significant portion of the variation explained by an archipelago-wide habitat effect
306 (MATS = 42.5, $P = 0.001$, $\eta^2 = 4.8\%$).

307

The small archipelago-wide effect of habitat-type was detected for PC1, PC2 and PC3 (mixed-effect ANOVA with island as a random effect, Table S8), but this effect was too small for *post hoc* tests to find which habitats differed. Archipelago-wide differences in dewlap coloration between habitats were also detected by SVMs trained on pooled data regardless of island identity, both for PCA data and reflectance scores (Fig. S6, S7). This seemed to be driven primarily by mangrove lizards being correctly reassigned more often than predicted by chance. Sensitivity analyses on these machines suggest that wavelengths from a large range, between 300 and 600nm, overall played a more important role in successful identification than wavelengths above 600nm (Fig. S9), and that PC3 and PC4 were more important than PC2 (Fig. S8). This suggests that archipelago-wide differences may involve fine deviations from the main axes of variation (such as PC2) in multiple wavelengths, and possibly nonlinear combinations of wavelengths, that only the SVMs, not the LDAs, could pick up (Fig. S10, S11). This pattern was weak, with machine accuracy scores narrowly distributed around about 50%, which is suggestive of only small deviations and a large degree of overlap in color space (Fig. S4 and S12).

Within islands, SVM classifiers correctly assigned individuals to their habitat of origin based solely upon dewlap coloration on five islands: Abaco, Bimini, Cayman Brac, Little Cayman, and Long island (Fig. 2). An LDA approach yielded similar success rates (Fig. S13), suggesting robust differences between these populations. Of the five islands, Little Cayman was the best discriminated with a mean SVM generalization success of 73.4% (Table S9). The results of the classification analyses on PCA data were very similar to results from SVMs and LDAs trained on reflectance values at 50nm-spaced wavelengths from 300 to 700nm (Fig. S14 and S15).

Differentiation in dewlap coloration occurred in multiple dimensions of color space. Moreover, the differences in dewlaps between habitats generally were not consistent among islands, thus, we will discuss the habitat-specific variation in dewlap coloration for each island where significant differences were detected in turn (Fig. 3, Tables 1, S10). Figure 3A provides a key to map principal component scores to the underlying wavelengths.

On Abaco, dewlaps did not differ in PC1, which represents brightness. Mangrove lizards had significantly lower PC2 scores, corresponding to higher ultraviolet reflectance and lower red reflectance. Coastal beach scrub lizards had lower scores on PC3, corresponding to lower ultraviolet reflectance and higher blue reflectance.

On Bimini, coastal beach scrub lizards had significantly brighter dewlaps than lizards from mangroves (PC1), but mangrove lizards had higher PC2 scores than beach scrub lizards, indicating higher violet and blue reflectance, and lower red reflectance. Lizards from primary coppice had higher PC3 scores overall, which correlated very positively with ultraviolet reflectance.

On Cayman Brac, coppice-lizard dewlaps were significantly less bright than lizards from the other habitats. Coastal beach scrub lizards had dewlaps that scored low on PC2, corresponding to lower violet-blue and more red, while the mangrove lizards exhibited the opposite: relatively higher levels of violet-blue and less red. In PC3 space we found that dewlaps from lizards in the coastal habitat had high ultraviolet reflectance, coppice lizards had intermediate levels, and mangrove lizards had relatively low levels.

On Little Cayman, the dewlaps of coppice lizards were significantly darker (PC1) than coastal-lizards. Mangrove lizards had less ultraviolet and redder dewlaps (PC2). The dewlaps of the coastal beach scrub lizards had higher levels of red and ultraviolet reflectance and less blue reflectance than the dewlaps of the other habitat-populations (PC3).

On Long Island, lizards from the coppice habitat had darker dewlaps than lizards from the other habitats (PC1). Coastal lizards had relatively more ultraviolet and less blue-green reflectance in their dewlaps (PC3). These coastal-habitat lizards also scored lower on PC4, corresponding to slightly more violet and green-yellow dewlaps, and less blue dewlaps, than the mangrove lizards on the island.

Sensitivity analyses on classifiers suggested an overall higher relative importance for PC2 and

366 PC3 in determining between-group differences on Abaco, both in SVM and LDA classifiers (Fig.
367 S16, S17), consistent with our ANOVA results (Fig. 3B). There was no strong signal of differences
368 in relative importance among principal components on the other islands. Sensitivity analyses of
369 SVMs trained on reflectance scores rather than principal components revealed, however, a consist-
370 ently higher importance of ultraviolet reflectance in between-group differences on all islands (Fig.
371 S18). This pattern was not recovered for LDAs trained on reflectance scores (Fig. S19).

372

373 We did not find significant spatial autocorrelation between the sampling sites on the islands
374 where we detected a significant habitat effect. We did, however, detect a significant positive sig-
375 nal of autocorrelation on Eleuthera (Table S11), suggesting possible color differentiation through
376 isolation-by-distance on this island.

377

378 In contrast, differences in dewlap coloration between habitats were often detected in close geo-
379 graphical proximity. Such differences were detected on Bimini, Cayman Brac, and Little Cayman
380 which were among the smallest islands in our study (Fig. S1). Indeed, most significant differences
381 in dewlap coloration involved sites that were 5-10km apart. Our most extreme case of significant
382 differences occurred for PC3 between a beach scrub site and a coppice site, separated from each
383 other by a few hundreds of meters at most on Bimini (multiple pairwise Wilcoxon-Mann-Whitney
384 tests, Fig. S20).

385

386 Patterns of differentiation were inconsistent across the five most significant islands. Contrasts
387 in principal components between habitats, calculated on pooled data from the whole archipelago,
388 were not similar, for any component, among islands (Fig. S21; MANOVA, Pillai's trace = 0.354,
389 $F(6, 32) = 1$, $P = 0.36$). No pattern of variation was shared by all five significant islands, along
390 any dimension. Some patterns did seem more common however, such as darker dewlaps among
391 coppice lizards (Cayman Brac, Little Cayman, and Long Island, Fig. 3) or the intermediate posi-
392 tion of coppice lizards in chromatic color space (Cayman Brac and Long Island). In other cases,
393 patterns of differentiation were reversed from one island to another, with more ultraviolet reflecting
394 dewlaps in mangroves than in coastal habitat on Abaco and Cayman Brac, but the opposite on
395 Little Cayman and Long Island. Overall, it seemed that patterns of heterogeneity of variance were
396 often driven by higher variances in coloration within beach scrub lizards (Fig. 3, Table 1). Yet
397 other patterns were idiosyncratic, such as the combination of higher red and ultraviolet reflectance
398 in coastal lizards on Little Cayman, where the rule seemed to be a negative correlation between
399 ultraviolet and red reflectance across every other island.

400

401 Discussion

402 **Dewlap coloration differs between habitats, but the differentiation is inconsistent with
403 adaptive convergence** Dewlaps are highly conspicuous in anoles and used as communication
404 signals in courtship (Sigmund, 1983; Driessens et al., 2014, 2015) and territorial displays (Losos,
405 1985; Macedonia and Stamps, 1994; Macedonia et al., 2013). On five of the nine islands we sampled
406 in the West Indies, we found that *A. sagrei* male dewlap coloration significantly varied between
407 individuals living in three different habitat types (beach scrub bush, primary coppice forest and
408 mangrove forest), even though those habitats were in close proximity to each other and the *sagrei*
409 populations are continuously distributed between the habitats.

410

411 One compelling indicator of adaptation by natural selection is the repetition of patterns of
412 differentiation across localities (Losos, 2009, 2011). In the context of this study, had we found con-
413 sistent patterns of dewlap differentiation between habitats, we would have concluded that there
414 was likely an adaptive basis for this pattern. However, the inconsistent and idiosyncratic patterns
415 of dewlap variation we observed contradict this prediction. What then explains the observed dif-
416 ferences among the populations?

417

418 One explanation is that this pattern is the result of multiple island colonizations of phenotypically
419 different individuals to these habitats. However, we reject this possibility because all of the
420 island populations in this study are strictly monophyletic, reflecting a single colonization event
421 per island (van de Schoot, unpublished thesis; Driessens et al. 2017; Reynolds et al. 2020). Other

drivers could be at work, including genetic drift, phenotypic plasticity, and divergent natural selection. Of these, we find divergent natural selection the most likely explanation, suggesting that future research with additional granularity on the drivers of dewlap coloration is needed. 422
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A role of neutral drift is unlikely Genetic drift may lead to patterns of trait divergence similar to local adaptation (Miles et al., 2019). We think this scenario is implausible, however, as we found significant differences in dewlap color over distances too small for genetic drift to have realistically counteracted the effect of homogenizing gene flow (Richardson et al., 2014). On Bimini, for example, we detected differences between sites only a few hundred meters away and *A. sagrei*, have previously been shown to be highly mobile (Kamath and Losos, 2018). Moreover, habitat-populations within each island were found to be non-monophyletic and often share identical haplotypes, based on phylogenetic analysis of mitochondrial DNA (van de Schoot et al. unpublished thesis). This is further evidence of gene flow between habitats. 425
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It is possible that complex drift scenarios other than simple distance-mediated, diffusion-like processes may be at play (Miles et al., 2019). For example, we did detect a significant signal of isolation-by-distance on Eleuthera, without finding differences in dewlap coloration between habitats, suggesting a possible role of drift on this island. Nonetheless, by and large our results align with previously documented cases of highly localized dewlap color divergence despite gene flow in multiple species of anoles. Ng et al. (2012) and Ng et al. (2016) found divergent dewlap coloration despite gene flow between subspecies of *A. distichus* on Hispaniola, and proposed this as a mechanism of reproductive isolation in the early stages of speciation (Ng and Glor, 2011; Lambert et al., 2013; Ng et al., 2017). Stapley et al. (2011) found that dewlap color polymorphism was maintained in the absence of genetic structure between populations of *A. apletophallus* from Panama. Thorpe and Stenson (2002) found that divergence in dewlap coloration matched habitat-type better than mitochondrial lineage in *A. roquet* on Martinique, and a convergent pattern was found in *A. trinitatis* on the featureless island of St Vincent (Thorpe, 2002). Finally, regionally-distinct body coloration, but not dewlap coloration, is present in *A. conspersus* on another small island, Grand Cayman, where no physical barriers to gene flow exist (Macedonia, 2001). 435
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A role of phenotypic plasticity is unlikely Differences in coloration between habitat populations may not be genetically determined and instead may be influenced by environmental factors. The yellow, orange and red coloration in anoline dewlaps are produced by pterins and carotenoids (Ortiz, 1962; Ortiz et al., 1962; Ortiz and Williams-Ashman, 1963; Ortiz and Maldonado, 1966; Macedonia et al., 2000; Steffen and McGraw, 2007, 2009). Animals lack the ability to synthesize carotenoids, and those must therefore be found in the diet, while pterins are synthesized from nucleotides (Goodwin, 1984; Hill et al., 2002; Hill and McGraw, 2006). However, experimental manipulation of dietary carotenoid content showed no effect on dewlap coloration in *A. sagrei* (Steffen et al., 2010) nor in *A. distichus* (Ng et al., 2013), which also has an orange-based dewlap. (Dewlap color has also been shown to change in relation to parasite load (Cook et al., 2013), though parasites were not investigated in this study. – to remove) These laboratory results suggest a plastic response in dewlap color to differences in diet across habitats unlikely, suggesting the differences we observed could therefore be genetically based. This hypothesis is further supported by Cox et al. (2017), who found a high degree of heritability of dewlap coloration in *A. sagrei*. 450
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Sensory Drive - maybe drop? One hypothesis that a relationship would exist between dewlap color and habitat stems from the idea that the communication signals evolve adaptively in response to light environment (the sensory drive hypothesis, Endler and McLellan 1988; Endler 1992, 1998). However, we find this explanation unlikely because the differences we observed were both inconsistent among islands and inconsistent with predictions of the sensory drive hypothesis. Previous studies have proposed that dewlap coloration may have evolved to be maximally detectable under local light conditions, primarily through UV contrast (i.e. UV-brighter dewlaps in UV-dark, mesic habitats and UV-darker dewlaps in UV-bright, xeric habitats), in *A. cristatellus* and *A. cooki* from Puerto Rico (Leal and Fleishman, 2002, 2004). On the contrary, we found no apparent habitat-dependent maximization of UV-contrast, or just any contrast in *A. sagrei*. Instead, we found for example the darkest dewlaps in the dark, mesic habitat – primary coppice forest – on three islands, and dewlaps often differed the most between beach scrub and mangrove forest, two xeric habitats with similar, high irradiance levels (Howard, 1950; Schoener, 1968). Studies of 464
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477 Jamaican and Hispaniolan anoles similarly found between-habitat differences in dewlap coloration
478 but no evidence for higher dewlap detectability in different habitats (Fleishman et al., 2009; Ng
479 et al., 2012). Our data are consistent with those previous results in suggesting that adaptation to
480 local light conditions, or at least broad habitat types, is not a major driver of the within-island
481 variation in dewlap coloration in *A. sagrei*.

482 **Fisherian sexual selection - maybe drop?** Selection, however, needs not necessarily be linked
483 to habitat type, and may take the form of arbitrary, "Fisherian" sexual selection, where female
484 preferences differ between localities for reasons unrelated to the environment, driving the divergent
485 evolution of male ornaments (Lande, 1981; Andersson, 1994; Higashi et al., 1999). This process
486 could account for the idiosyncratic patterns of within-island divergence we report, where initial
487 differences in female preferences could have arisen for nonselective reasons (e.g. plasticity or random
488 drift). Substantial levels of promiscuity in *A. sagrei* suggest ample opportunity for female mate
489 choice (Kamath and Losos, 2018), and are in line with this scenario. However, (Baeckens et al.,
490 2018) found no link between *A. sagrei* dewlap coloration and size dimorphism (a proxy for sexual
491 selection) in an among-island study of the same archipelagos.

492 **Natural selection may proceed differently across islands and habitats** A further po-
493 tential explanation for the inconsistencies in dewlap coloration among habitats between islands
494 is that the observed differences in dewlap color are the result of divergent natural selection. If
495 that is the case, however, either the habitats we have identified are not as similar as we thought,
496 such that the same habitat type on different islands favors different colors, or, alternatively, the
497 selective pressures causing dewlap divergence are related to some environmental factor other than
498 habitat type that we did not measure. The habitats on different islands may, for example, differ
499 in densities of predators or congeners, which have been shown to affect among-island dewlap di-
500 versity Vanhooydonck et al. (2009); Baeckens et al. (2018). In particular, Baeckens et al. (2018)
501 recently showed that dewlaps with spotted patterns occurred more often in *A. sagrei* on islands
502 with more coexisting species of anoles. Therefore, if local adaptation occurs, it may be more likely
503 to involve components of the environment that do not encompass our broad habitat categories
504 leading to island-specific differences between habitats rather than parallel differences expected if
505 habitat identity was the primary selective pressure driving divergence.

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507 One last possibility to explain idiosyncratic patterns of between-habitat differences we observe
508 is that, if island populations are different in color on average, they may be experiencing differ-
509 ent portions of a within-island fitness landscape whose shape changes depending on the starting
510 phenotype. In this study, we indeed found that dewlap coloration differed much more between
511 than within islands. So, a population may not respond to selection in the same manner depend-
512 ing on its starting conditions. This is true not only in terms of phenotype (average dewlap color
513 differs among islands) but also genetic composition (e.g. independent colonizations may have im-
514 portant genetic founder effects, Reynolds et al. 2020), and so the selective pressures applying to
515 populations with different starting phenotypes may drive these divergent outcomes. For example,
516 Fleishman et al. (2020) found that yellow stimuli are less detectable than red stimuli in high-light
517 environments while both colors are readily detectable in low-light environments. Consequently, the
518 environmental pressures applying to yellower dewlaps may differ than those for redder dewlaps, as
519 red dewlaps should be always equally or more detectable than yellow dewlaps across light environ-
520 ments, whereas yellow dewlaps could experience more directional selection towards more red when
521 in a high-light environments.

522 **Conclusion** We identified patterns of dewlap color differentiation in populations of *A. sagrei*
523 from different habitat-types on multiple small islands of the West Indies. However, our main
524 hypothesis to explain our findings – local adaptation and sensory drive – received little support from
525 our multiple-island dataset. We also found other mechanisms such as drift or plasticity unlikely
526 candidates to explain the observed patterns. This suggests that combinations of multiple factors
527 interacting in subtle ways, and not necessarily in parallel across islands, may have contributed to
528 present-day dewlap color diversity.

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⁵³⁹ **Figures**

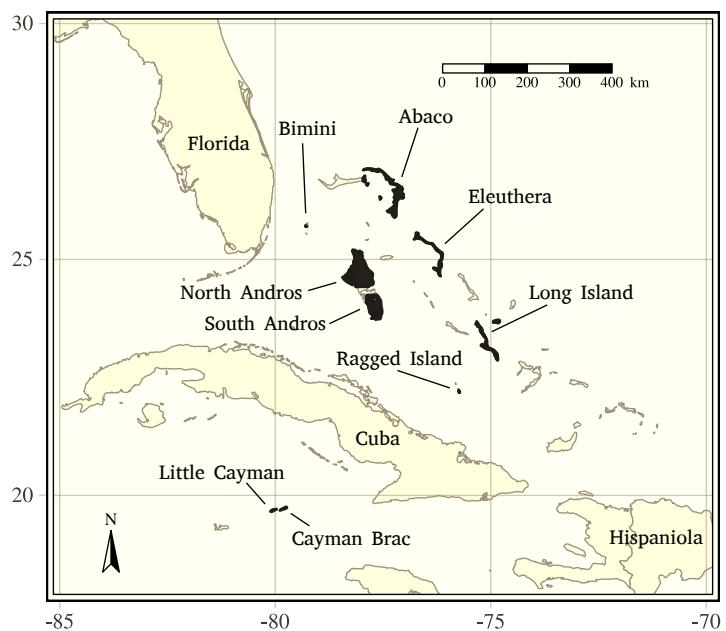


Figure 1: Map of the West Indies with sampled islands highlighted in black.

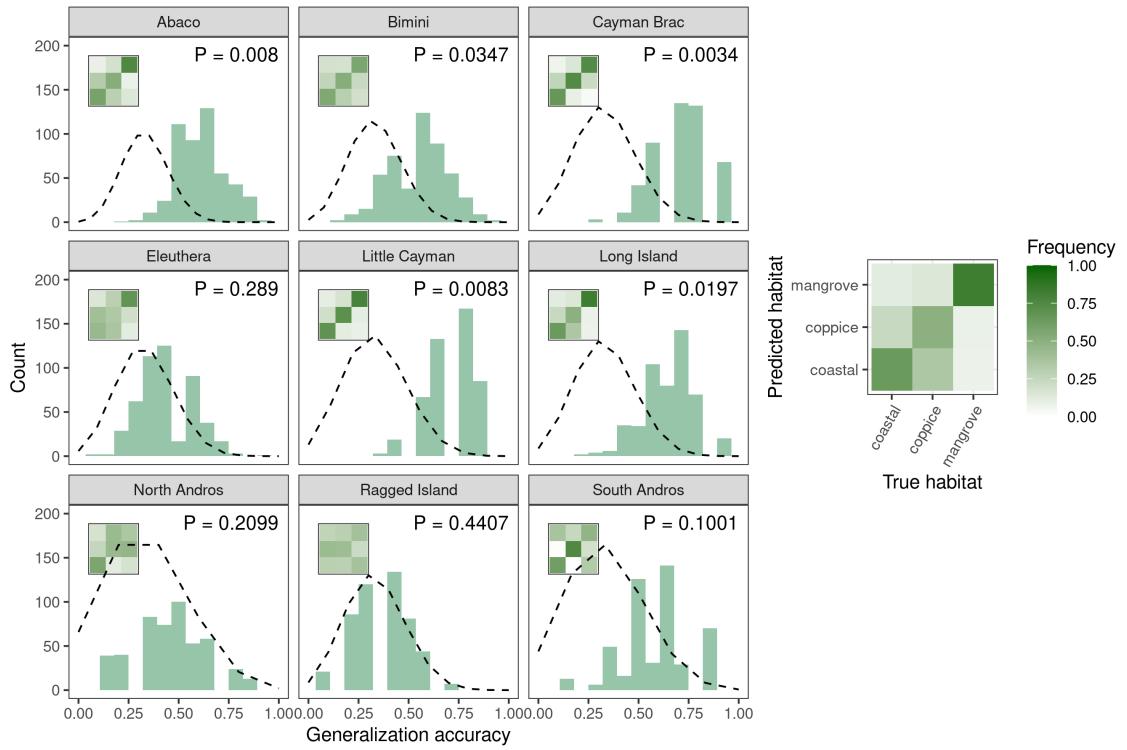


Figure 2: SVM classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The dashed line is the density of a corresponding null binomial distribution, which would be expected under random guessing (testing sets with 20% of the observations for each island and success probability of 1/3). Inset plots show the corresponding average confusion matrices and represent the proportion of lizards from each habitat (columns) reassigned in each other habitat (rows), with an interpretation guide in the right panel. Binomial test P-values indicate deviations of the mean classification accuracy to the null distribution. *, $P < 0.05$.

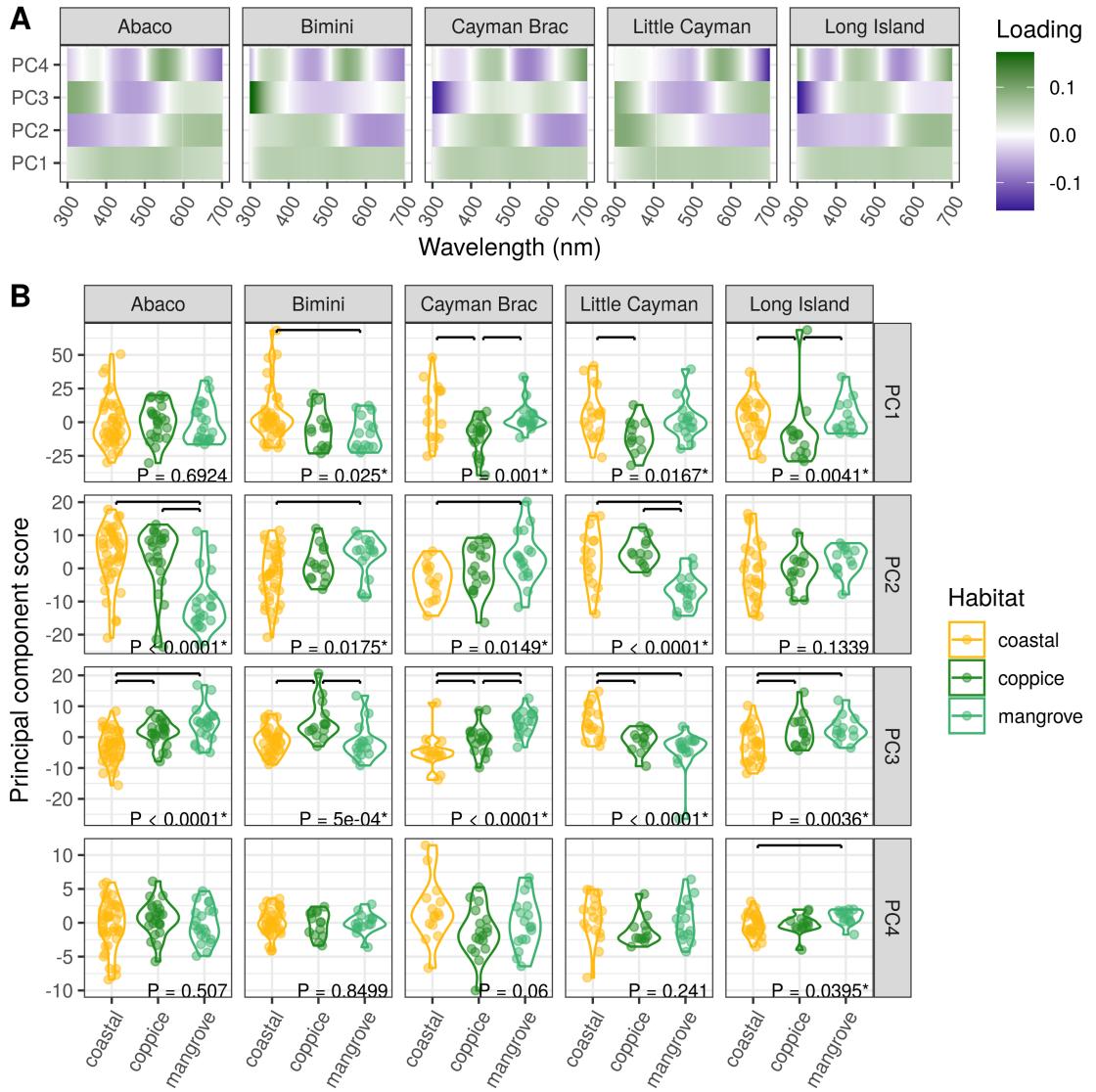


Figure 3: Dewlap color variation between habitat-types across the most significant islands. (A) Mapping of reflectance at various wavelengths onto the principal components (loadings from the PCA rotation matrix). (B) Distribution of PC scores between habitats along the first four PCs on each island where significant between-habitat differences were detected using SVMs. P-values are reported for univariate ANOVA (or Kruskal-Wallis tests when applicable, see Methods). Post hoc significant differences at a 0.05 error rate are indicated with horizontal bars. *, $P < 0.05$.

Tables

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Table 1: Significance of habitat differences in dewlap coloration, using ANOVA for all islands where significant multivariate differences in dewlap coloration were detected by SVMs. Best fitting model: 1, OLS; 2, GLS. df, degrees of freedom. ΔAICc , difference in AICc between the best fitting model and the OLS-model. AICcw, AICc weight. LRT, likelihood ratio test. Log-lik., log-likelihood. χ^2 , likelihood ratio. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Island	Variable	Best fit	df	AICc	ΔAICc	AICcw	ΔAICc	dfLRT	Log-lik.	χ^2	P
Abaco	PC1	1	4	710.4	0.0	0.746	2	-357.0	0.14	0.9308	***
Abaco	PC2	1	4	620.1	0.0	0.882	2	-310.2	31.74	0.0000	***
Abaco	PC3	1	4	517.8	0.0	0.732	2	-257.2	27.37	0.0000	***
Abaco	PC4	1	4	440.6	0.0	0.596	2	-217.2	1.36	0.5070	*
Bimini	PC1	1	4	561.3	0.0	0.595	2	-283.1	7.40	0.0248	*
Bimini	PC2	1	4	448.1	0.0	0.656	2	-223.8	8.09	0.0175	*
Bimini	PC3	2	6	405.3	-0.2	0.529	2	-199.2	10.39	0.0056	**
Bimini	PC4	1	4	274.2	0.0	0.854	2	-132.7	0.33	0.8499	***
Cayman Brac	PC1	2	6	402.8	-4.1	0.884	2	-200.9	13.81	0.0010	**
Cayman Brac	PC2	1	4	332.1	0.0	0.853	2	-165.9	8.41	0.0149	*
Cayman Brac	PC3	1	4	295.8	0.0	0.800	2	-146.6	27.16	0.0000	***
Cayman Brac	PC4	1	4	279.2	0.0	0.897	2	-137.8	5.63	0.0600	*
Little Cayman	PC1	1	4	367.2	0.0	0.777	2	-186.0	8.18	0.0167	*
Little Cayman	PC2	2	6	287.6	-3.6	0.859	2	-140.5	29.76	0.0000	***
Little Cayman	PC3	1	4	277.7	0.0	0.669	2	-138.1	21.34	0.0000	***
Little Cayman	PC4	1	4	226.7	0.0	0.780	2	-110.7	2.85	0.2410	
Long Island	PC1	2	6	442.3	-2.1	0.740	2	-221.2	2.91	0.2331	
Long Island	PC2	2	6	351.4	-3.1	0.823	2	-172.6	4.52	0.1043	
Long Island	PC3	1	4	322.1	0.0	0.862	2	-160.0	11.24	0.0036	**
Long Island	PC4	1	4	195.5	0.0	0.767	2	-92.9	6.46	0.0395	*

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Supplementary Figures

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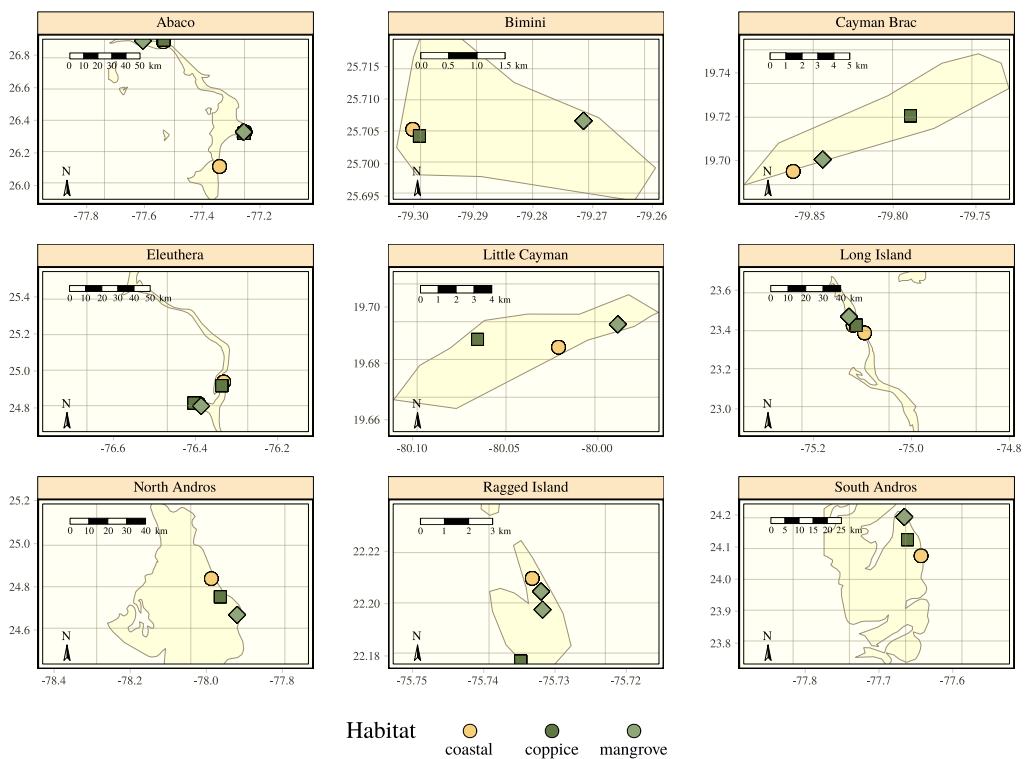


Figure S1: Map of the sampling sites and corresponding habitats across nine islands of the West Indies.

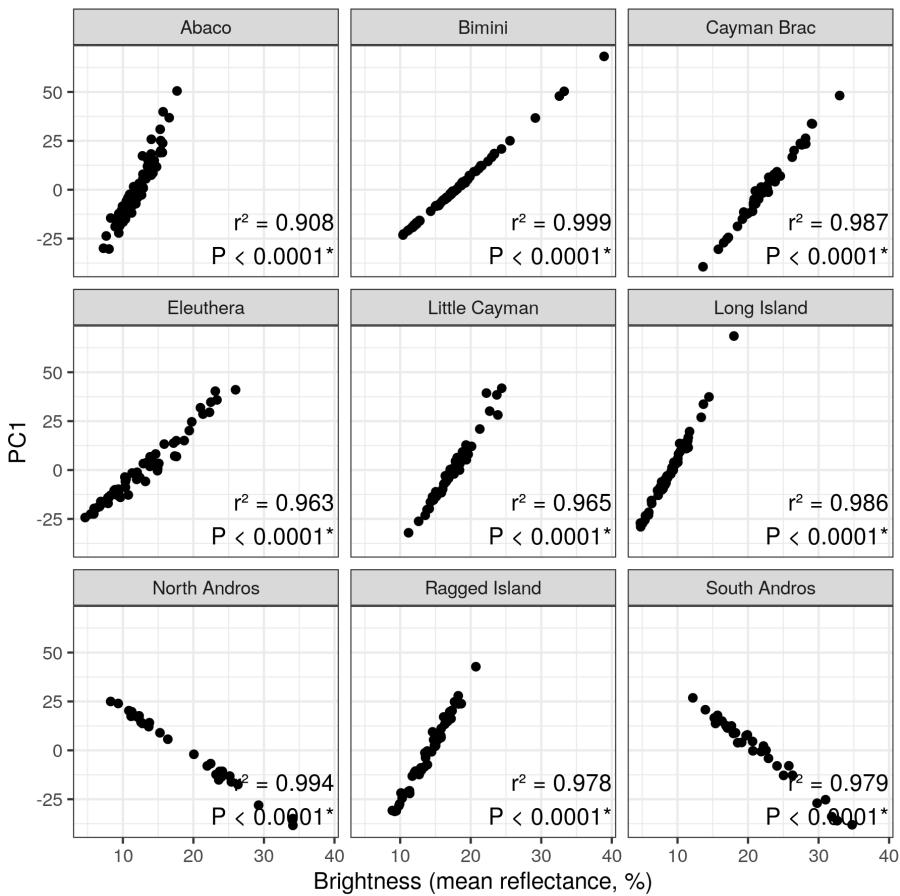


Figure S2: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score for each island. Pearson's squared correlation coefficients are reported. *, $P < 0.05$.

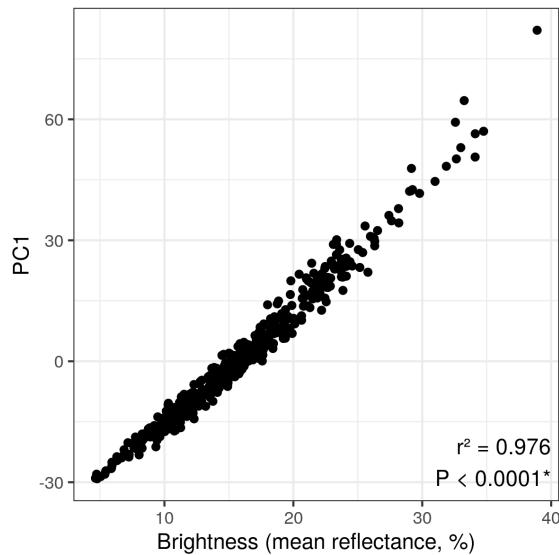


Figure S3: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across the whole archipelago. Pearson's squared correlation coefficient is reported. *, $P < 0.05$.

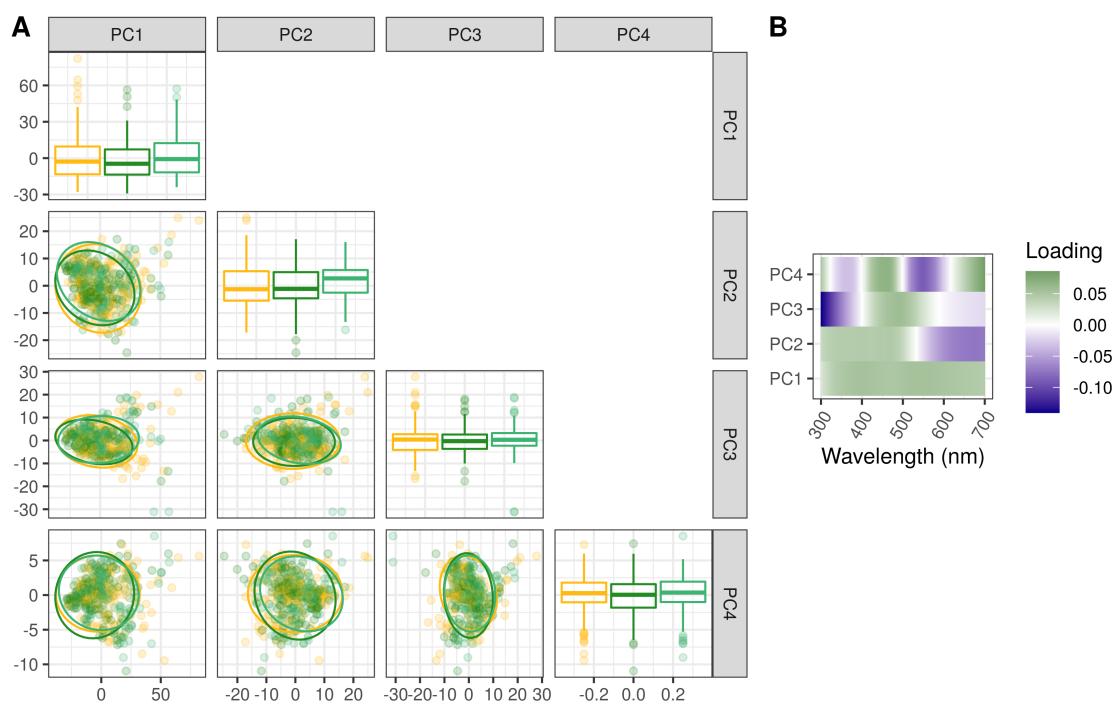


Figure S4: (A) Principal component scores and 5% confidence ellipses across habitats for the whole archipelago. The principal component analysis was performed on reflectance data from all islands pooled together. (B) PCA rotation matrix showing the loadings of each wavelength from 300 to 700nm onto the principal components.

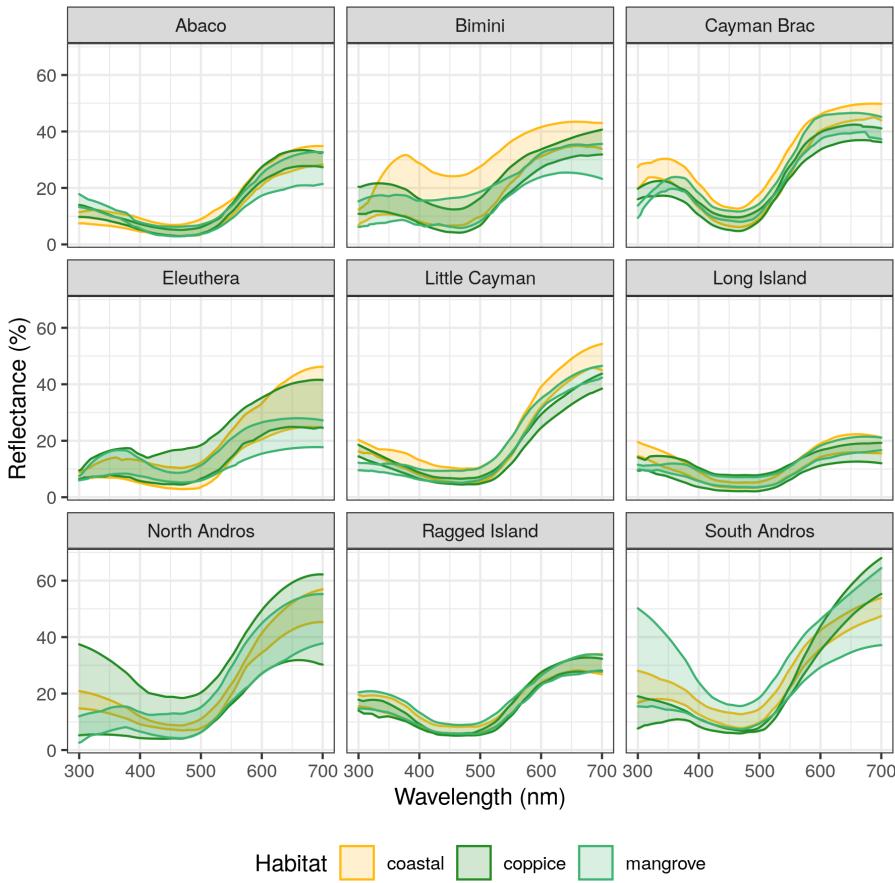


Figure S5: 5-95th percentile range of lizard dewlap reflectance values (in % of incoming light) across wavelengths for each island and each habitat.

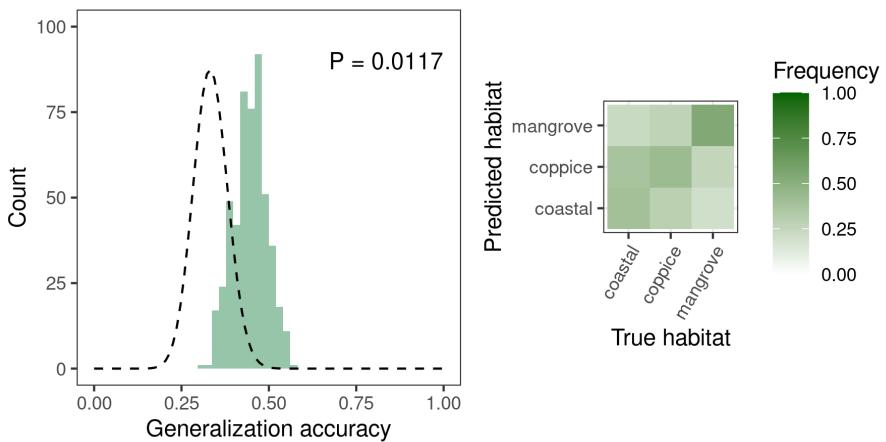


Figure S6: Archipelago-wide SVM classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 2.

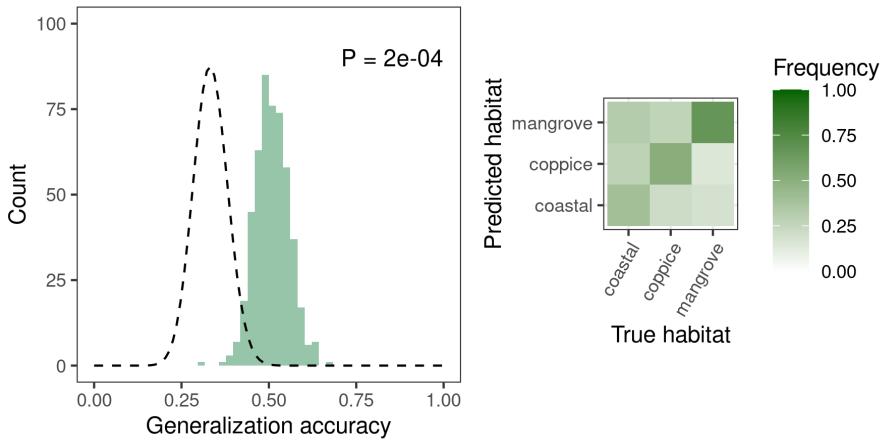


Figure S7: Archipelago-wide SVM classification accuracy based on reflectance data at 50nm-intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 2.

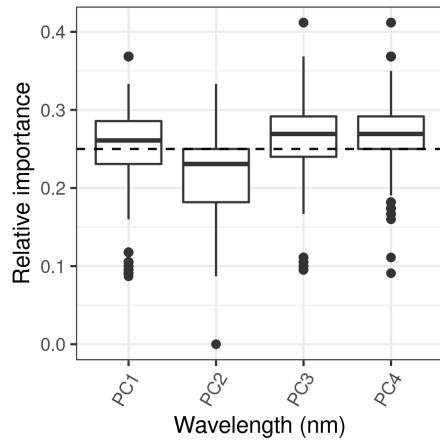


Figure S8: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on principal component data (Figure S6), with relative importance computed for every machine.

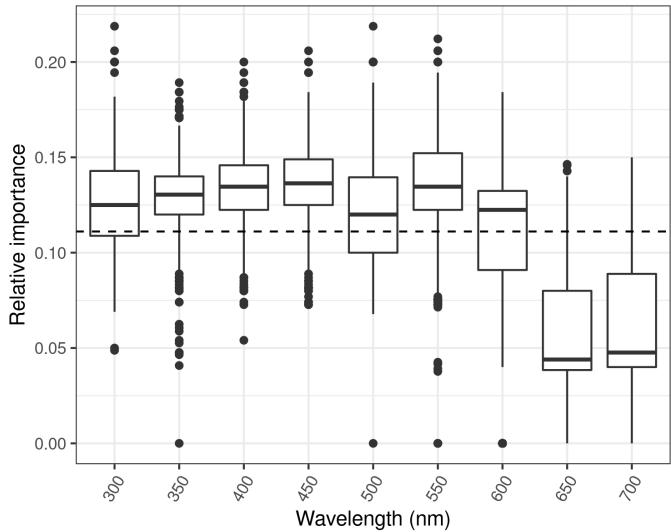


Figure S9: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance data at 50nm-intervals in wavelength (Figure S7), with relative importance computed for every machine.

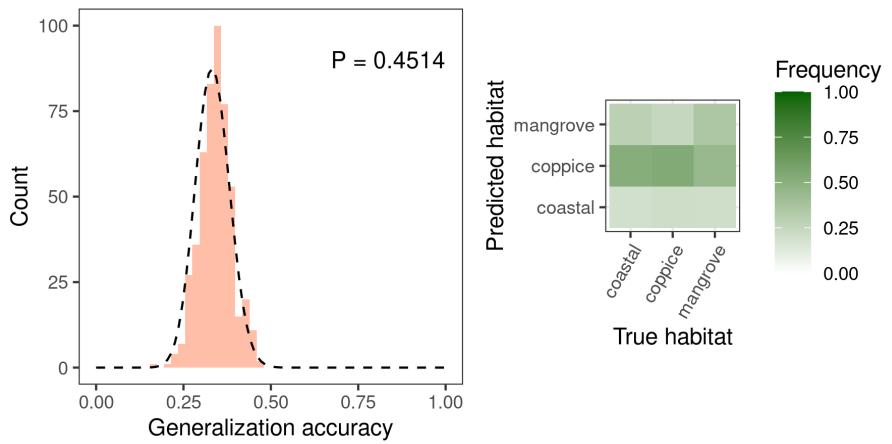


Figure S10: Archipelago-wide LDA classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 2.

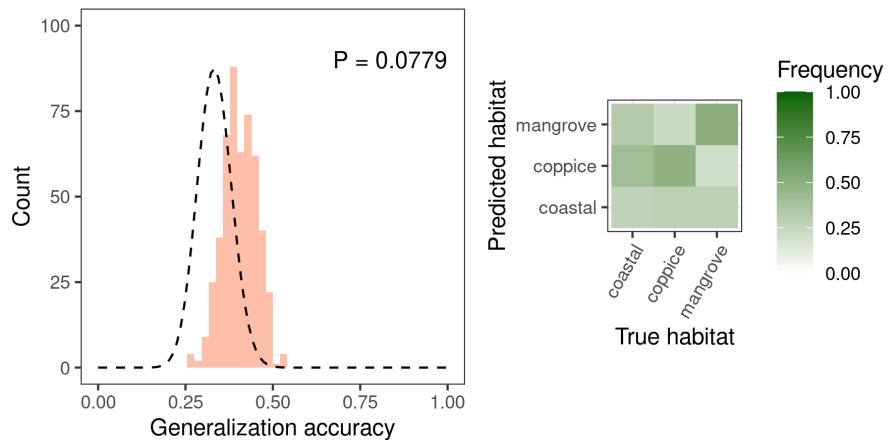


Figure S11: Archipelago-wide LDA classification accuracy based on reflectance data at 50nm-intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 2.

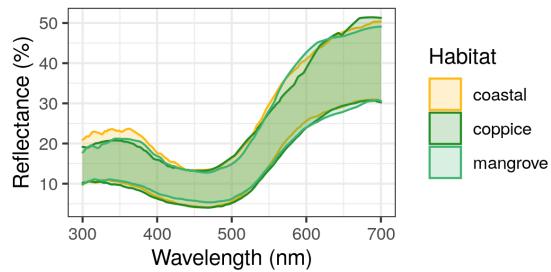


Figure S12: 5-95th percentile range of lizard dewlap reflectance values (in % of incoming light) across wavelengths for each habitat throughout the whole archipelago.

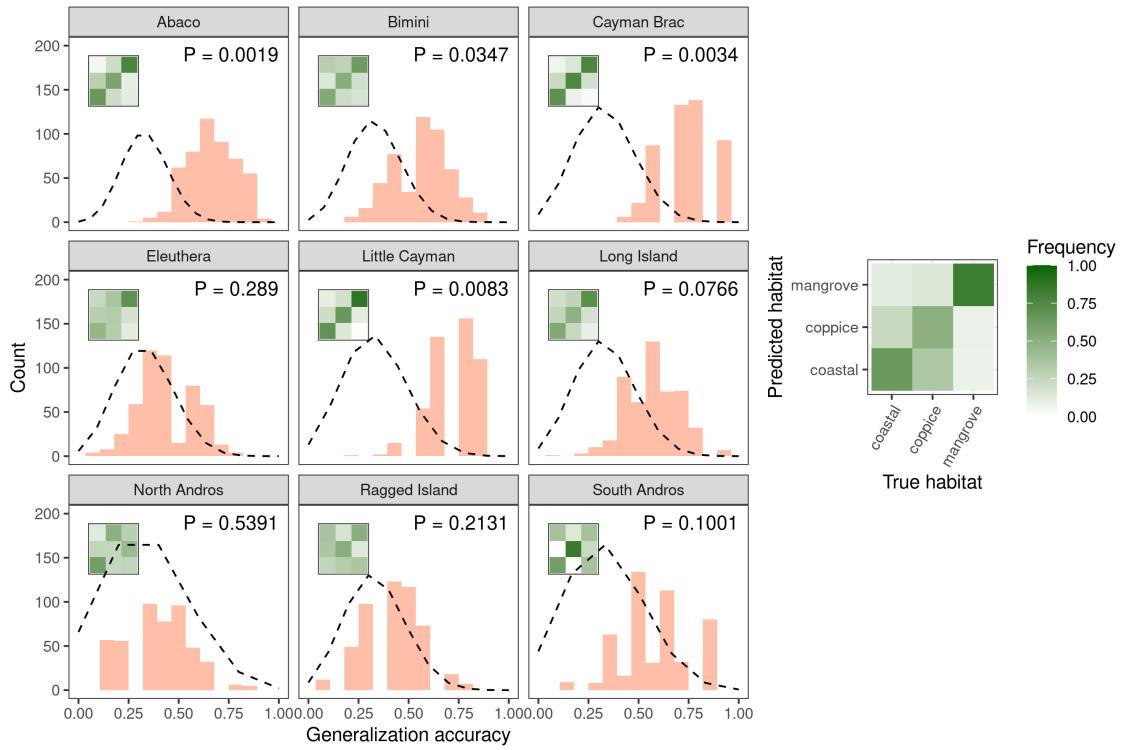


Figure S13: LDA classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

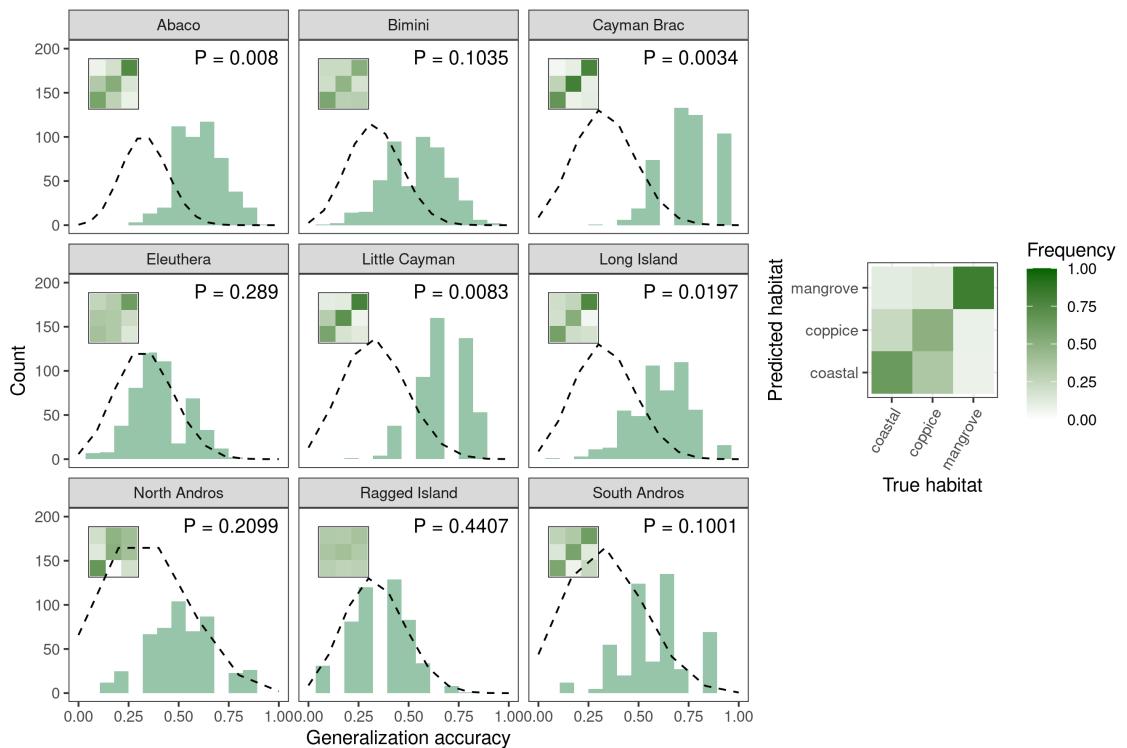


Figure S14: SVM classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

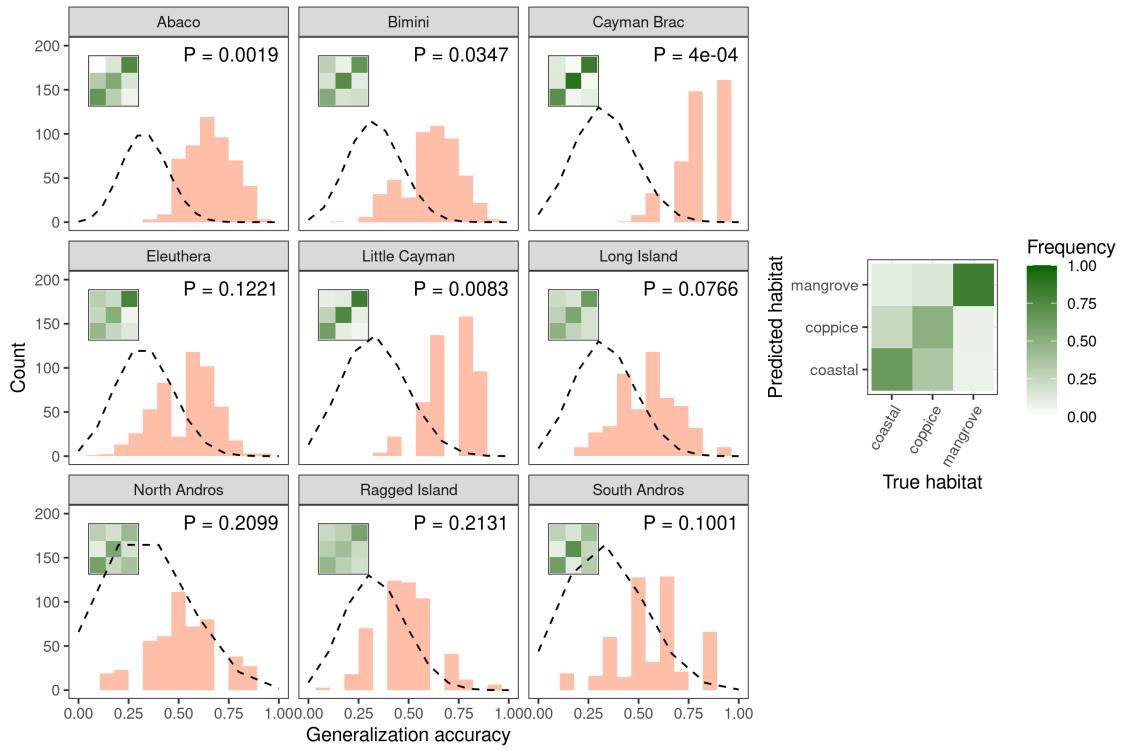


Figure S15: LDA classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

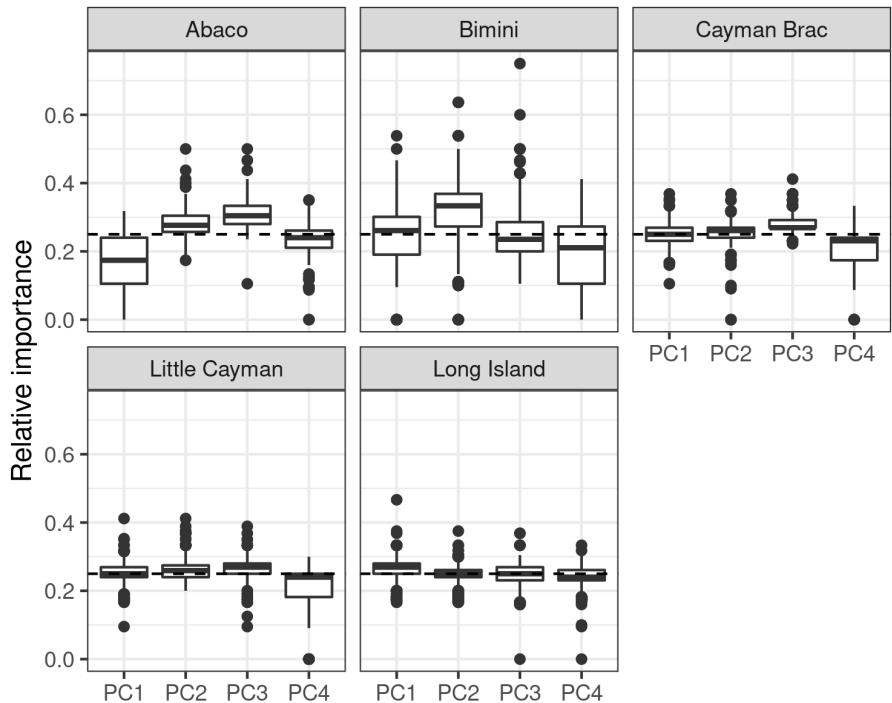


Figure S16: Sensitivity analyses of the different input variables in the within-island SVM classification on principal component data (Figure ??), with relative importance computed for every machine.

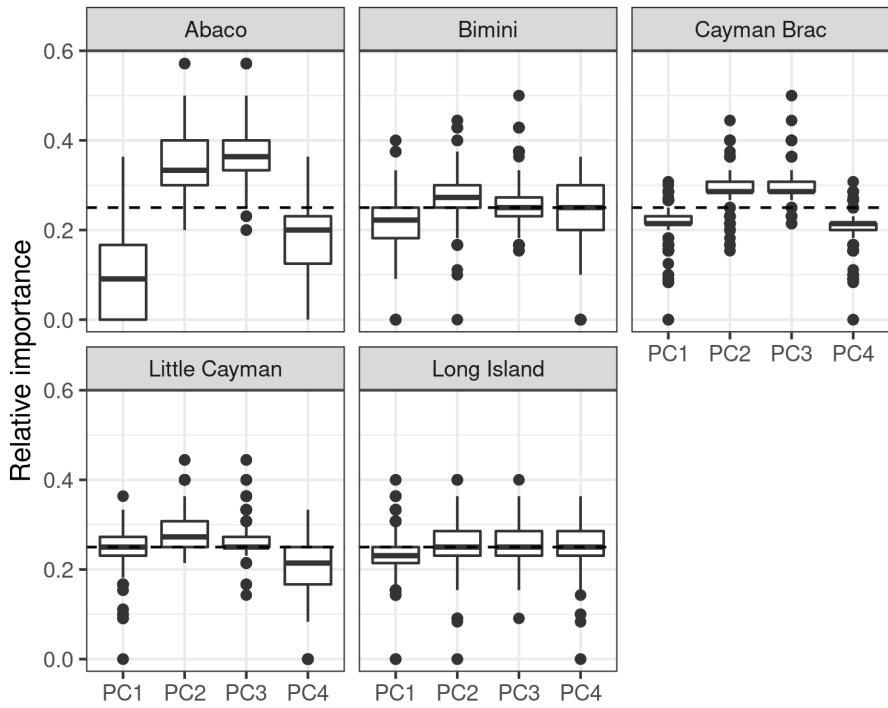


Figure S17: Sensitivity analyses of the different input variables in the within-island LDA classification on principal component data (Figure S13), with relative importance computed for every machine.

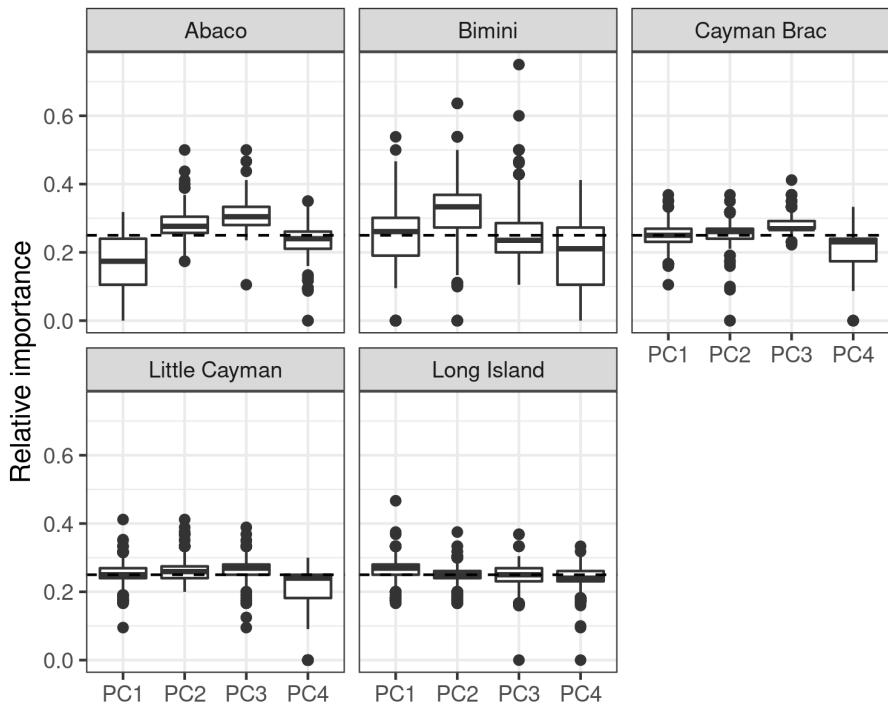


Figure S18: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance at 50nm-intervals in wavelength (Figure S14), with relative importance computed for every machine.

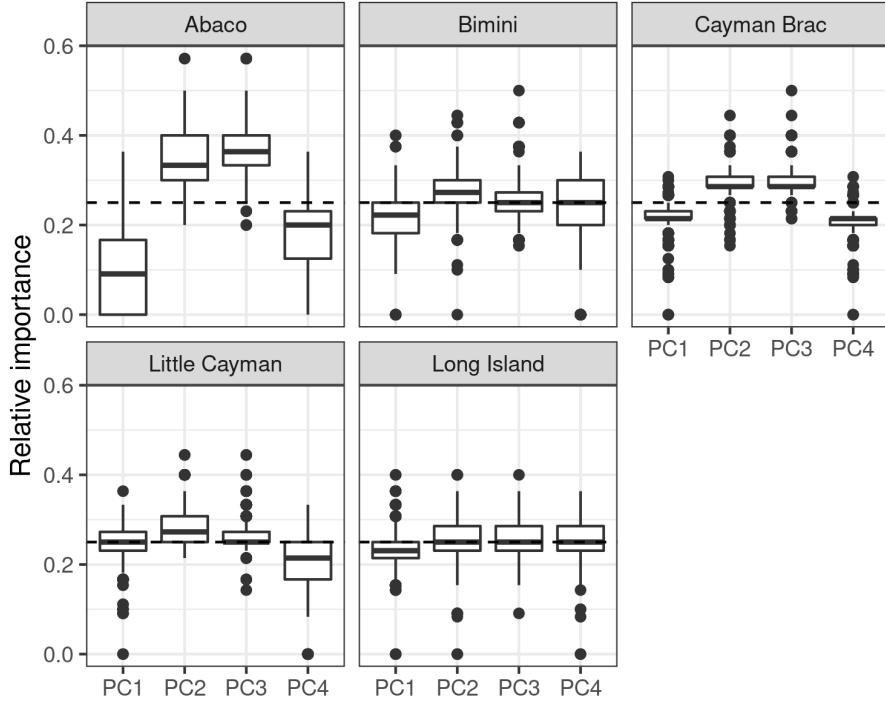


Figure S19: Sensitivity analyses of the different input variables in the archipelago-wide LDA classification on reflectance at 50nm-intervals in wavelength (Figure S15), with relative importance computed for every machine.

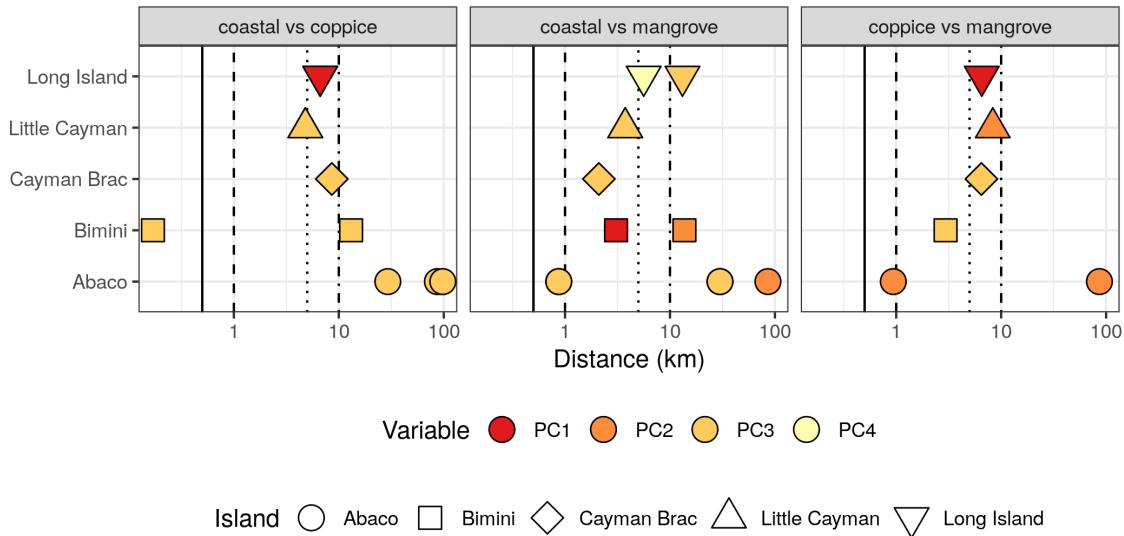


Figure S20: Spatial scale of between-habitat differences in dewlap coloration. For each variable and each pair of habitats where significant differences were detected (Figure 3), we performed multiple post hoc pairwise comparisons between the sites involved (Figure S1, Table S2), using nonparametric Wilcoxon-Mann-Whitney tests. Here we report, for each pair of habitats, the distances between sites that significantly differed in dewlap coloration at an error rate of 0.05 (P-values corrected with the Benjamini-Hochberg procedure for multiple testing).

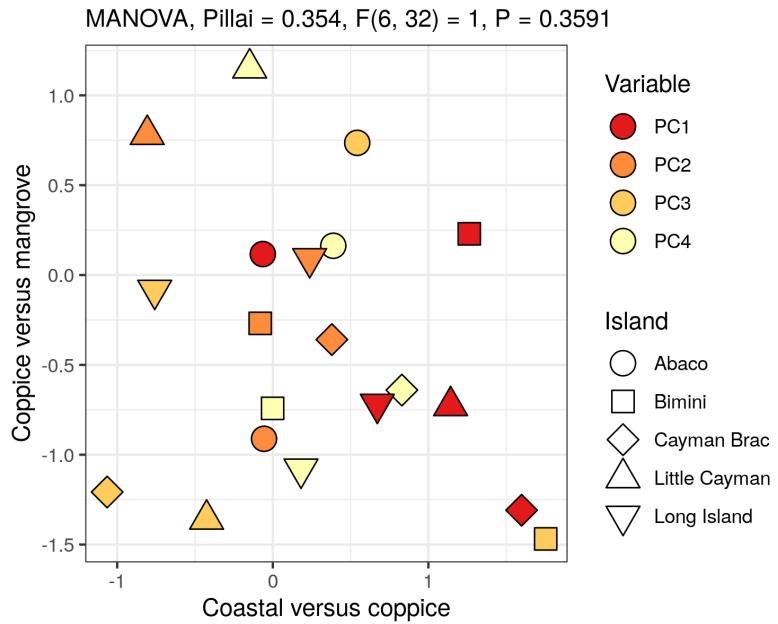


Figure S21: Test of parallel divergence between islands. Differences in habitat-means, or contrasts, are shown for two pairs of habitats for each principal component on each island, rescaled so the standard deviation of the means along each principal component is one. The contrasts represent the patterns of between-habitat variation on each island, for a given principal component. The absence of clustering of islands by variable indicates that islands differ in their between-habitat divergence patterns. This is confirmed by a non-significant MANOVA test of the between versus within-variable variance in contrasts.

Supplementary Tables

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

Island	Longitude	Latitude	Habitat	PC1	PC2	PC3	PC4
Abaco	-77.7256	26.9083	mangrove	-5.4905	1.3541	-0.4741	0.0083
Abaco	-77.5800	26.9020	coastal	1.8633	0.0365	-0.4475	0.0033
Abaco	-77.5763	26.9128	coppice	-1.6738	-1.7793	-0.0499	0.0012
Abaco	-77.1784	26.1045	coastal	1.1863	2.0408	-0.3468	0.0022
Abaco	-77.0055	26.3254	mangrove	-9.0319	-2.7460	0.4687	0.0077
Abaco	-77.0039	26.3170	coppice	0.9967	0.5161	-0.0267	-0.0118
Abaco	-76.9968	26.3260	coastal	7.6077	0.3186	0.1771	-0.0008
Bimini	-79.3022	25.5859	coastal	5.7537	-0.1593	-0.2505	0.0001
Bimini	-79.3014	25.7052	coastal	-3.1822	1.6617	-0.0460	0.0024
Bimini	-79.3002	25.7042	coppice	-1.3514	-3.8786	0.1027	-0.0027
Bimini	-79.2709	25.7066	mangrove	3.3656	0.6244	0.1569	-0.0021
Cayman Brac	-79.8627	19.6878	coastal	6.6606	-2.5670	0.0166	-0.0007
Cayman Brac	-79.8441	19.6949	mangrove	-1.0914	4.3607	0.0855	0.0001
Cayman Brac	-79.7887	19.7209	coppice	-4.5197	-1.9793	-0.0946	0.0004
Eleuthera	-76.3347	24.8146	coppice	3.2669	-1.2404	0.1018	-0.0085
Eleuthera	-76.3058	24.8127	coastal	0.4216	-3.5133	-0.0567	0.0009
Eleuthera	-76.2901	24.7981	mangrove	2.1881	0.7517	0.3957	-0.0055
Eleuthera	-76.1616	24.9129	coppice	-1.9136	1.0868	-0.4978	-0.0092
Eleuthera	-76.1492	24.9335	coastal	-3.1863	2.4270	0.1881	0.0218
Little Cayman	-80.0660	19.6906	coppice	0.8021	-1.9569	-0.0760	-0.0068
Little Cayman	-80.0205	19.6865	coastal	-6.6917	-1.2615	0.0659	0.0057
Little Cayman	-79.9871	19.6986	mangrove	6.5083	2.8079	-0.0129	-0.0010
Long Island	-75.2299	23.4740	mangrove	-1.2873	1.9371	-0.1880	-0.0029
Long Island	-75.2063	23.4282	coastal	2.3686	-0.9033	0.0215	0.0096
Long Island	-75.1884	23.4292	coppice	-4.6266	0.5060	0.1049	-0.0070
Long Island	-75.1408	23.3883	coastal	3.6139	-1.4521	0.0475	0.0025
North Andros	-77.8908	24.8391	coastal	-2.1881	-1.1236	0.0397	-0.0060
North Andros	-77.8428	24.7516	coppice	-1.8115	0.0012	-0.1678	0.0024
North Andros	-77.7540	24.6644	mangrove	3.5997	1.0101	0.1153	0.0033
Ragged Island	-75.7364	22.1768	coppice	3.2851	-0.3274	0.1911	-0.0013
Ragged Island	-75.7314	22.2097	coastal	-0.6412	-0.8878	-0.1293	-0.0033
Ragged Island	-75.7276	22.2045	mangrove	-2.9188	1.5792	-0.0034	0.0099
Ragged Island	-75.7270	22.1973	mangrove	-1.2210	0.7285	-0.0721	-0.0028
South Andros	-77.6050	24.2027	mangrove	-3.9253	0.4734	0.0477	-0.0005
South Andros	-77.5936	24.1289	coppice	6.1152	-0.4925	0.0349	0.0012
South Andros	-77.5453	24.0764	coastal	-0.7933	-0.1248	-0.0887	-0.0004

Table S3: Proportion of variance explained by the first four principal components on each island, as well as across the whole archipelago.

Island	PC1	PC2	PC3	PC4	Total
Abaco	0.400	0.279	0.147	0.079	0.906
Bimini	0.502	0.208	0.160	0.051	0.921
Cayman Brac	0.438	0.190	0.155	0.105	0.888
Eleuthera	0.490	0.233	0.138	0.066	0.926
Little Cayman	0.441	0.212	0.176	0.078	0.907
Long Island	0.515	0.205	0.161	0.043	0.925
North Andros	0.560	0.170	0.152	0.054	0.937
Ragged Island	0.483	0.226	0.127	0.072	0.907
South Andros	0.488	0.247	0.146	0.067	0.948
Archipelago	0.473	0.197	0.164	0.079	0.913

Table S4: Pearson's correlation test between dewlap brightness, as measured by the average reflectance between 300 and 700nm in wavelength, and PC1 scores, for all islands and across the whole archipelago. ***, $P < 0.001$.

Island	r^2	P	
Abaco	0.908	< 0.0001	***
Bimini	0.999	< 0.0001	***
Cayman Brac	0.987	< 0.0001	***
Eleuthera	0.963	< 0.0001	***
Little Cayman	0.965	< 0.0001	***
Long Island	0.986	< 0.0001	***
North Andros	0.994	< 0.0001	***
Ragged Island	0.978	< 0.0001	***
South Andros	0.979	< 0.0001	***
Archipelago	0.976	< 0.0001	***

Table S5: Henze-Zirkler's test of multivariate normality, performed on principal components in each habitat and on each island. HZ, test statistic. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Island	Habitat	HZ	P	
Abaco	coastal	1.10	0.0027	**
Abaco	coppice	1.07	0.0022	**
Abaco	mangrove	1.06	0.0023	**
Bimini	coastal	1.28	0.0001	***
Bimini	coppice	0.85	0.0482	*
Bimini	mangrove	1.19	0.0001	***
Cayman Brac	coastal	0.65	0.5311	
Cayman Brac	coppice	0.70	0.3940	
Cayman Brac	mangrove	0.66	0.5357	
Eleuthera	coastal	1.61	0.0000	***
Eleuthera	coppice	1.48	0.0000	***
Eleuthera	mangrove	0.73	0.1423	
Little Cayman	coastal	0.62	0.6599	
Little Cayman	coppice	0.64	0.4867	
Little Cayman	mangrove	0.87	0.0413	*
Long Island	coastal	0.82	0.1468	
Long Island	coppice	0.92	0.0150	*
Long Island	mangrove	0.77	0.1289	
North Andros	coastal	0.66	0.3174	
North Andros	coppice	0.76	0.0900	
North Andros	mangrove	0.67	0.3185	
Ragged Island	coastal	0.76	0.2268	
Ragged Island	coppice	0.80	0.1115	
Ragged Island	mangrove	0.54	0.9022	
South Andros	coastal	0.66	0.3451	
South Andros	coppice	0.66	0.3154	
South Andros	mangrove	0.91	0.0144	*

Table S6: Box's M-test of homogeneity of covariance matrices across habitats on each island. χ^2 , test statistic. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Island	χ^2	df	P	
Abaco	47.1	20	0.0006	***
Bimini	36.0	20	0.0152	*
Cayman Brac	36.9	20	0.0120	*
Eleuthera	44.6	20	0.0013	**
Little Cayman	32.8	20	0.0356	*
Long Island	56.2	20	0.0000	***
North Andros	33.7	20	0.0283	*
Ragged Island	29.3	20	0.0824	
South Andros	46.5	20	0.0007	***

Table S7: Shapiro-Wilk's test of univariate normality performed on each island where significant differences were detected by SVM classification, in each habitat where deviations from multivariate normality were detected. W , test statistic. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Island	Habitat	Variable	W	P
Abaco	coastal	PC1	0.954	0.0941
Abaco	coastal	PC2	0.927	0.0112 *
Abaco	coastal	PC3	0.973	0.4228
Abaco	coastal	PC4	0.955	0.1027
Abaco	coppice	PC1	0.970	0.6776
Abaco	coppice	PC2	0.816	0.0005 ***
Abaco	coppice	PC3	0.930	0.0976
Abaco	coppice	PC4	0.941	0.1711
Abaco	mangrove	PC1	0.881	0.0155 *
Abaco	mangrove	PC2	0.869	0.0093 **
Abaco	mangrove	PC3	0.986	0.9873
Abaco	mangrove	PC4	0.939	0.2044
Bimini	coastal	PC1	0.821	0.0000 ***
Bimini	coastal	PC2	0.960	0.1854
Bimini	coastal	PC3	0.856	0.0002 ***
Bimini	coastal	PC4	0.945	0.0611
Bimini	coppice	PC1	0.911	0.1648
Bimini	coppice	PC2	0.958	0.6927
Bimini	coppice	PC3	0.953	0.6146
Bimini	coppice	PC4	0.971	0.8953
Bimini	mangrove	PC1	0.884	0.0536
Bimini	mangrove	PC2	0.976	0.9363
Bimini	mangrove	PC3	0.982	0.9805
Bimini	mangrove	PC4	0.975	0.9232
Eleuthera	coastal	PC1	0.909	0.0461 *
Eleuthera	coastal	PC2	0.886	0.0157 *
Eleuthera	coastal	PC3	0.906	0.0390 *
Eleuthera	coastal	PC4	0.962	0.5293
Eleuthera	coppice	PC1	0.922	0.0567
Eleuthera	coppice	PC2	0.954	0.3055
Eleuthera	coppice	PC3	0.781	0.0001 ***
Eleuthera	coppice	PC4	0.901	0.0188 *
Little Cayman	mangrove	PC1	0.907	0.1024
Little Cayman	mangrove	PC2	0.904	0.0924
Little Cayman	mangrove	PC3	0.739	0.0005 ***
Little Cayman	mangrove	PC4	0.973	0.8802
Long Island	coppice	PC1	0.686	0.0003 ***
Long Island	coppice	PC2	0.848	0.0210 *
Long Island	coppice	PC3	0.931	0.3188
Long Island	coppice	PC4	0.904	0.1280
South Andros	mangrove	PC1	0.787	0.0067 **
South Andros	mangrove	PC2	0.861	0.0500 *
South Andros	mangrove	PC3	0.697	0.0008 ***
South Andros	mangrove	PC4	0.950	0.6411

Table S8: Univariate ANOVAs performed on each principal component across the whole archipelago. Legend is the same as for Table 1, except that best fitting models 3 and 4 refer to the mixed effect equivalents to the OLS and GLS model, with island as a random effect (see Methods).

Variable	Best fit	df	AICc	ΔAICc	AICcw	df _{LRT}	Log-lik.	χ^2	P
PC1	3	5	3749.9	-228.3	0.613	2	-1874.7	8.69	0.0130 *
PC2	4	7	3002.2	-162.3	0.976	2	-1496.2	17.76	0.0001 ***
PC3	4	7	2826.3	-175.4	0.968	2	-1407.8	7.03	0.0298 *
PC4	4	7	2015.7	-305.8	0.519	2	-1000.1	0.47	0.7914

Table S9: Mean SVM classification accuracy per island, over all replicates and cross-validation bins. N , number of observations per island; p_{test} , proportion of the data sampled to form the training set; n_{test} , number of observations in the testing set. P-values indicate deviations from the expected null binomial distribution, with n_{test} events per island and random guess success probability 1/3. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

Island	Accuracy	N	p_{test}	n_{test}	P
Abaco	0.612	86	0.2	17	0.0080 **
Bimini	0.547	67	0.2	13	0.0347 *
Cayman Brac	0.721	50	0.2	10	0.0034 **
Eleuthera	0.437	56	0.2	11	0.2890
Little Cayman	0.734	45	0.2	9	0.0083 **
Long Island	0.651	53	0.2	10	0.0197 *
North Andros	0.453	28	0.2	5	0.2099
Ragged Island	0.364	50	0.2	10	0.4407
South Andros	0.600	31	0.2	6	0.1001

Table S10: Results of nonparametric Kruskal-Wallis tests performed on each variable on each island where deviations from normality were detected.

Island	Variable	χ^2	df	P
Abaco	PC1	0.74	2	0.6924
Abaco	PC2	23.13	2	0.0000 ***
Bimini	PC1	7.38	2	0.0250 *
Bimini	PC3	15.17	2	0.0005 ***
Little Cayman	PC3	19.95	2	0.0000 ***
Long Island	PC1	10.98	2	0.0041 **
Long Island	PC2	4.02	2	0.1339

Table S11: Individual-based permutation tests of spatial autocorrelation within islands. P-values were computed from 1,000 permutations of individual site-labels. Pearson's coefficient r measures the correlation between distances in color space and geodesic distances among the sites. N , number of sites. *, $P < 0.05$.

Island	r	P	N
Abaco	-0.213	0.817	7
Bimini	0.044	0.510	4
Cayman Brac	-0.010	0.465	3
Eleuthera	0.816	0.015	5 *
Little Cayman	-0.688	0.684	3
Long Island	-0.189	0.579	4
North Andros	0.730	0.199	3
Ragged Island	0.706	0.114	4
South Andros	-0.852	0.776	3