

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

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## Abstract

Animal signals evolve in an ecological context. Moreover, locally adapting animal sexual signals can be especially important for initiating or reinforcing reproductive isolation during the early stages of speciation. Dewlap color in *Anolis* lizards can be highly variable between populations in relation to both biotic and abiotic adaptive drivers, albeit at relatively large geographical scales. Here, we investigated local adaptation of the dewlap across habitat-types at a small spatial scale, as this may give an indication of how conditions for the early stages of speciation may be met. 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. Passive divergence in dewlap phenotype associated with isolation-by-distance did not explain our results. Instead, the observed patterns in dewlap coloration are more consistent with an adaptive explanation in these *A. sagrei* populations, as one would otherwise expect differences within islands to be erased by gene flow at such small geographical scales. These habitat-specific dewlap differences varied in magnitude and direction across islands, and may suggest a role of sexual selection in driving these local patterns. While at present, populations from these different habitats probably experience too much gene flow to follow distinct evolutionary lineages, should additional barriers arise between habitat-specific populations, the observed disruptive selection on dewlap coloration may facilitate ecological speciation.

**Keywords** — *Anolis*, reflectance, local adaptation, sexual signal, supervised machine learning

## 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, for example, 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 recipient(s) (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;

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37 Halfwerk et al., 2014). This selective pressure may drive the local adaptation of communication  
38 signals.

39 One potential barrier to the maintenance of localized signal divergence is the homogenizing  
40 effect of gene flow. Population genetics theory suggests that gene flow may counteract local  
41 adaptation between localities and prevent divergence altogether, especially at small spatial scales,  
42 because of the inflow of maladapted alleles or because of the breaking of linkage between coevolving  
43 loci (Felsenstein, 1976; García-Ramos and Kirkpatrick, 1997; Dieckmann and Doebeli, 1999;  
44 Lenormand, 2002; Hendry et al., 2007a). This has been confirmed empirically in systems such as  
45 stick-insects (Nosil and Crespi, 2004) and sticklebacks (Hendry et al., 2007b). Yet, examples of  
46 microgeographic adaptation, i.e. adaptation at smaller scales than the range of dispersal, exist,  
47 highlighting a high potential of some organisms to respond to selection in the face of gene flow  
48 (see Richardson et al. 2014 and references therein). Examples include small scale adaptation in  
49 fragmented areas in Australian fruit flies (Willi and Hoffmann, 2012), or local adaptation to pre-  
50 dation pressure in North American salamanders (Richardson and Urban, 2013). Therefore, despite  
51 evidence that local adaptation may be particularly difficult at small spatial scales where gene flow  
52 tends to cause adjoining populations to remain genetically homogeneous, the potential adaptive  
53 response of species traits, in particular communication signals, to localized differences in habitats  
54 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  
59 as a communication signal in courtship (Sigmund, 1983; Driessens et al., 2014, 2015), 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 differences  
70 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.,  
72 2012, 2013, 2016; Vanhooydonck et al., 2009; Driessens et al., 2017). Some studies suggest that  
73 those differences may be adaptive, and that dewlaps may have evolved to maximize detectability  
74 given local light conditions (Fleishman and Persons, 2001; Leal and Fleishman, 2002, 2004). Other  
75 studies testing this hypothesis, however, found no pattern (Fleishman et al., 2009; Ng et al., 2012;  
76 Macedonia et al., 2014).

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

85  
86 The species *Anolis sagrei* is widespread across islands of the West Indies (Reynolds et al.,  
87 2020). It has been the subject of study concerning local adaptation (Losos et al., 1994, 1997, 2001;  
88 Kolbe et al., 2012), biological invasion (Kolbe et al., 2008), sexual selection (Tokarz, 2002; Tokarz  
89 et al., 2005; Tokarz, 2006; Driessens et al., 2014; Steffen and Guyer, 2014; Driessens et al., 2015)  
90 and many other topics. Between-island variation in the mainly orange-red color of its dewlap was  
91 shown to be better explained by climatic variables (Driessens et al., 2017) than by proxies for biotic  
92 factors such as sexual selection or predation pressure (Vanhooydonck et al., 2009; Baeckens et al.,  
93 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  
95  
despite gene flow.  
96  
97

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 *Anolis 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 their 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). 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.

146 **Analysis**

147 All analyses in this study were performed in R 3.6.1 ([R Core Team, 2019](#)).

148 **Dimensionality reduction**

149 Reflectance curves were smoothed using the R package [pavo](#) ([Maia et al., 2013](#)) as well as with  
150 custom R functions, down to one reflectance value at each nanometer in wavelength from 300  
151 to 700nm. Because neighboring wavelengths are highly collinear in reflectance, we reduced the  
152 dimensionality of the data using principal component analysis (PCA), as per [Cuthill et al. \(1999\)](#)  
153 and [Leal and Fleishman \(2002\)](#). We performed PCA on each island separately and systematically  
154 retained the first four principal components (PC), which together always explained more than  
155 88.8% of the variance across islands (Table [S3](#)). PC1 explained between 40 and 56% of the variance  
156 across islands; PC2 explained 17.4–27.9%; PC3 12.7–17.6% and PC4 4.3–10.5%. The first four PCs  
157 explained similar proportions of variance when calculated for all islands together (Table [S3](#)). PCs  
158 need not represent the same wavelengths across islands because they are fitted on different datasets.  
159 Nevertheless, PC1 was very collinear with brightness for all islands (Figure [S2](#), Table [S4](#)). PC2  
160 correlated highly with the red and ultraviolet ends of the spectrum, which were inversely correlated  
161 with each other (Fig. [3A](#)). Higher PCs corresponded to various combinations of wavelengths.  
162 Because PC1 correlated uniformly with all wavelengths across the spectrum we considered PC2  
163 onwards to capture the chromatic dimensions of color space, i.e. the relative contributions of the  
164 wavelengths regardless of brightness.

165 **Pooled analyses**

166 In addition to within-island PCA, we performed a PCA on pooled data from the whole archipelago.  
167 The first four principal components explained 91.3% of the variance (Table [S3](#)). Again PC1 strongly  
168 correlated with brightness (Fig. [S3](#), Table [S4](#)). PC2 was positively correlated to short wavelengths  
169 (ultraviolet to blue) and negatively correlated to long wavelengths (green to red, Fig. [S4B](#)). PC3  
170 was strongly negatively correlated with UV reflectance and positively correlated with blue-green.  
171 PC4 was made of a mosaic of wavelengths, correlating positively with blue and red but negatively  
172 with ultraviolet and yellow.

173

174 We used this dataset to partition the variance in dewlap coloration among islands, habitats  
175 and habitats within islands, using a two-way multivariate analysis of variance (MANOVA) with  
176 an interaction term. However, because the assumptions of parametric MANOVA were violated  
177 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,  
178 Box's M-test, [Box 1949](#); [Morrison 1988](#), R package [heplots](#), [Fox et al. 2018](#), Table [S6](#)), we used  
179 a semi-parametric MANOVA instead (R package [MANOVA.RM](#), [Friedrich et al. 2018](#)), with P-  
180 values calculated from a bootstrap procedure with 1,000 iterations. We calculated the proportion  
181 of variance explained by islands, habitats and the habitat-by-island interaction using partial effect  
182 sizes  $\eta^2$  on a MANOVA-approximation of the analysis (R package [heplots](#), [Fox et al. 2018](#)).

184 **Machine learning**

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

188

189 Machine learning for group comparison has become more common in ecology and evolution  
190 in recent years (e.g. [Pigot et al. 2020](#)). In particular, SVMs are designed to find the best pos-  
191 sible nonlinear boundaries between labelled groups of points in multidimensional spaces, without  
192 assumptions about the distribution of the data ([Cortes and Vapnik, 1995](#); [Cristianini and Shawe-  
193 Taylor, 2000](#); [Kim and von Oertzen, 2018](#)). This makes them well suited to field biological data,  
194 which often violate the assumptions of classical linear modeling ([Kim and von Oertzen, 2018](#)) and  
195 can be, as in the case of coloration, inherently highly multivariate ([Cuthill et al., 1999](#)). First,  
196 a machine is trained to recognize differences between groups within a subset of the data called  
197 the training set. Significance of differences is then assessed by testing the accuracy of that fitted  
198 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  $\sigma$  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, 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 tested the assumptions of the parametric ANOVA for each island included in the univariate analyses. For all islands where deviations from multivariate normality were detected in at least one habitat (Table S5), we assessed univariate normality for each principal component (Shapiro-Wilk's test, Table S7). For skewed PCs that deviated significantly from normality, we repeated the analysis using a nonparametric Kruskal-Wallis test (Hollander et al., 2013). We found no multivariate outliers based on the Mahalanobis distance (package MVN, Korkmaz et al. 2014). We used the cases of better fit of the GLS model relative to the OLS model as evidence for heterogeneity of

256 variances, which were then accounted for by the GLS approach (Table 1).

257  
258 Significant *post hoc* contrasts were assessed using Tukey's Honest Significant Difference (HSD)  
259 test whenever the assumptions of normality and homogeneity of variances was met (Tukey, 1949),  
260 Dunnett's T3 method when only homogeneity of variances was violated but not normality (Dun-  
261 nett, 1980), and Nemenyi's test when normality was violated (Nemenyi, 1963). All *post hoc* tests  
262 were performed with the R package PMCMRplus (Pohlert, 2020).

263  
264 We used the same procedure to investigate which variables, if any, were involved in archipelago-  
265 wide multivariate differences between habitats detected in our two-way MANOVA design (see  
266 Pooled analyses). However, in the first step or our model comparison procedure, we added mixed-  
267 effect equivalents of our OLS and GLS models, this time with island as a random effect. The  
268 resulting four models were compared and the best fitting variance structure was retained as ex-  
269 plained above.

## 270 Spatial autocorrelation

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

## 281 Site differences

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

## 288 Results

289 We tested for variation in *A. sagrei* dewlap coloration between populations living in three charac-  
290 teristic habitat types across nine islands that span the West Indian range of the brown anole (Fig  
291 1, S1). We found that most of the variation in coloration is partitioned between islands (two-way  
292 semi-parametric MANOVA, modified ANOVA-type statistic (MATS) = 2009.6,  $P < 0.001$ , Fig.  
293 S5, explained variance  $\eta^2 = 44.3\%$ , MANOVA approximation). Nonetheless, we did find evidence  
294 for differences in dewlap coloration between habitat-types, and those were mostly island-specific  
295 (habitat-by-island interaction term, MATS = 384.4,  $P < 0.001$ , explained variance  $\eta^2 = 11.4\%$ ),  
296 with a small but significant portion of the variation explained by an archipelago-wide habitat effect  
297 (MATS = 42.5,  $P = 0.001$ ,  $\eta^2 = 4.8\%$ ).

298  
299 The small archipelago-wide effect of habitat-type was detected for PC1, PC2 and PC3 (mixed-  
300 effect ANOVA with island as a random effect, Table S8), but this effect was too small for *post hoc*  
301 tests to find which habitats differed. Archipelago-wide differences in dewlap coloration between  
302 habitats were also detected by SVMs trained on pooled data regardless of island identity, both for  
303 PCA data and reflectance scores (Fig. S6, S7). This pattern seemed to be driven by mangrove  
304 lizards being correctly reassigned more often than predicted by chance. Sensitivity analyses on  
305 these machines suggest a relatively small role of long wavelengths (red reflectance) in driving this  
306 pattern (Fig. S9) – **be more explicit about how the colors vary among habitats** –, but did  
307 not reveal strong differences between the PCs in relative importance (Fig. S8). Archipelago-wide

differences were not detected by LDA classifiers at all (Fig. S10, S11). 308

Within islands, SVM classifiers correctly assigned individuals to their habitat of origin based 309  
solely upon dewlap coloration on five islands: Abaco, Bimini, Cayman Brac, Little Cayman, and 310  
Long island (Fig. 2). An LDA approach yielded similar success rates (Fig. S12), suggesting robust 311  
differences between these populations. Of the five islands, Little Cayman was the best discrimi- 312  
nated with a mean SVM generalization success of 73.4% (Table S9). The results of the classification 313  
analyses on PCA data were very similar to results from SVMs and LDAs trained on reflectance 314  
values at 50nm-spaced wavelengths from 300 to 700nm (Fig. S13 and S14). 315

Differentiation in dewlap coloration occurred in multiple dimensions of color space. Moreover, 316  
the differences in dewlaps between habitats generally were not consistent among islands, thus, we 317  
will discuss the habitat-specific variation in dewlap coloration for each island where significant 318  
differences were detected in turn (Fig. 3, Tables 1, S10). Figure 3A provides a key to map principal 319  
component scores to the underlying wavelengths. 320

On Abaco, dewlaps did not differ in PC1, which represents brightness. Mangrove lizards had 321  
significantly lower PC2 scores, corresponding to higher ultraviolet reflectance and lower red 322  
reflectance. Coastal beach scrub lizards had lower scores on PC3, corresponding to lower ultraviolet 323  
reflectance and higher blue reflectance. 324

On Bimini, coastal beach scrub lizards had significantly brighter dewlaps than lizards from 325  
mangroves (PC1), but mangrove lizards had higher PC2 scores than beach scrub lizards, indicat- 326  
ing higher violet and blue reflectance, and lower red reflectance. Lizards from primary coppice had 327  
higher PC3 scores overall, which correlated very positively with ultraviolet reflectance. 328

On Cayman Brac, coppice-lizard dewlaps were significantly less bright than lizards from the 329  
other habitats. Coastal beach scrub lizards had dewlaps that scored low on PC2, corresponding 330  
to lower violet-blue and more red, while the mangrove lizards exhibited the opposite: relatively 331  
higher levels of violet-blue and less red. In PC3 space we found that dewlaps from lizards in the 332  
coastal habitat had high ultraviolet reflectance, coppice lizards had intermediate levels, and man- 333  
grove lizards had relatively low levels. 334

On Little Cayman, the dewlaps of coppice lizards were significantly darker (PC1) than coastal- 335  
lizards. Mangrove lizards had less ultraviolet and redder dewlaps (PC2). The dewlaps of the 336  
coastal beach scrub lizards had higher levels of red and ultraviolet reflectance and less blue re- 337  
flectance than the dewlaps of the other habitat-populations (PC3). 338

On Long Island, lizards from the coppice habitat had darker dewlaps than lizards from the other 339  
habitats (PC1). Coastal lizards had relatively more ultraviolet and less blue-green reflectance in 340  
their dewlaps (PC3). These coastal-habitat lizards also scored lower on PC4, corresponding to 341  
slightly more violet and green-yellow dewlaps, and less blue dewlaps, than the mangrove lizards 342  
on the island. 343

Sensitivity analyses on classifiers suggested an overall higher relative importance for PC2 and 344  
PC3 in determining between-group differences on Abaco, both in SVM and LDA classifiers (Fig. 345  
S15, S16), consistent with our ANOVA results (Fig. 3B). There was no strong signal of differences 346  
in relative importance among principal components on the other islands. Sensitivity analyses of 347  
SVMs trained on reflectance scores rather than principal components revealed, however, a consis- 348  
tently higher importance of ultraviolet reflectance in between-group differences on all islands (Fig. 349  
S17). This pattern was not recovered for LDAs trained on reflectance scores (Fig. S18). 350

We did not find significant spatial autocorrelation between the sampling sites on the islands 351  
where we detected a significant habitat effect. We did, however, detect a significant positive sig- 352  
nal of autocorrelation on Eleuthera (Table S11), suggesting possible color differentiation through 353  
isolation-by-distance on this island. 354

In contrast, differences in dewlap coloration between habitats were often detected in close ge- 355

366 ographical proximity. For example, Bimini, Cayman Brac, and Little Cayman were among the  
367 smallest islands in our study (Fig. S1). Indeed, most significant differences in dewlap coloration  
368 involved sites that were 5-10km apart. Our most extreme case of significant differences occurred  
369 for PC3 between a beach scrub site and a coppice site, separated from each other by a few hundreds  
370 of meters at most on Bimini (multiple pairwise Wilcoxon-Mann-Whitney tests, Fig. S19).

371       Patterns of differentiation were inconsistent across the five most significant islands. Contrasts  
372 in principal components between habitats, calculated on pooled data from the whole archipelago,  
373 were not similar, for any component, among islands (Fig. S20; MANOVA, Pillai's trace = 0.354,  
374  $F(6, 32) = 1$ , P = 0.36). No pattern of variation was shared by all five significant islands, along  
375 any dimension. Some patterns did seem more common however, such as darker dewlaps among  
376 coppice lizards (Cayman Brac, Little Cayman, and Long Island, Fig. 3) or the intermediate posi-  
377 tion of coppice lizards in chromatic color space (Cayman Brac and Long Island). In other cases,  
378 patterns of differentiation were reversed from one island to another, with more ultraviolet reflecting  
379 dewlaps in mangroves than in coastal habitat on Abaco and Cayman Brac, but the opposite on  
380 Little Cayman and Long Island. Overall, it seemed that patterns of heterogeneity of variance were  
381 often driven by higher variances in coloration within beach scrub lizards (Fig. 3, Table 1). Yet  
382 other patterns were idiosyncratic, such as the combination of higher red and ultraviolet reflectance  
383 in coastal lizards on Little Cayman, where the rule seemed to be a negative correlation between  
384 ultraviolet and red reflectance across every other island.

386

## 387 Discussion

388 **Jonathan's take: too much push in favor of selection, without really having much**  
389 **evidence. Simple models of genetic drift are ruled out and we don't think it is plastic-**  
390 **ity. However, our primary test for adaptation—looking for parallel responses across**  
391 **islands—also was falsified. We suggest, however, that selection could be involved, and**  
392 **suggest several ways this might have happened.**

393 **Dewlap coloration differs between habitat-types** We found that male dewlap coloration in  
394 *A. sagrei* significantly varied between fine-scale habitat-types (beach scrub bush, primary coppice  
395 forest and mangrove forest) on five islands of the West Indies: Abaco, Bimini, Cayman Brac, Little  
396 Cayman and Long Island. However, the habitat-specific variation in dewlaps was not consistent  
397 between these islands. Although those results are consistent with selection acting at a very local  
398 scale, other evolutionary drivers could be at work, such as phenotypic plasticity, random drift, or  
399 multiple colonization events. We reject this last explanation because all of the island populations  
400 in this study are strictly monophyletic, implying a single colonization event per island (van de  
401 Schoot, unpublished thesis; Driessens et al. 2017; Reynolds et al. 2020).

402 **A role of neutral drift is unlikely** Differences in organismal traits between environments are  
403 not necessarily proof of adaptation or selection, and genetic drift may result in patterns similar  
404 to local adaptation (Miles et al., 2019). Nevertheless, two lines of evidence from our data suggest  
405 that this scenario may be implausible. First, we found little evidence for a role of phenotypic  
406 isolation-by-distance (spatial autocorrelation) in explaining the differences we report. We did de-  
407 tect a significant signal of isolation-by-distance on Eleuthera, but there were no differences in  
408 dewlap coloration between habitats on this island. Second, we detected differences between habi-  
409 tats at relatively small spatial scales, most of the time between sites 5-10km apart, sometimes a few  
410 hundred meters away, on Bimini for example. Such small-scale differences would be unlikely under  
411 strong gene flow (Richardson et al., 2014). Our study islands lack geographic barriers to the move-  
412 ment of *A. sagrei*, which have been shown to be highly mobile (Kamath and Losos, 2018), implying  
413 widespread gene flow across sites and habitats. Moreover, habitat-populations within each island  
414 were found to be non-monophyletic and often share identical haplotypes, based on phylogenetic  
415 analysis of mitochondrial DNA sequences (van de Schoot et al. unpublished thesis), suggesting  
416 gene flow between habitats may be widespread. Our results align with previous documented cases  
417 of persistent dewlap color divergence despite gene flow in multiple species of anoles, sometimes in  
418 relation to environmental conditions. Ng et al. (2012) and Ng et al. (2016) found divergent dewlap

419 coloration in the face of gene flow between subspecies of *A. distichus* across Hispaniola, and pro-  
posed 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 polymor-  
phism 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 pat-  
tern 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 an-  
other small island, Grand Cayman, where no physical barriers to gene flow exist (Macedonia, 2001).

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**No conclusive evidence for local adaptation of dewlap coloration** One of the most infor-  
mative tests for adaptation is the convergence of differentiation patterns across replicate islands or  
localities (Losos, 2009, 2011). Previous studies have described convergent patterns of dewlap color  
evolution in similar environments across islands and species (Thorpe and Stenson, 2002; Thorpe,  
2002). Others have suggested that dewlap coloration may have evolved to be maximally detectable  
under local light conditions imposed by the environment, 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). Although UV  
reflectance was commonly involved in between-habitat divergence in *A. cooki* and *A. cristatellus*,  
we found no such patterns in *A. sagrei*, where instead, we found 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). The inconsistent and idiosyncratic patterns we observed suggest  
that dewlap color variation between habitats cannot be predicted by habitat identity alone, and  
the test of convergence across islands is rejected. Studies of Jamaican and Hispaniolan anoles  
similarly found between-habitat differences in dewlap coloration but no evidence for higher dewlap  
detectability in different habitats (Fleishman et al., 2009; Ng et al., 2012). Habitats on different  
islands may also differ in aspects other than light conditions, such as densities of predators or  
congeners, which have been shown to affect among-island dewlap diversity (Vanhooijdonck et al.,  
2009; Baekens et al., 2018). In particular, Baekens et al. (2018) recently showed that dewlaps  
with spotted patterns occurred more often in *A. sagrei* on islands with more coexisting species of  
anolles. Our data are consistent with those previous results in suggesting that adaptation to local  
light conditions, or at least broad habitat types, is not a major driver of the within-island variation  
in dewlap coloration in *A. sagrei*.

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**Sexual selection could be at play** Selection, however, needs not necessarily be linked to habi-  
tat type, and may take the form of arbitrary, "Fisherian" sexual selection, where female preferences  
differ between localities for reasons unrelated to the environment, driving the divergent evolution  
of male ornaments (Andersson, 1994). This process could account for the idiosyncratic patterns of  
within-island divergence we report, where initial differences in female preferences could have arisen  
for nonselective reasons (e.g. plasticity or random drift). Substantial levels of promiscuity in *A.*  
*sagrei* suggest ample opportunity for female mate choice (Kamath and Losos, 2018), and are in  
line with this scenario. However, Baekens et al. (2018) found no link between *A. sagrei* dewlap  
coloration and size dimorphism (a proxy for sexual selection) in an among-island study of the same  
archipelagos. Another form of sexual selection is the "good genes" model, where the cue under  
sexual selection is an indicator of individual quality (e.g. better immune response to disease) and  
of indirect benefit to the offspring (Andersson, 1994). Several studies suggest that this possibility  
could be the case for anoles. For example, Cook et al. (2013) found lower orange reflectance in  
dewlaps with heavily parasitized *A. brevirostris*, suggesting a trade-off in carotenoid use between  
the immune response and pigment deposition. Steffen and Guyer (2014) found that lower UV and  
orange-red reflectance predict contest-winning success between *A. sagrei* males, while Driessens  
et al. (2015) further found that more yellow and red dewlaps (relative to UV) predict better body  
condition, and that higher yellow and UV reflectance at the margin of the dewlap predict higher  
hematocrit (the concentration of red blood cells), indicating a better health. Other aspects of the  
dewlap than color have also been found to be indicators of individual quality, such as dewlap size  
(Vanhooijdonck et al., 2005, 2009), but not dewlap display frequency (Tokarz, 2002; Tokarz et al.,  
2005; Driessens et al., 2015). If dewlap coloration is indeed an indicator of male quality in *A.*

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*sagrei*, however, this would explain the observed patterns only if cues associated with good genes differ between sites, which is unlikely as it implies an association of male fitness with different dewlap coloration in different habitats, the evolution of which may be counteracted by gene flow (citation to say that if gene flow counters the divergence of one trait, then it is even worse for the association of two traits). Basically, one would expect good genes-sexual selection to favor the same coloration across the archipelago, and would therefore not result in local differences between habitats.

**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 such as parasite load, as mentioned above (Cook et al., 2013). 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. This makes a plastic response to differences in diet across habitats unlikely. Furthermore, developmental plasticity during the ontogeny is also unlikely because dewlap coloration develops at sexual maturity in anoles (Ng et al., 2013). 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*. Response to parasite load is definitely an example of plasticity!

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**Implications in the context of speciation** Local adaptation can be a precursor to ecological speciation, a process that may have given rise to the adaptive radiation of *Anolis* lizards (Harmon et al., 2003; Gavrilets and Losos, 2009). Ecologically-mediated divergence of a sexual signal may be a potent path to the evolution of reproductive isolation through divergent sexual selection (Reynolds and Fitzpatrick, 2007; Servedio et al., 2011). Evidence suggests that dewlap coloration could take this role in anoles (Ng and Glor, 2011; Lambert et al., 2013; Geneva et al., 2015; Ng et al., 2017), or at least that it is frequently involved in species recognition (Williams, 1969; Williams and Rand, 1977; Losos, 1985; Macedonia and Stamps, 1994; Fleishman, 2000; Macedonia et al., 2013; Ingram et al., 2016; Baeckens et al., 2018). Although a correlation between dewlap coloration and reproductive isolation is not detected at the phylogenetic scale of the whole genus (Nicholson et al., 2007; Harrison and Poe, 2012; Ingram et al., 2016), sexual signals are often evolutionarily very labile (Kraaijeveld et al., 2011), and the anole dewlap in particular is capable of rapid macroevolution throughout its macroevolutionary history (Nicholson et al., 2007). For example, *A. conspersus* on Grand Cayman evolved a UV-blue dewlap from an ancestral orange dewlap in 2 to 3 million years (Macedonia, 2001). We present evidence of multiple cases of potentially adaptive maintenance of habitat-associated dewlap divergence over small geographical scales in *A. sagrei* across the West Indies. While these intra-island populations do not appear to be in the process of speciation, our results suggest that the anoline dewlap has enough micro-scale, local adaptive potential to contribute to reproductive isolation, should it be recruited for assortative mating.

## 517 Acknowledgements

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

**526 Figures**

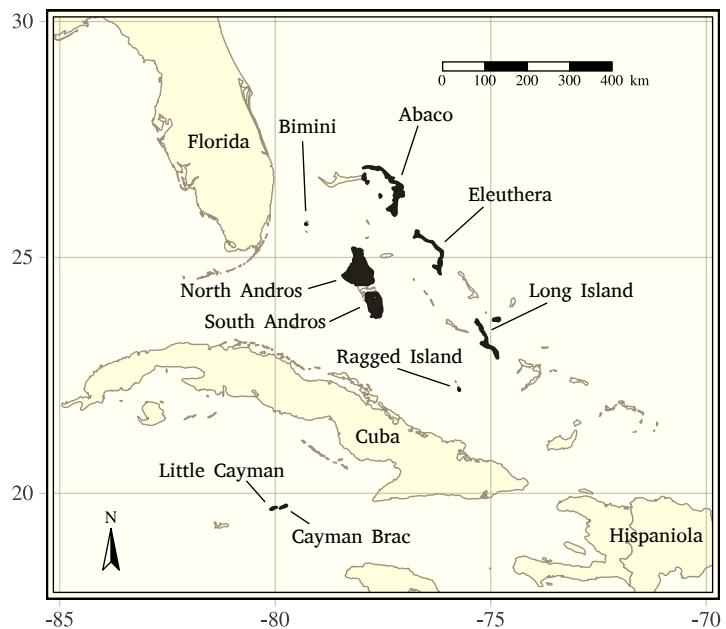


Figure 1: Map of the West Indies with sampled islands highlighted in black.

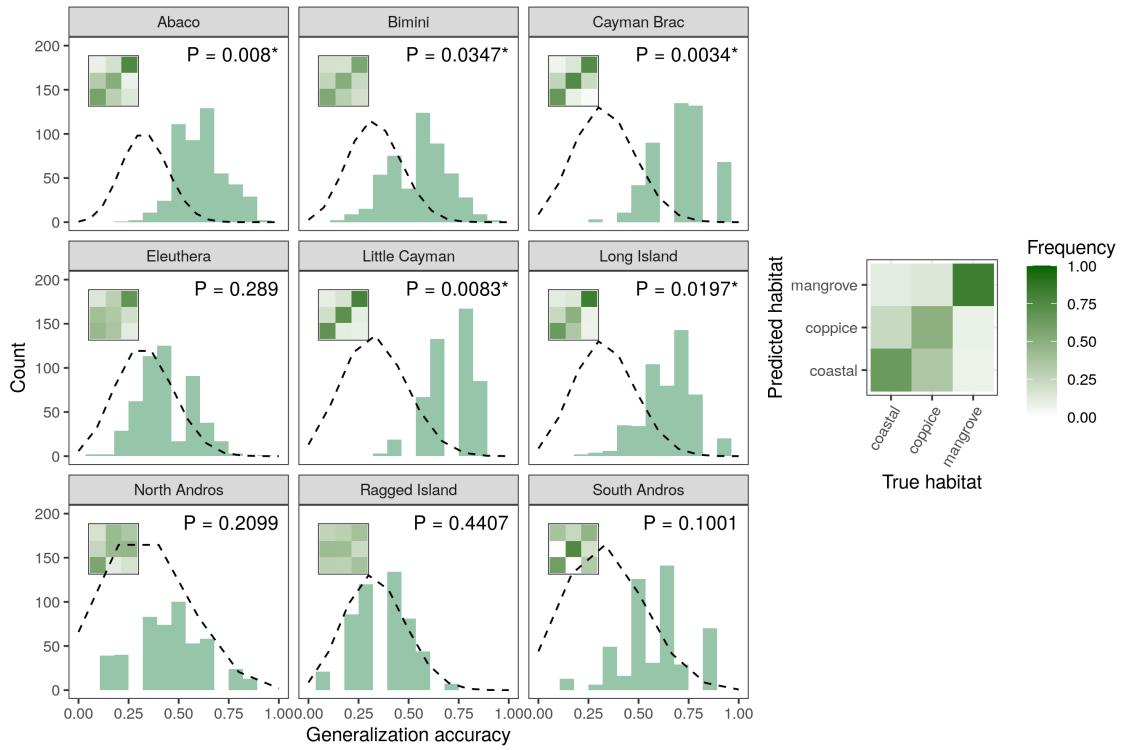


Figure 2: SVM classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The dashed line is the density of a corresponding null binomial distribution, which would be expected under 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. \*,  $P < 0.05$ .

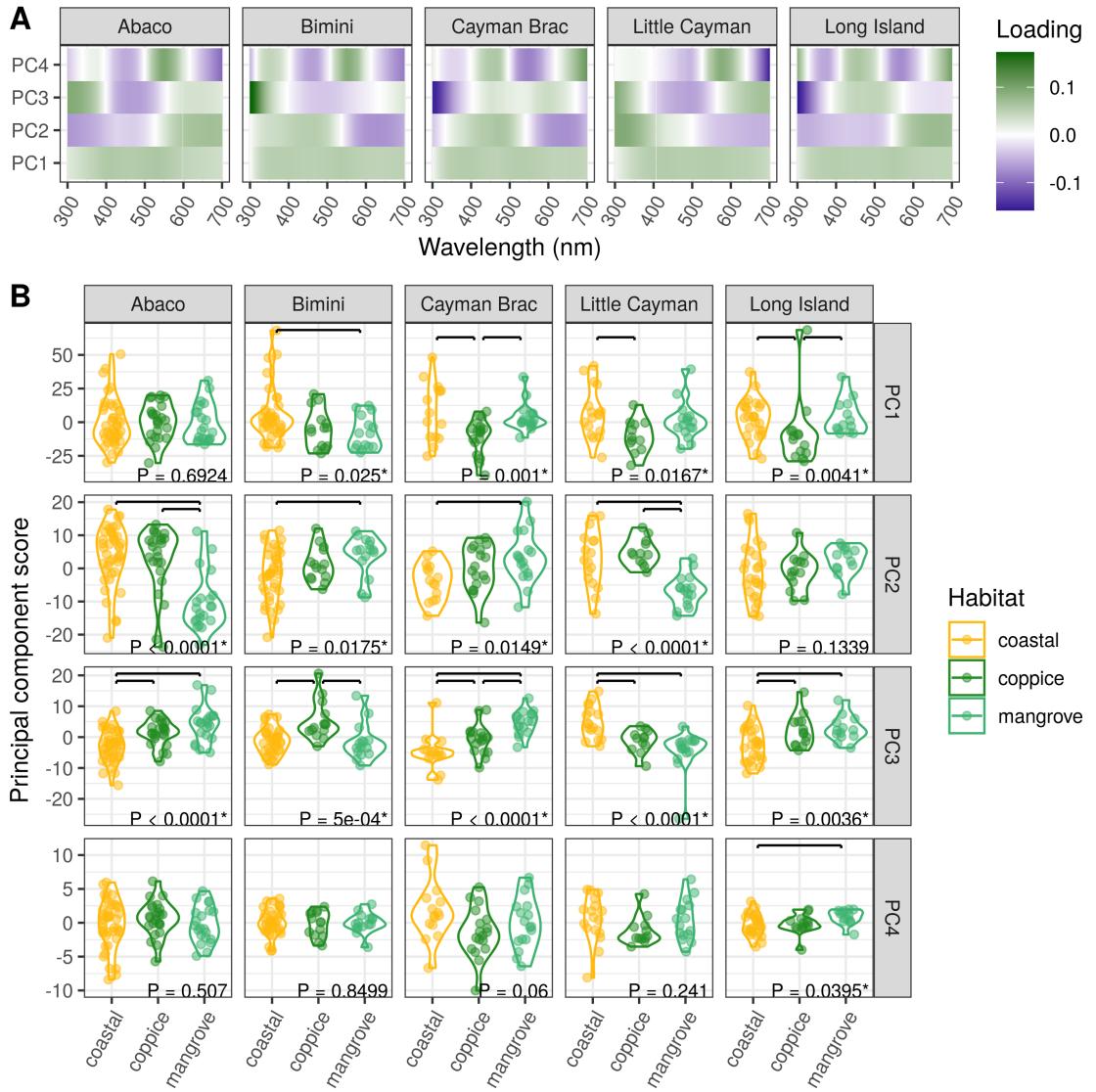


Figure 3: Dewlap color variation between habitat-types across the most significant islands. (A) Mapping of reflectance at various wavelengths onto the principal components (loadings from the PCA rotation matrix). (B) Distribution of PC scores between habitats along the first four PCs on each island where 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. \*,  $P < 0.05$ .

527 **Tables**

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.  $\Delta \text{AICc}$ , difference in AICc between the best fitting model and the OLS-model. AICcw, AICc weight. LRT, likelihood ratio test. Log-lik., log-likelihood.  $\chi^2$ , likelihood ratio. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Island	Variable	Best fit	df	AICc	$\Delta \text{AICc}$	AICcw	$\Delta \text{AICc}$	dfLRT	Log-lik.	$\chi^2$	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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**814 Supplementary Figures**

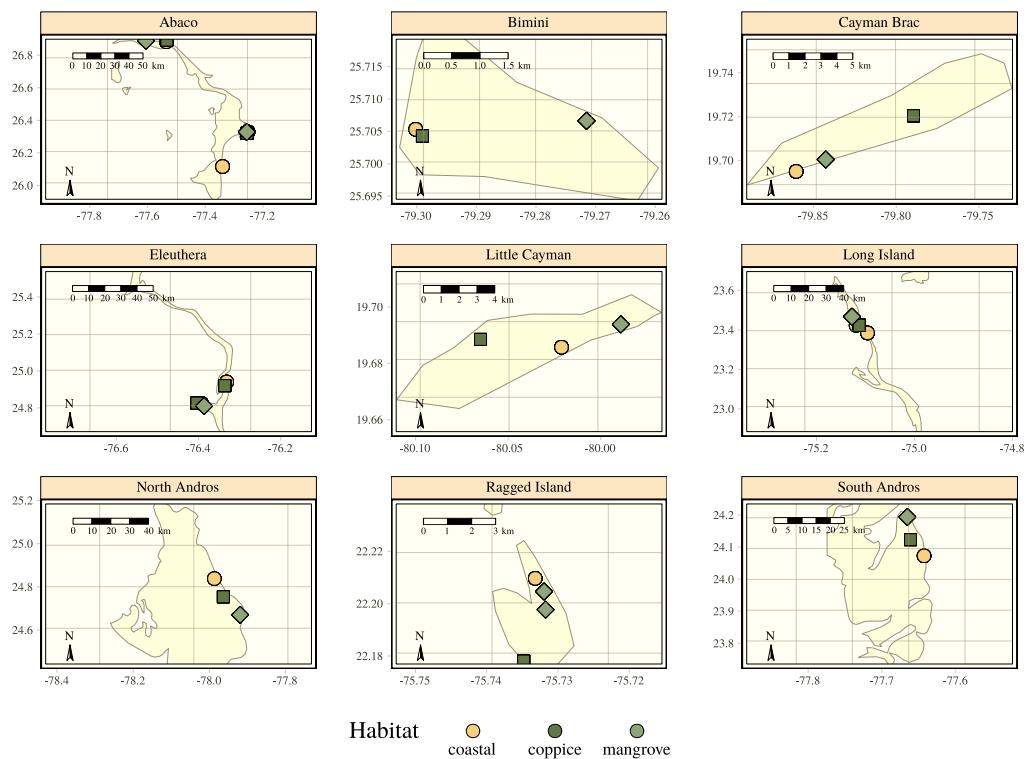


Figure S1: Map of the sampling sites and corresponding habitats across nine islands of the West Indies.

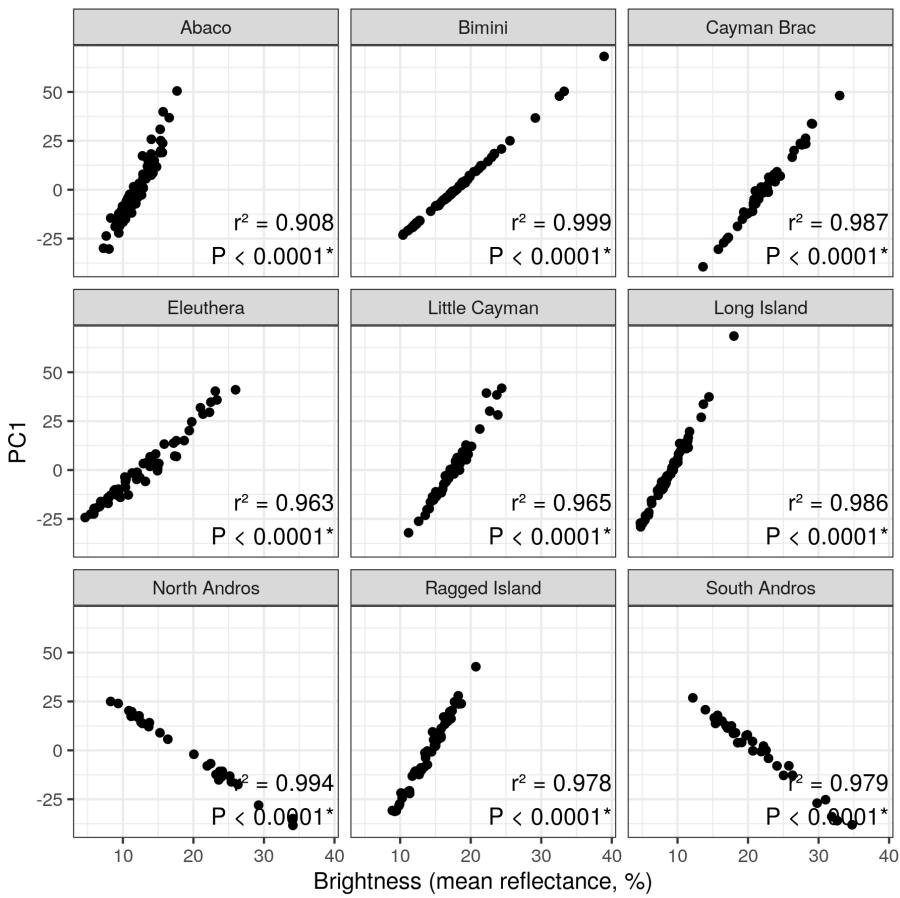


Figure S2: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score for each island. Pearson's squared correlation coefficients are reported. \*,  $P < 0.05$ .

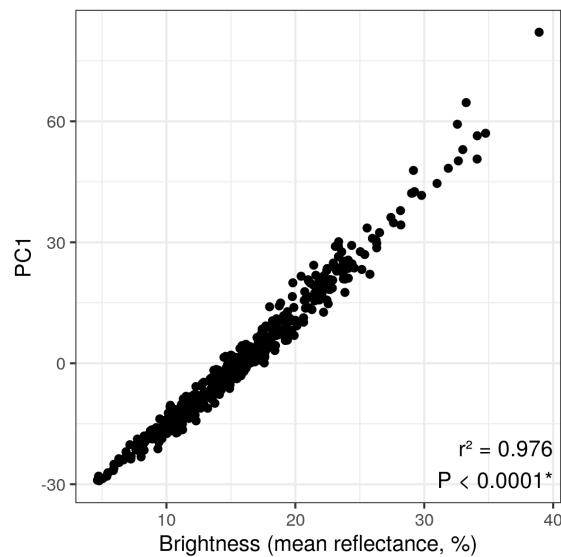


Figure S3: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across the whole archipelago. Pearson's squared correlation coefficient is reported. \*,  $P < 0.05$ .

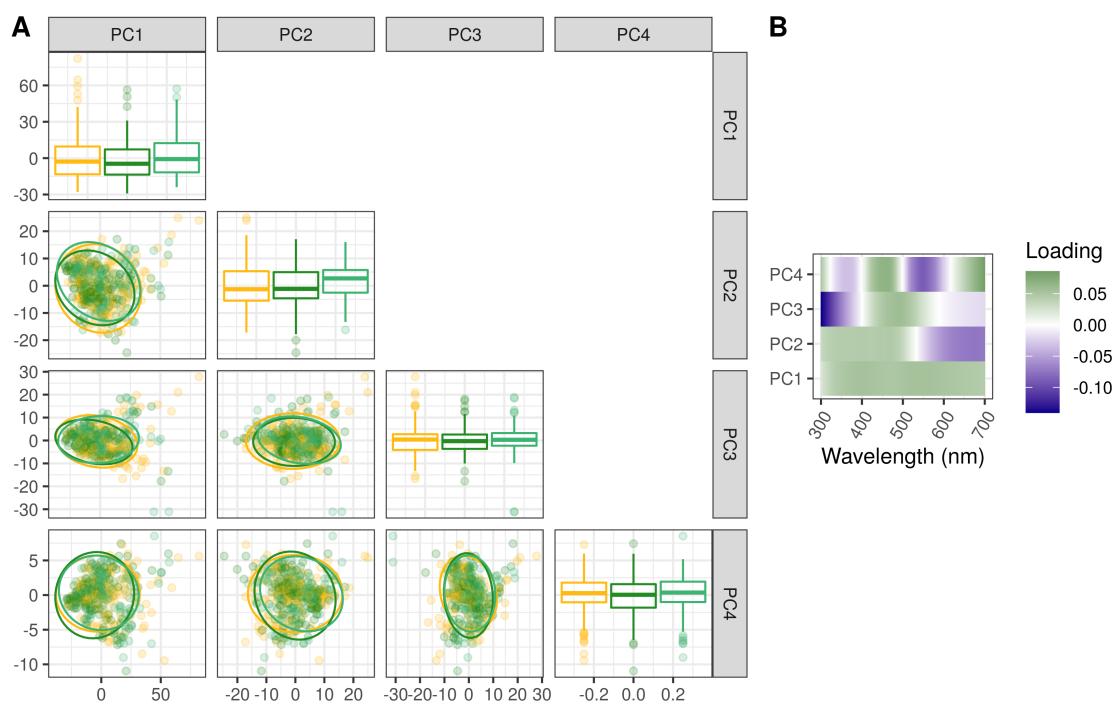


Figure S4: (A) Principal component scores and 5% confidence ellipses across habitats for the whole archipelago. The principal component analysis was performed on reflectance data from all islands pooled together. (B) PCA rotation matrix showing the loadings of each wavelength from 300 to 700nm onto the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

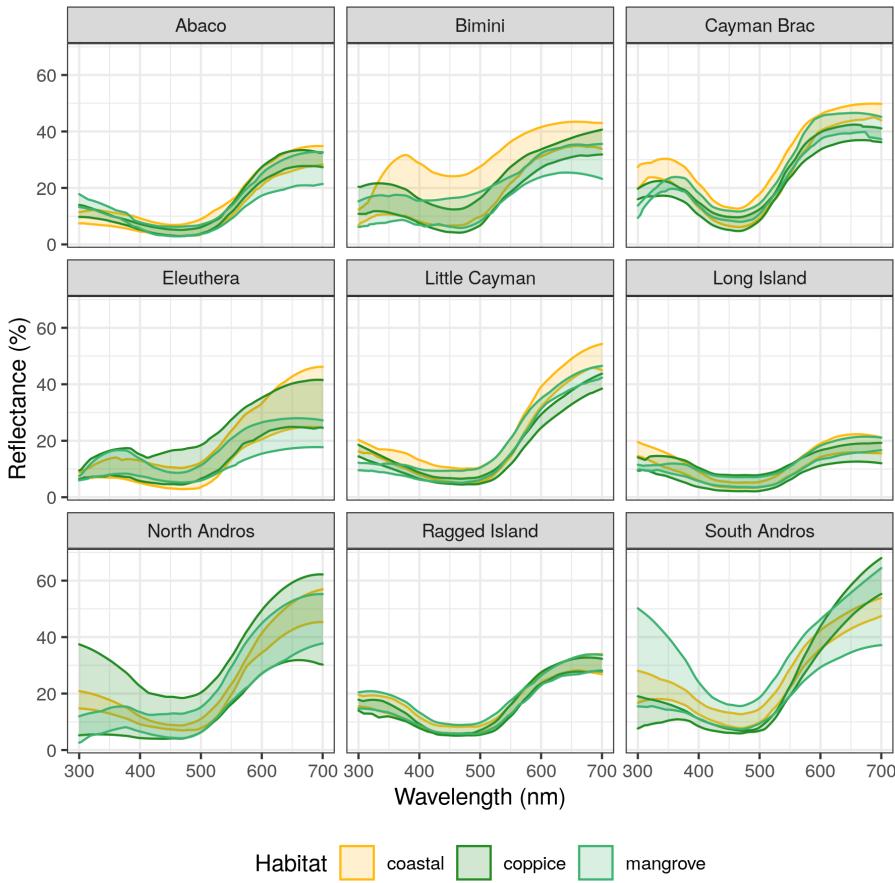


Figure S5: 5-95th percentile range of lizard dewlap reflectance values (in % of incoming light) across wavelengths for each island and each habitat.

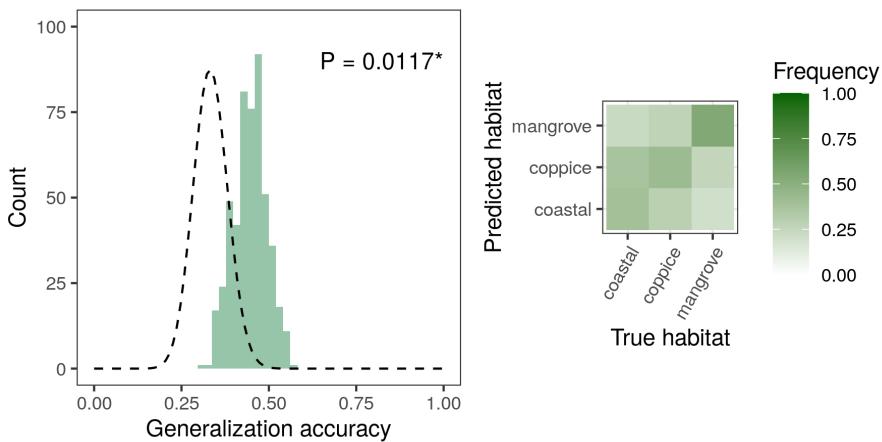


Figure S6: Archipelago-wide SVM classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure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

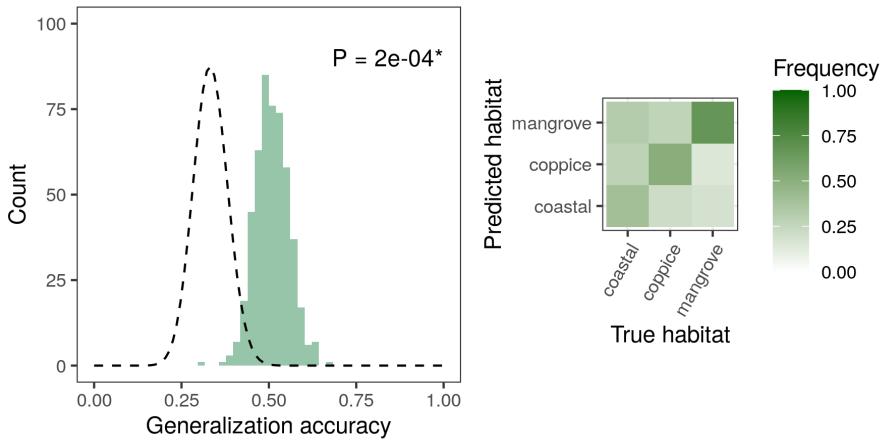


Figure S7: Archipelago-wide SVM classification accuracy based on reflectance data at 50nm-intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation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

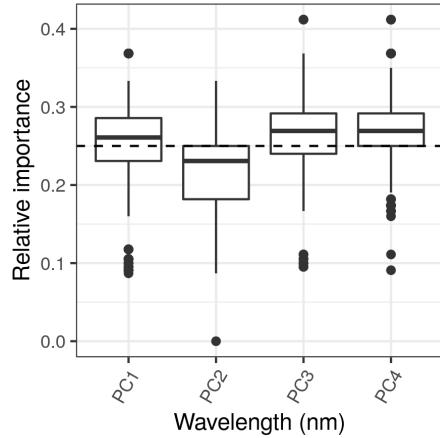


Figure S8: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on principal component data (Figure S6), with relative importance computed for every machine.

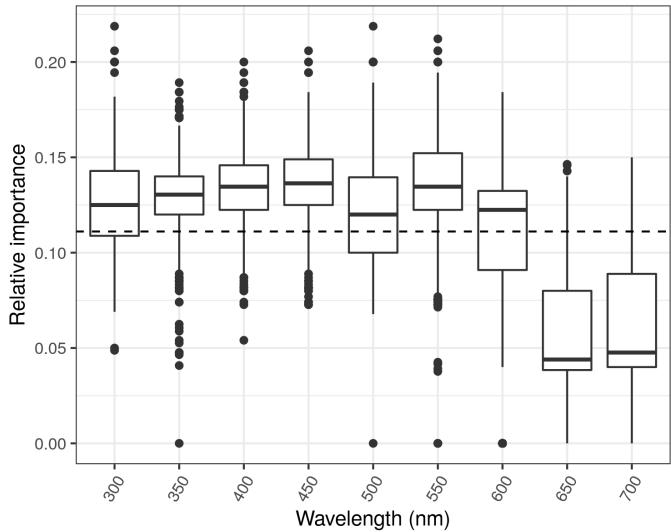


Figure S9: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance data at 50nm-intervals in wavelength (Figure S7), with relative importance computed for every machine.

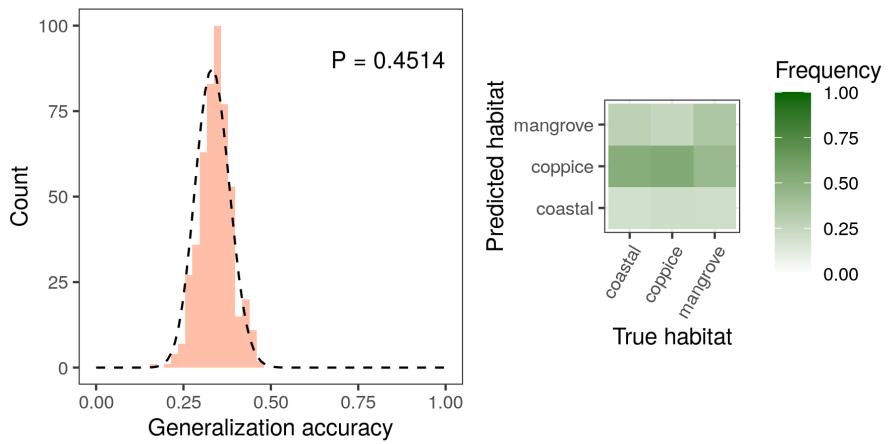


Figure S10: Archipelago-wide LDA classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure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

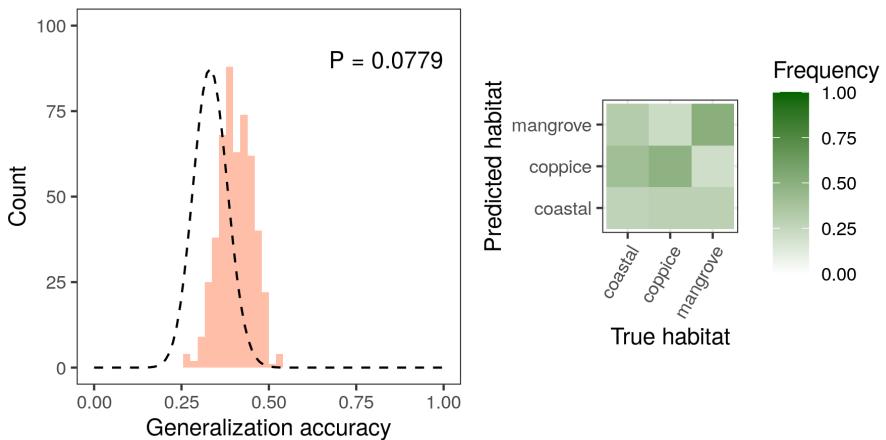


Figure S11: Archipelago-wide LDA classification accuracy based on reflectance data at 50nm intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation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

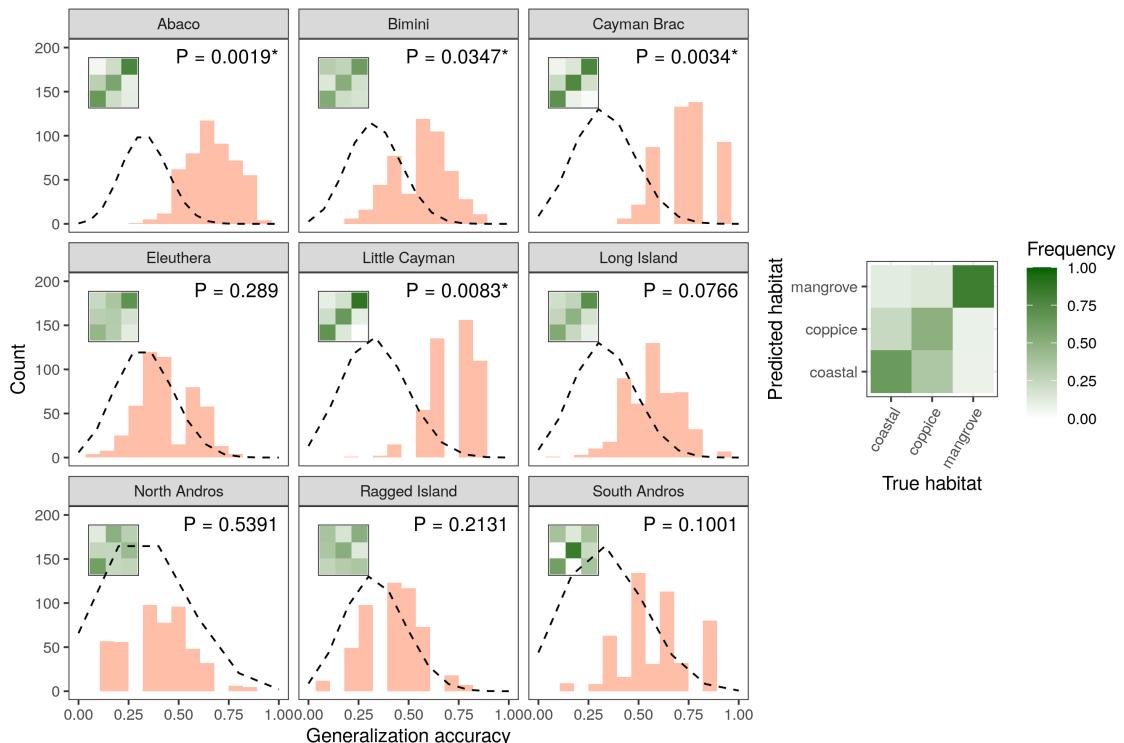


Figure S12: LDA classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

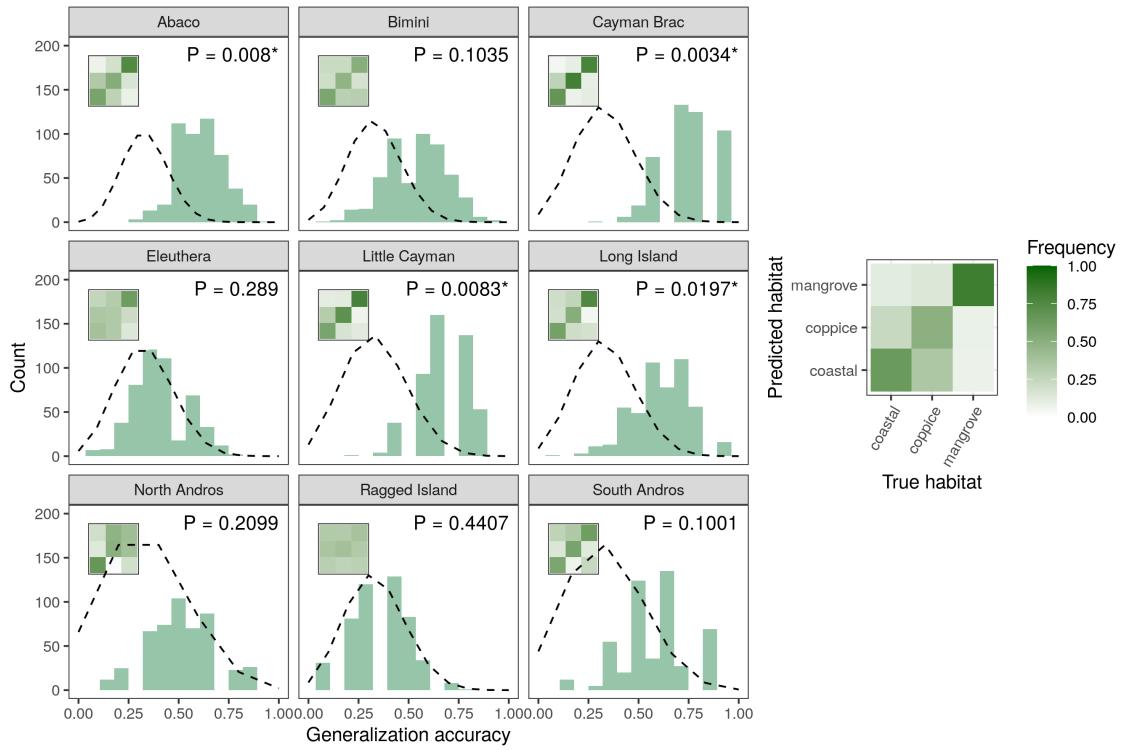


Figure S13: SVM classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

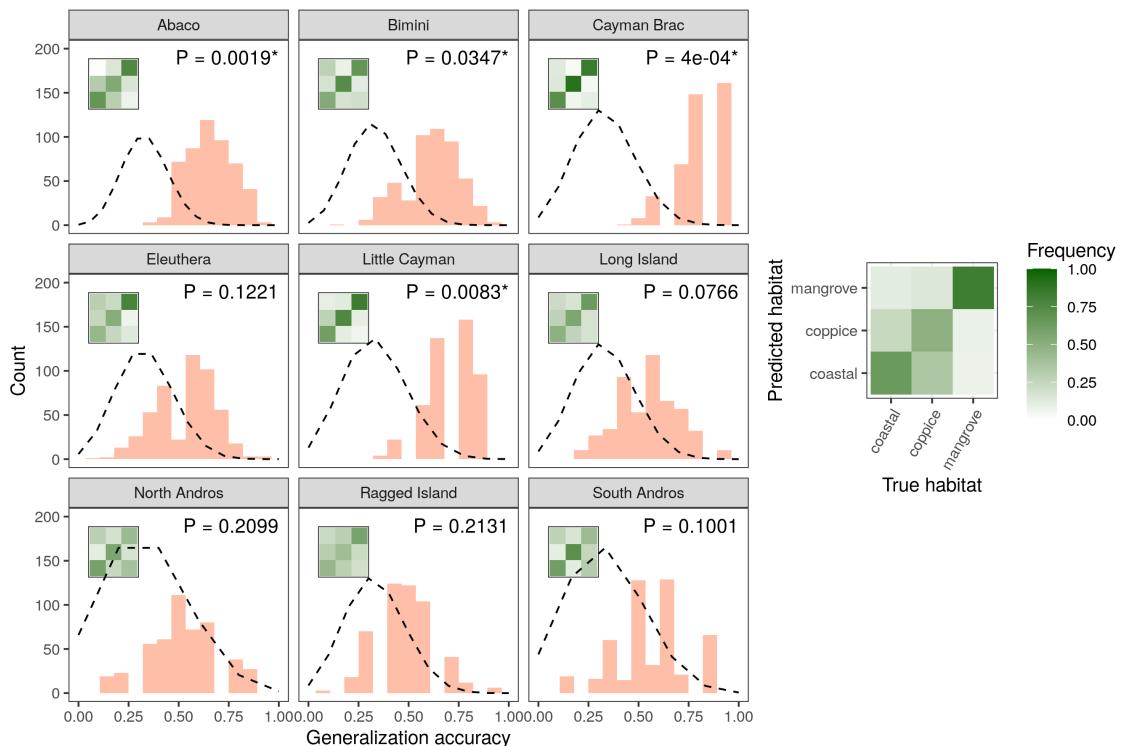


Figure S14: LDA classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

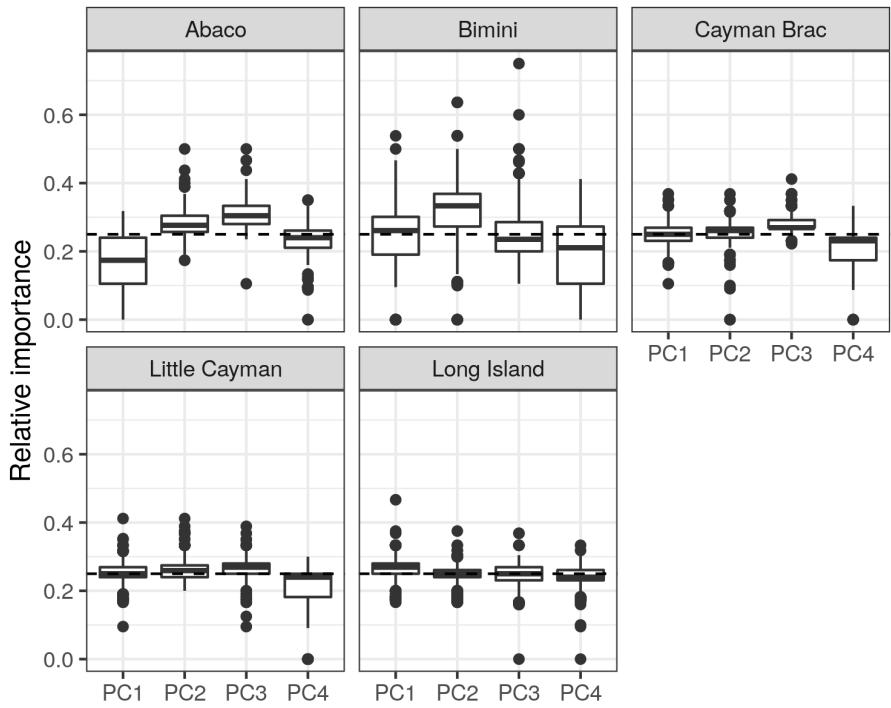


Figure S15: Sensitivity analyses of the different input variables in the within-island SVM classification on principal component data (Figure ??), with relative importance computed for every machine.

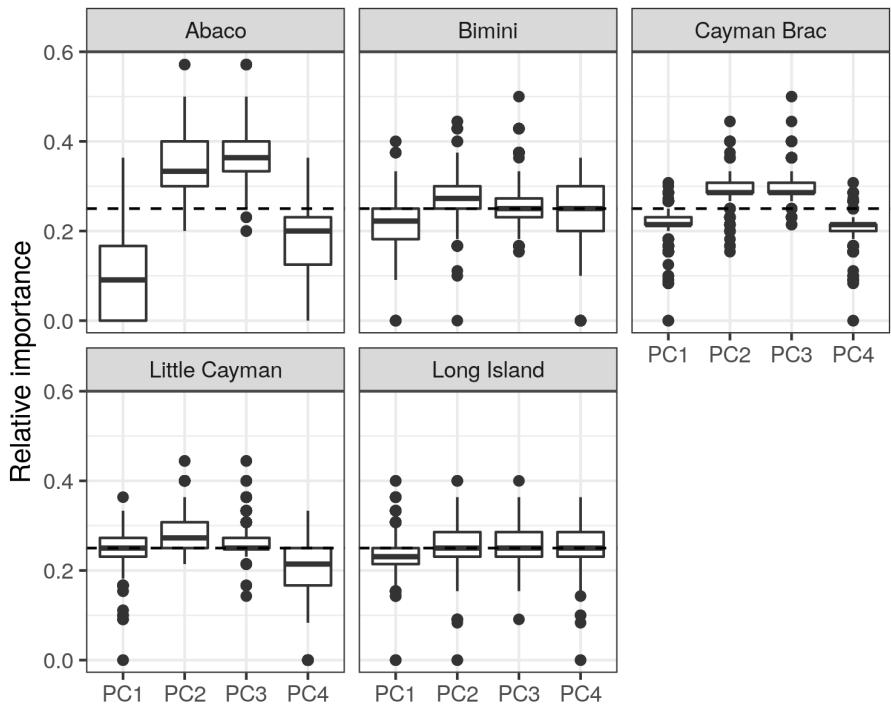


Figure S16: Sensitivity analyses of the different input variables in the within-island LDA classification on principal component data (Figure S12), with relative importance computed for every machine.

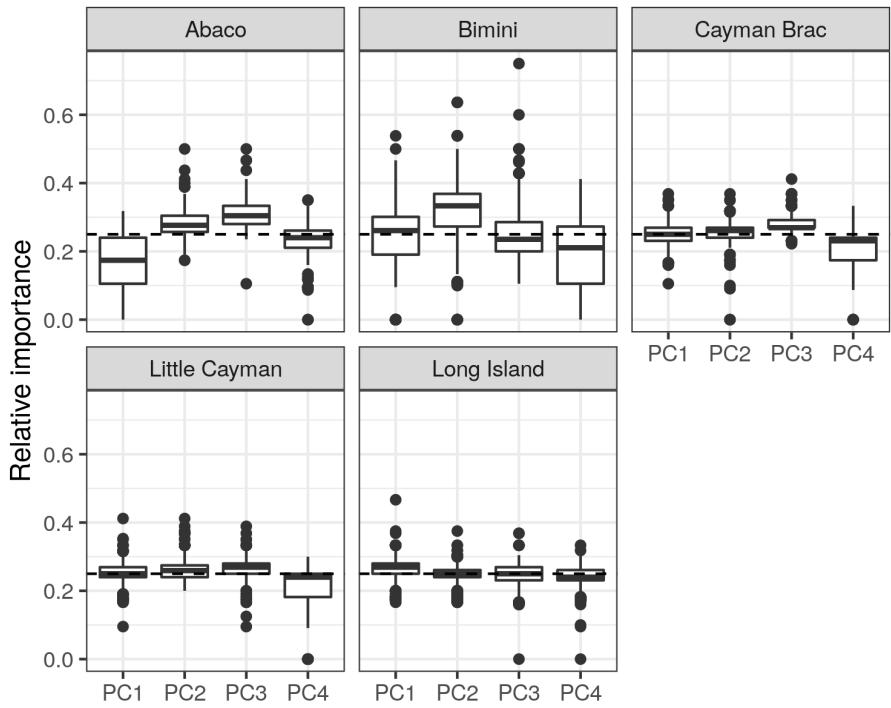


Figure S17: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance at 50nm-intervals in wavelength (Figure S13), with relative importance computed for every machine.

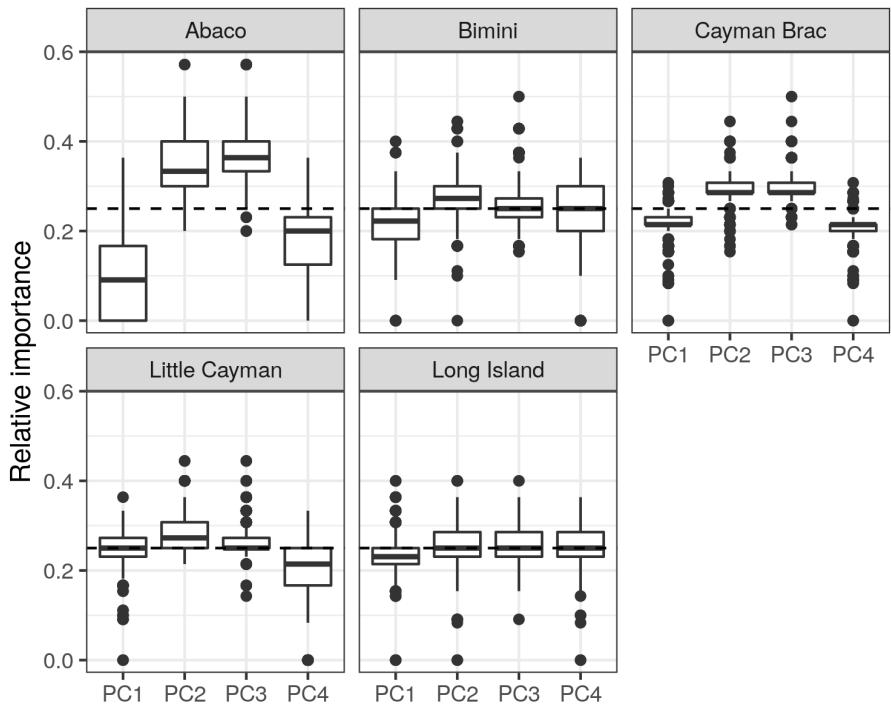


Figure S18: Sensitivity analyses of the different input variables in the archipelago-wide LDA classification on reflectance at 50nm-intervals in wavelength (Figure S14), with relative importance computed for every machine.

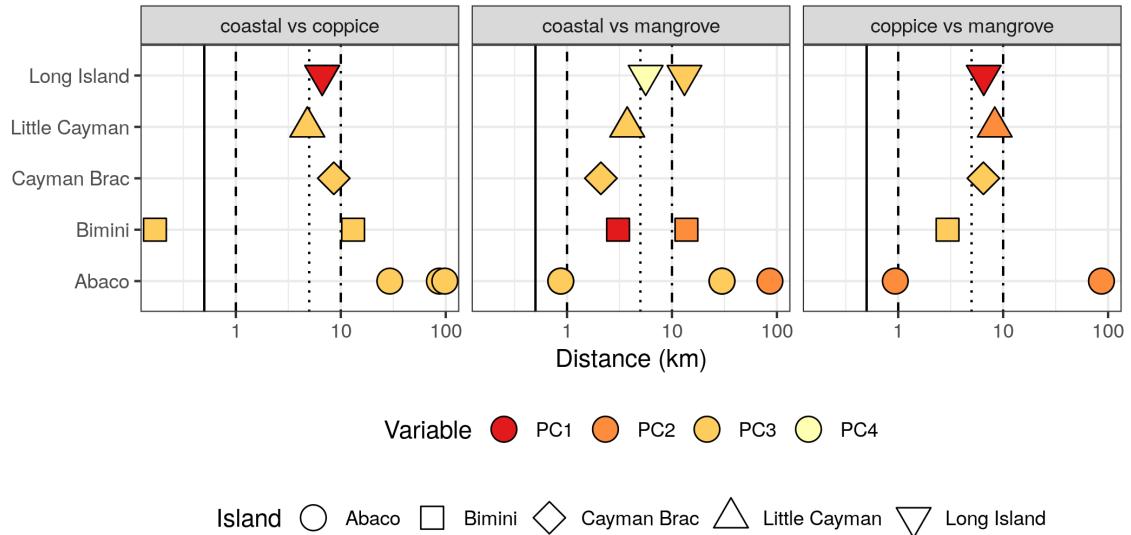


Figure S19: Spatial scale of between-habitat differences in dewlap coloration. For each variable and each pair of habitats where significant differences were detected (Figure 3), we performed multiple post hoc pairwise comparisons between the sites involved (Figure S1, Table S2), using nonparametric 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).

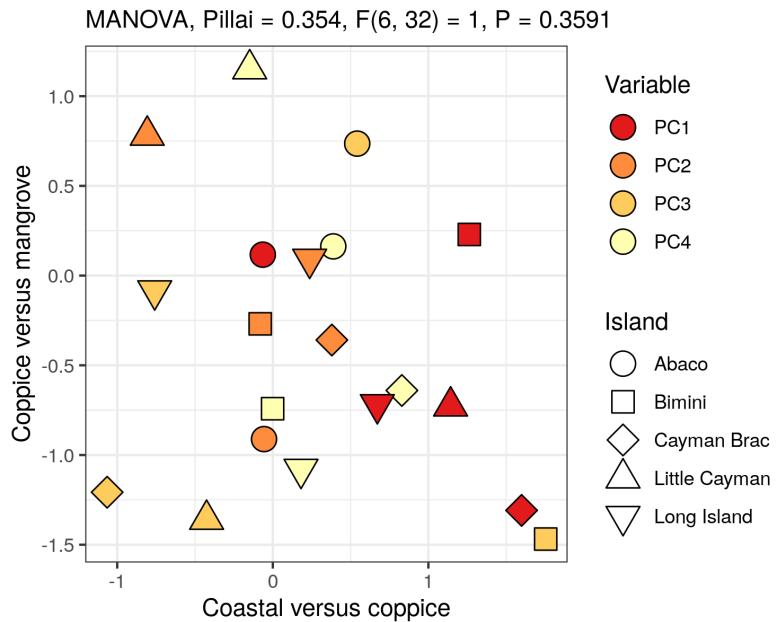


Figure S20: Test of parallel divergence between islands. Differences in habitat-means, or contrasts, are shown for two pairs of habitats for each principal component on each island, rescaled so the standard deviation of the means along each principal component is one. The contrasts represent the patterns 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.

## 815    Supplementary Tables

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.  $\chi^2$ , test statistic. \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

Island	$\chi^2$	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	$\Delta\text{AICc}$	AICcw	df <sub>LRT</sub>	Log-lik.	$\chi^2$	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;  $p_{\text{test}}$ , proportion of the data sampled to form the training set;  $n_{\text{test}}$ , number of observations in the testing set. P-values indicate deviations from the expected null binomial distribution, with  $n_{\text{test}}$  events per island and random guess success probability 1/3. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ .

Island	Accuracy	$N$	$p_{\text{test}}$	$n_{\text{test}}$	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	$\chi^2$	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