

# 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 some consistent 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. Although these habitat-specific dewlap differences vary in magnitude and direction across islands, and islands themselves differ substantially, we found a suite of consistent archipelago-wide differences between habitat types, suggesting parallel responses to similar selective pressures. 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

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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 signals.

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

Lizards of the neotropical genus *Anolis* are a model system for studying the eco-evolutionary dynamics of local adaptation and natural selection (Losos, 2009). A particularly conspicuous trait of anoles is their dewlap; an extensible flap of skin that is typically sexually dimorphic and used as a communication signal in courtship (Sigmund, 1983; Driessens et al., 2014, 2015), competition (Losos, 1985; Macedonia and Stamps, 1994; Macedonia et al., 2013) as well as in predator deterrence (Leal and Rodríguez-Robles, 1995, 1997; Leal and Rodriguez-Robles, 1997). Dewlap characteristics vary widely among the approximately 400 species of the genus (Nicholson et al., 2007). Interspecific variation in dewlap coloration is implicated in species recognition (Rand and Williams, 1970; Williams, 1969; Williams and Rand, 1977; Losos, 1985; Macedonia and Stamps, 1994; Fleishman, 2000; Macedonia et al., 2013), and possibly involved in speciation (Lambert et al., 2013; Geneva et al., 2015; Ng et al., 2017).

Within species, studies have shown a link between variation in dewlap coloration and differences in habitats or climatic conditions (Macedonia, 2001; Leal and Fleishman, 2002; Thorpe and Stenson, 2002; Thorpe, 2002; Leal and Fleishman, 2004; Vanhooydonck et al., 2009; Ng et al., 2012, 2013, 2016; Vanhooydonck et al., 2009; Driessens et al., 2017). Some studies suggest that those differences may be adaptive, and that dewlaps may have evolved to maximize detectability given local light conditions (Fleishman and Persons, 2001; Leal and Fleishman, 2002, 2004). Other studies testing this hypothesis, however, found no pattern (Fleishman et al., 2009; Ng et al., 2012; Macedonia et al., 2014).

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

The species *Anolis sagrei* is widespread across islands of the West Indies (Reynolds et al., 2020). It is a model organism in studies of local adaptation (Losos et al., 1994, 1997, 2001; Kolbe et al., 2012), biological invasion (Kolbe et al., 2008) and sexual selection (Tokarz, 2002; Tokarz et al., 2005; Tokarz, 2006; Driessens et al., 2014; Steffen and Guyer, 2014; Driessens et al., 2015). Between-island variation in the mainly orange-red color of its dewlap was shown to be better explained by climatic variables (Driessens et al., 2017) than 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 adaptation despite gene flow.

The island bank systems of the Bahamas and Cayman Islands 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). Each of these island groups (Caymans and Bahamas) has been colonized independently by *A. sagrei* from Cuba (Driessens et al. 2017; 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.

Here, we analyzed the color characteristics *A. sagrei* dewlaps within nine islands in the Bahamas and Cayman Islands, combining reflectance spectrometry and supervised machine learning. Our sampling design included sites in close proximity (the median distance between two sites within an island was 11.2km). 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, contrary 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). We found strong support for fine-scale, within-island differences in coloration between lizards inhabiting the three habitat-types in several color space dimensions, suggesting a potentially strong effect of divergent selection. However, the divergence patterns we observed did not match our *a priori* predictions and were inconsistent between islands. We found no evidence of isolation-by-distance as an explanation for the observed differences. Our results are nevertheless consistent with small-scale adaptive maintenance of signal polymorphism despite presumed considerable opportunity for gene flow, and might suggest that idiosyncrasies of local drift, selection, and gene flow contribute to differing outcomes in different populations.

## 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 and island banks 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 11.18km, with some islands being sampled at smaller or larger scales (Figure S1, Table S2). 80.3% of all pairwise distances within islands were below 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

148 taken with an Ocean Optics USB4000 spectrometer, a pulsed Xenon light source (PX-2, Ocean  
149 Optics, Largo, FL, USA) and a reflectance probe protected by a black anodized aluminum sheath.  
150 Measurements were taken with a 45-degree inclination to prevent specular reflection (Endler, 1990).  
151 The device was regularly standardized with a Spectralon white standard (Labsphere, North Sutton,  
152 NH, USA). Reflectance was measured at the center of the dewlap.

## 153 Analysis

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

### 155 Dimensionality reduction

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

### 172 Pooled analyses

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

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

### 191 Machine learning

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

195 Machine learning for group comparison has become more popular in ecology and evolution in  
196 recent years (e.g. Pigot et al. 2020). In particular, SVMs are designed to find the best possi-  
197 ble 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  $\sigma$  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.

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

264

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

270

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

## 277 Spatial autocorrelation

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

## 288 Site differences

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

## 295 Results

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

305

306 The small archipelago-wide effect of habitat-type was detected for PC1, PC2 and PC3 (mixed-  
307 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 pattern seemed to be driven by mangrove lizards being correctly reassigned more often than predicted by chance. Sensitivity analyses on these machines suggest a relatively small role of long wavelengths (red reflectance) in driving this pattern (Fig. S9), but did 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).

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. S12), 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. S13 and S14).

Differentiation in dewlap coloration occurred in multiple dimensions of color space. Moreover, the differences in dewlaps between habitats were not always consistent between 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 PC3 in determining between-group differences on Abaco, both in SVM and LDA classifiers (Fig. S15, S16), consistent with our ANOVA results (Fig. 3B). There was no strong signal of differences in relative importance among principal components on the other islands. Sensitivity analyses of SVMs trained on reflectance scores rather than principal components revealed, however, a consistently higher importance of ultraviolet reflectance in between-group differences on all islands (Fig. S17). This pattern was not recovered for LDAs trained on reflectance scores (Fig. S18).

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

**370**

**371** In contrast, differences in dewlap coloration between habitats were often detected in close ge-  
**372**ographical proximity. For example, Bimini, Cayman Brac, and Little Cayman were among the  
**373**smallest islands in our study (Fig. S1). Indeed, most significant differences in dewlap coloration  
**374**involved sites that were 5-10km apart. Our most extreme case of significant differences occurred for  
**375**PC3 between the beach scrub site and a coppice site, separated from each other by a few hundreds  
**376**of meters at most on Bimini (multiple pairwise Wilcoxon-Mann-Whitney tests, Fig. S19).

**377**

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

**392**

## **393** Discussion

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

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

**419**

Our results align with previous documented cases of persistent dewlap color divergence despite gene flow in multiple species of anoles, sometimes in relation to environmental conditions. Ng et al. (2012) and Ng et al. (2016) found divergent dewlap coloration in the face of gene flow between subspecies of *A. distichus* across 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).

**Dewlap coloration could be locally adapted** Although phenotypic divergence at small spatial scales may be an indicator of local adaptation, the inconsistency of the between-habitat divergence patterns we observed across islands implies that neutral processes cannot be definitely ruled out (Losos, 2011). Conversely, the absence of parallel divergence across islands does not rule out local adaptation either (Losos, 2011). If selection has an effect, it may be dependent on aspects of the environment that are not encompassed by our broad habitat-type classification into coastal dry scrub, primary coppice forest and mangrove forest, and dewlap coloration may be influenced by a mosaic of interacting local environmental factors, which need not be the same across islands.

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. 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 (Vanhoydonck et al., 2009; Baeckens et al., 2018). In particular, Baeckens et al. (2018) recently showed that dewlaps with spotted patterns occurred more often in *A. sagrei* on islands with more coexisting species of anoles. Our data are consistent with those previous results in suggesting that adaptation to local light conditions, or at least broad habitat types, may not be a major driver of the variation in dewlap coloration in *A. sagrei*.

**Sexual selection could be at play** Sexual selection is an important component of an adaptive, sensory drive hypothesis of dewlap color divergence across environmental conditions, because in this scenario habitat-dependent selection is executed by the signal recipients (Endler and McLellan, 1988), such as mating partners (Driessens et al., 2014). An alternative yet non-mutually exclusive explanation for the observed idiosyncratic patterns of within-island divergence is that of "Fisherian" sexual selection (Andersson, 1994). Under the Fisherian model of sexual selection, arbitrary female preferences (i.e. independent of the environment) for certain male ornaments may drive divergent evolution of male signals, such as dewlap coloration, if female preferences differ between localities for other reasons than environment-dependent perception abilities (a situation that could have arisen e.g. in an initial phase of genetic 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 explanation. Therefore, arbitrary, habitat-independent female preferences could fur-

476 ther explain the inconsistent patterns of divergence across islands. In contrast with this, however,  
477 Baeckens et al. (2018) found no link between *A. sagrei* dewlap coloration and size dimorphism (a  
478 proxy for sexual selection) in an among-island study of the same archipelagos.

479       Besides, although this process is well-established in some systems such as African cichlids (e.g.  
480 Seehausen 1997), previous work suggests that dewlap coloration in *Anolis* may act as a mating cue  
481 according to a "good genes" model of sexual selection (Andersson, 1994), rather than a Fisherian  
482 process. In the good genes model, the cue under sexual selection is an indicator of individual  
483 quality (e.g. better immune response to disease) and of indirect benefit to the offspring. For ex-  
484 ample, Cook et al. (2013) found lower orange reflectance in dewlaps with heavily parasitized *A.*  
485 *brevirostris*, suggesting a trade-off in carotenoid use between the immune response and pigment  
486 deposition. Steffen and Guyer (2014) found that lower UV and orange-red reflectance predict  
487 contest-winning success between *A. sagrei* males, while Driessens et al. (2015) further found that  
488 more yellow and red dewlaps (relative to UV) predict better body condition, and that higher yellow  
489 and UV reflectance at the margin of the dewlap predict higher hematocrit (the concentration of  
490 red blood cells), indicating a better health. Other aspects of the dewlap than color have also been  
491 found to be indicators of individual quality, such as dewlap size (Vanhooydonck et al., 2005, 2009),  
492 but not dewlap display frequency (Tokarz, 2002; Tokarz et al., 2005; Driessens et al., 2015).  
493

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495       **A role of phenotypic plasticity is unlikely** Differences in coloration between habitat popu-  
496 lations may not be genetically determined, and may be influenced by environmental factors such  
497 as parasite load, as mentioned above (Cook et al., 2013). The yellow, orange and red coloration in  
498 anoline dewlaps are produced by pterins and carotenoids (Ortiz, 1962; Ortiz et al., 1962; Ortiz and  
499 Williams-Ashman, 1963; Ortiz and Maldonado, 1966; Macedonia et al., 2000; Steffen and McGraw,  
500 2007, 2009). Animals lack the ability to synthesize carotenoids, and those must therefore be found  
501 in the diet, while pterins are synthesized from nucleotides (Goodwin, 1984; Hill et al., 2002; Hill  
502 and McGraw, 2006). However, experimental manipulation of dietary carotenoid content showed  
503 no effect on dewlap coloration in *A. sagrei* (Steffen et al., 2010) nor in *A. distichus* (Ng et al.,  
504 2013), which also has an orange-based dewlap. This makes a plastic response to differences in  
505 diet across habitats unlikely. Furthermore, developmental plasticity during the ontogeny is also  
506 unlikely because dewlap coloration develops at sexual maturity in anoles (Ng et al., 2013). The  
507 differences we observed could therefore be heritable. This hypothesis is further supported by Cox  
508 et al. (2017), who found a high degree of heritability of dewlap coloration in *A. sagrei*. Although  
509 most studies used one or two-generation common garden experiments and thus could not rule out  
510 transgenerational plastic effects (Tariel et al., 2020), dewlap coloration generally seems to not be a  
511 plastic trait. This further reinforces an adaptive explanation, where dewlap color could be under  
512 differential natural and/or sexual selection in these different habitats.

513

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

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532

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**541 Figures**

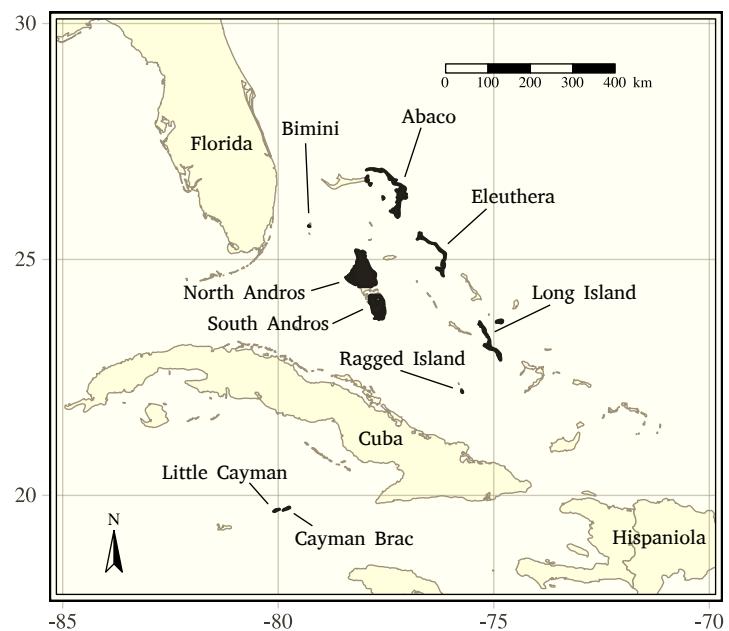


Figure 1: Map of the West Indies with sampled islands highlighted in black.

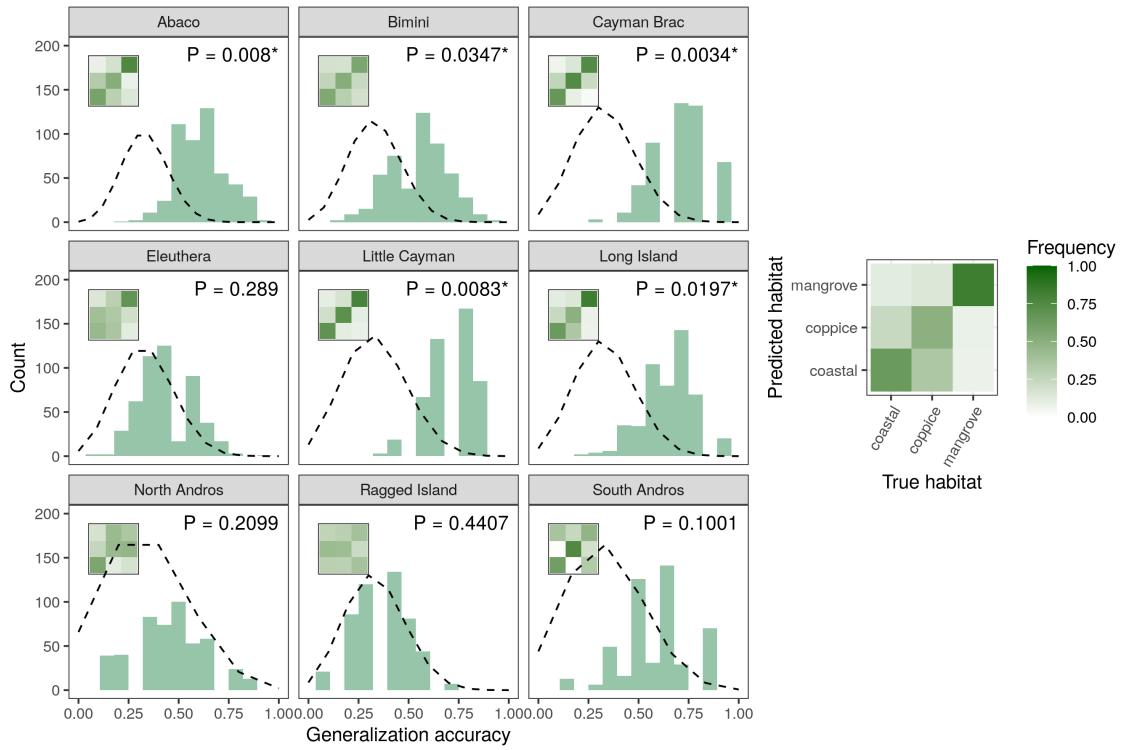


Figure 2: SVM classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The dashed line is the density of a corresponding null binomial distribution, which would be expected under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

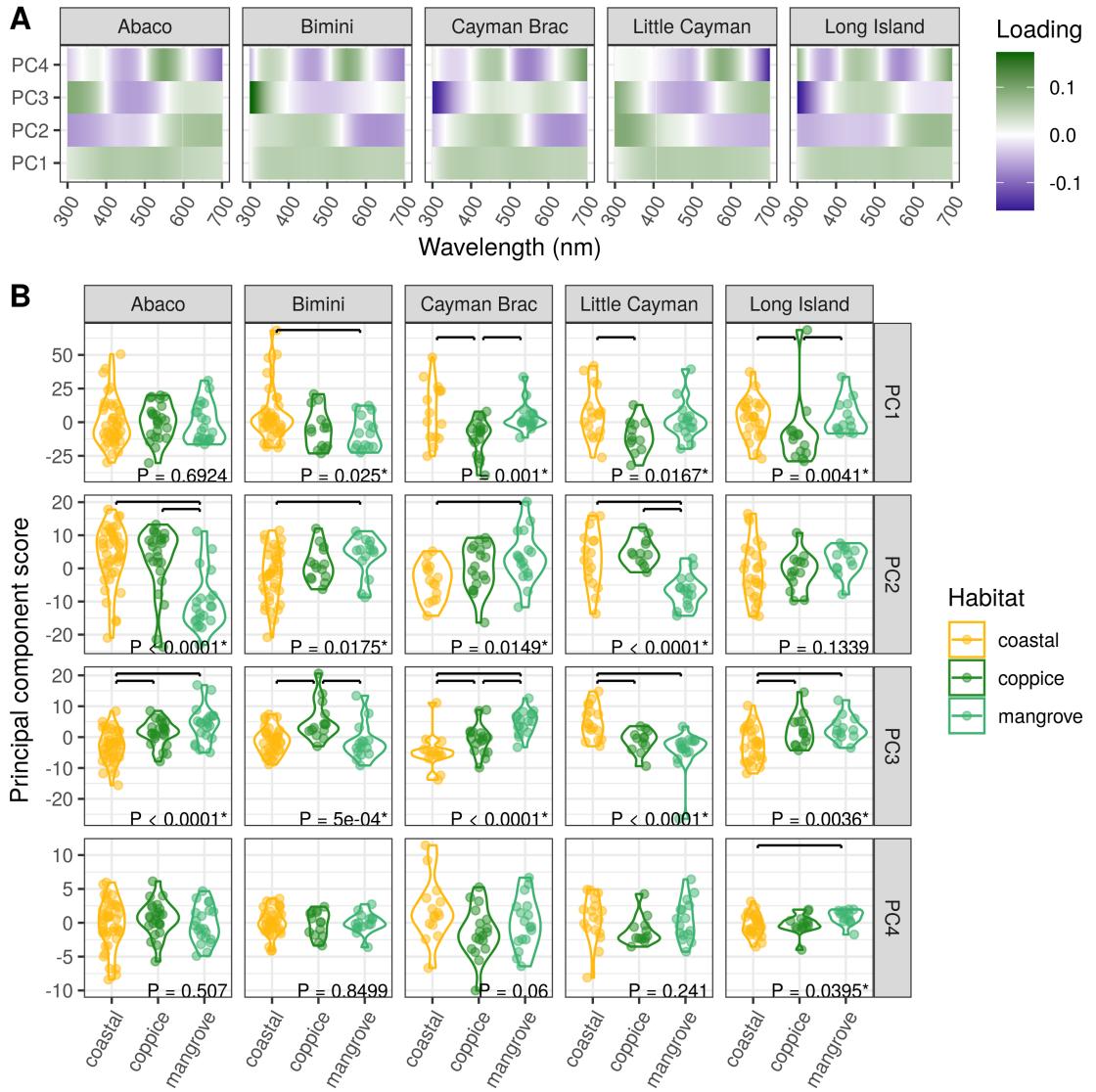


Figure 3: Dewlap color variation between habitat-types across the most significant islands. (A) Mapping of reflectance at various wavelengths onto the principal components (loadings from the PCA rotation matrix). (B) Distribution of PC scores between habitats along the first four PCs on each island where 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.  $\Delta \text{AICc}$ , 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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## 829 Supplementary Figures

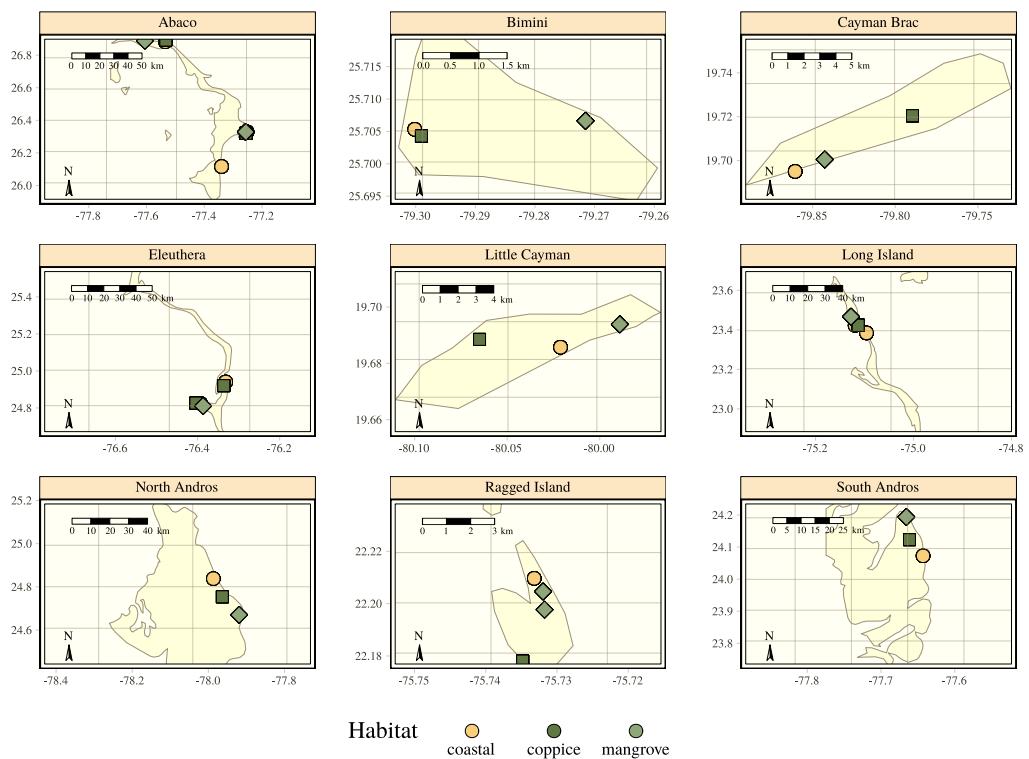


Figure S1: Map of the sampling sites and corresponding habitats across nine islands of the West Indies.

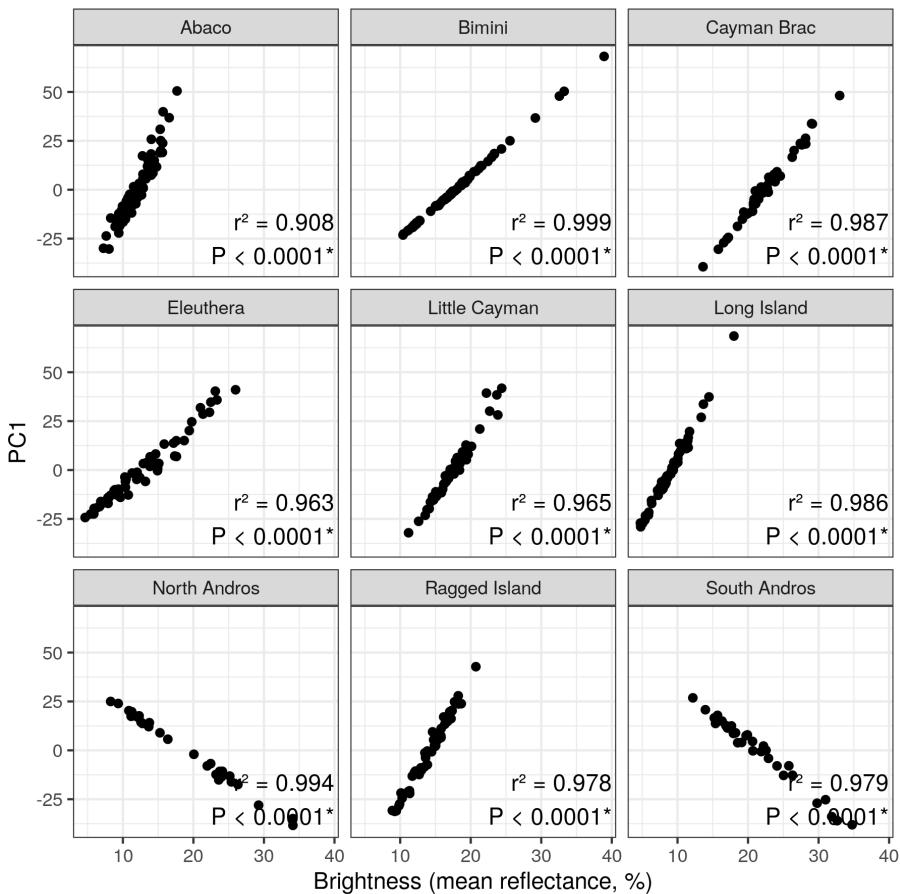


Figure S2: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score for each island. Pearson's squared correlation coefficients are reported. \*,  $P < 0.05$ .

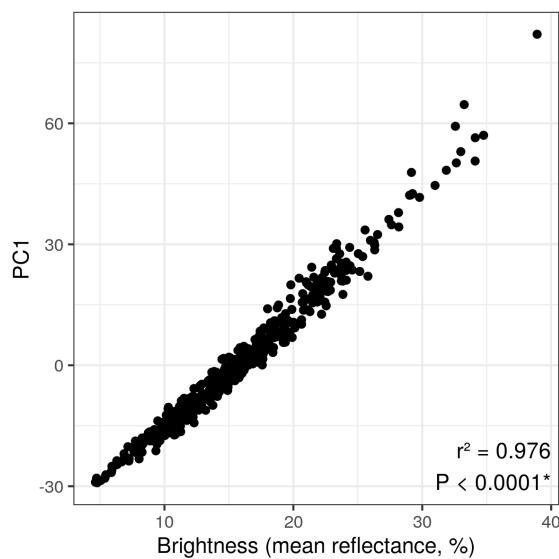


Figure S3: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across the whole archipelago. Pearson's squared correlation coefficient is reported. \*,  $P < 0.05$ .

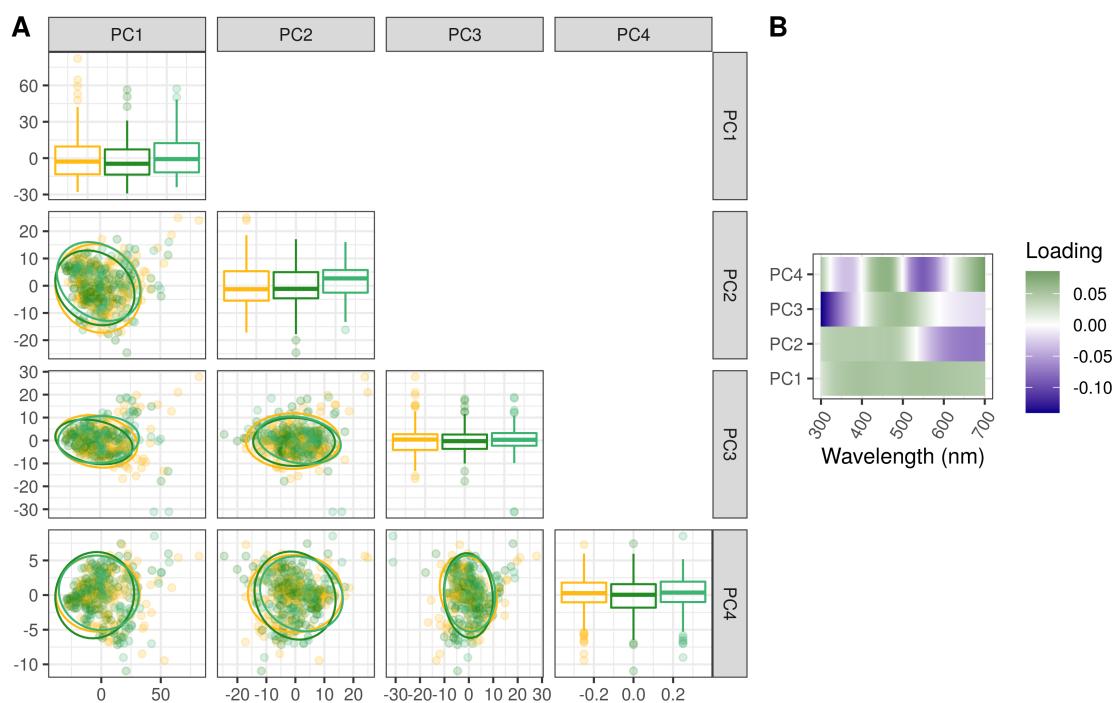


Figure S4: (A) Principal component scores and 5% confidence ellipses across habitats for the whole archipelago. The principal component analysis was performed on reflectance data from all islands pooled together. (B) PCA rotation matrix showing the loadings of each wavelength from 300 to 700nm onto the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

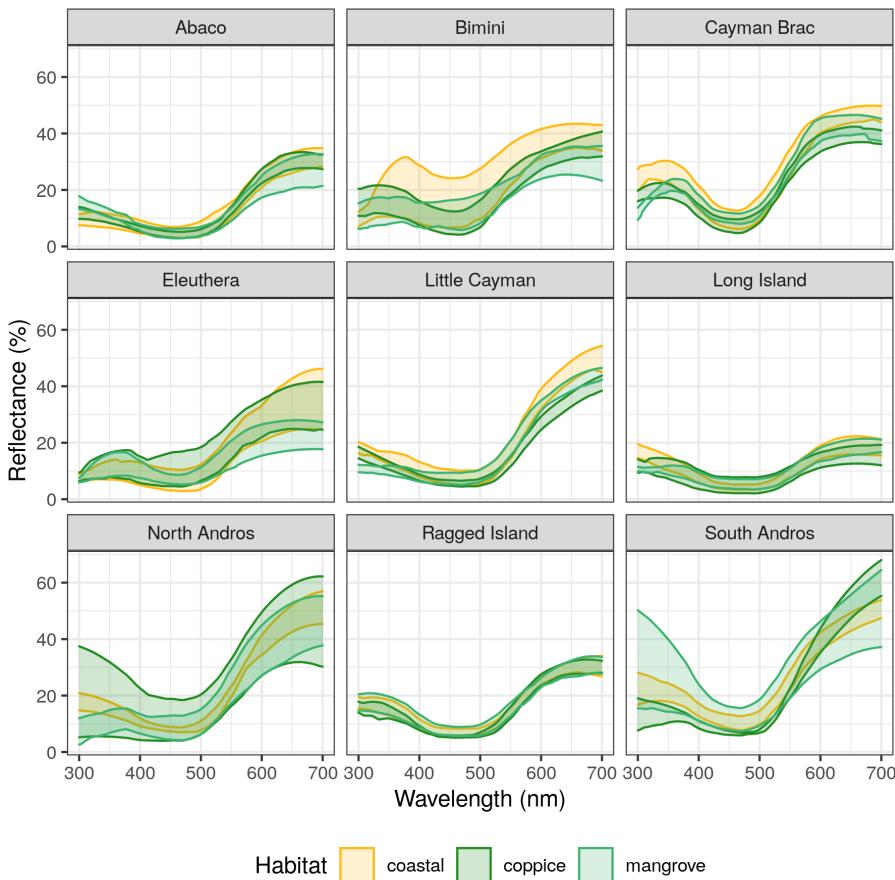


Figure S5: 5-95th percentile range of lizard dewlap reflectance values (in % of incoming light) across wavelengths for each island and each habitat.

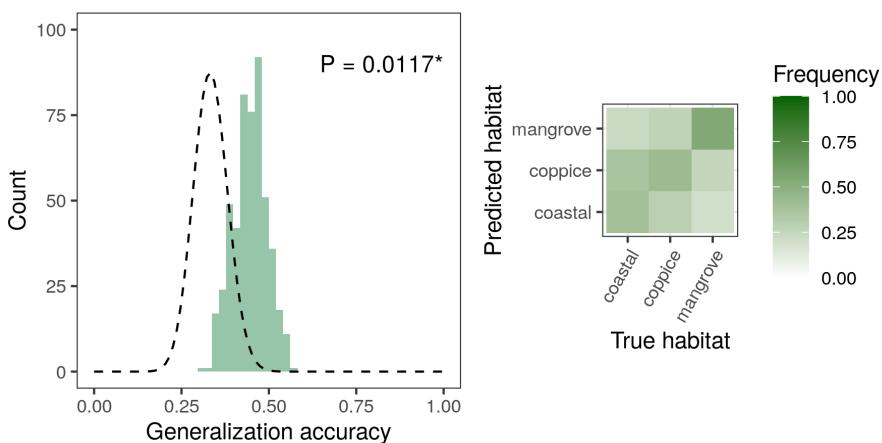


Figure S6: Archipelago-wide SVM classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure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

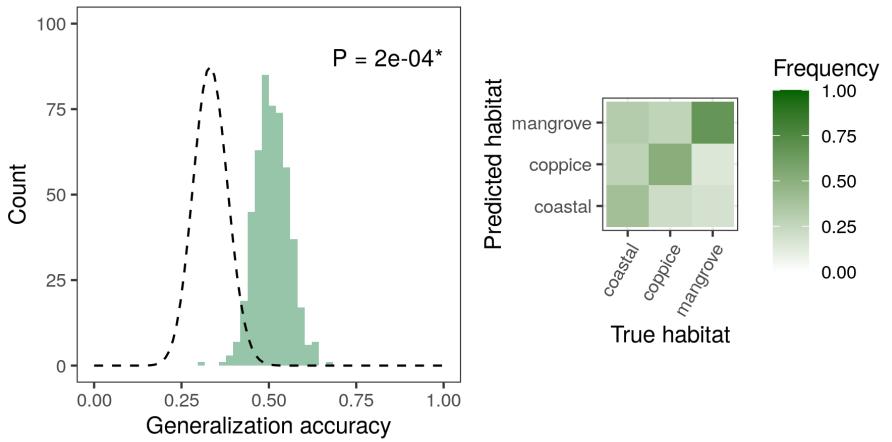


Figure S7: Archipelago-wide SVM classification accuracy based on reflectance data at 50nm-intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation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

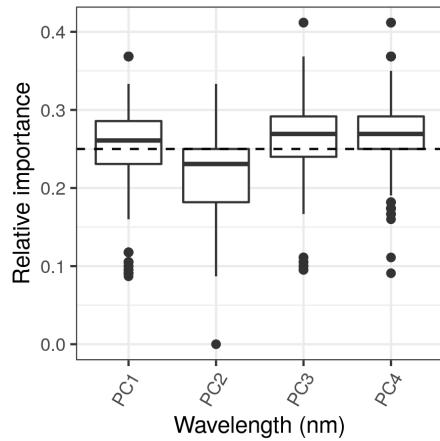


Figure S8: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on principal component data (Figure S6), with relative importance computed for every machine.

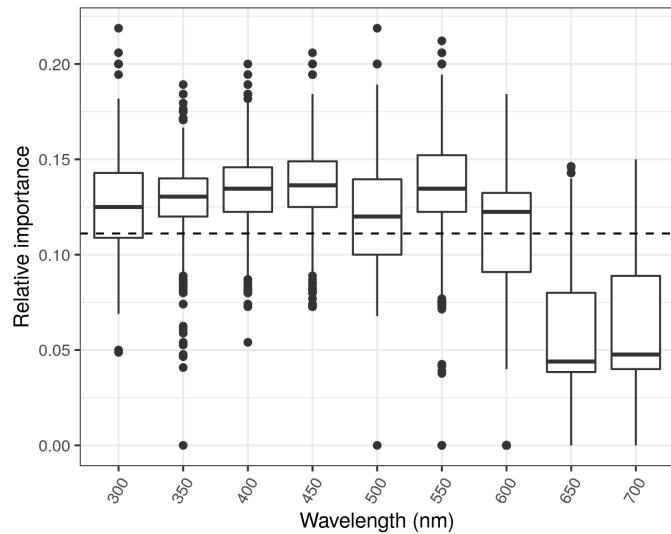


Figure S9: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance data at 50nm-intervals in wavelength (Figure S7), with relative importance computed for every machine.

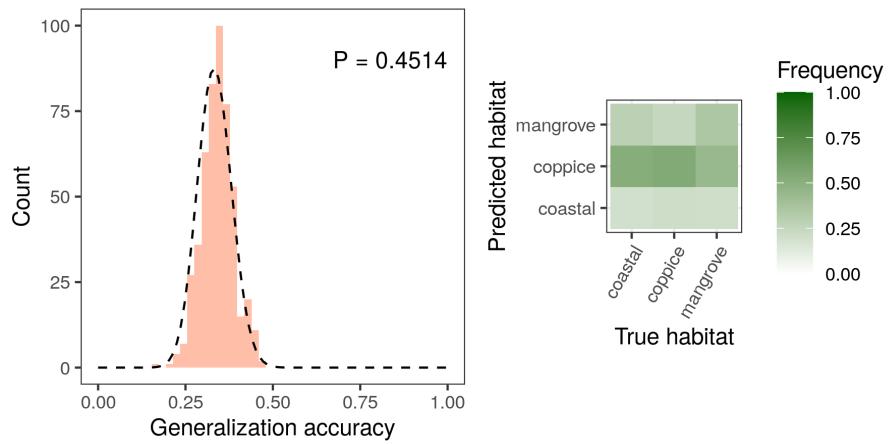


Figure S10: Archipelago-wide LDA classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure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

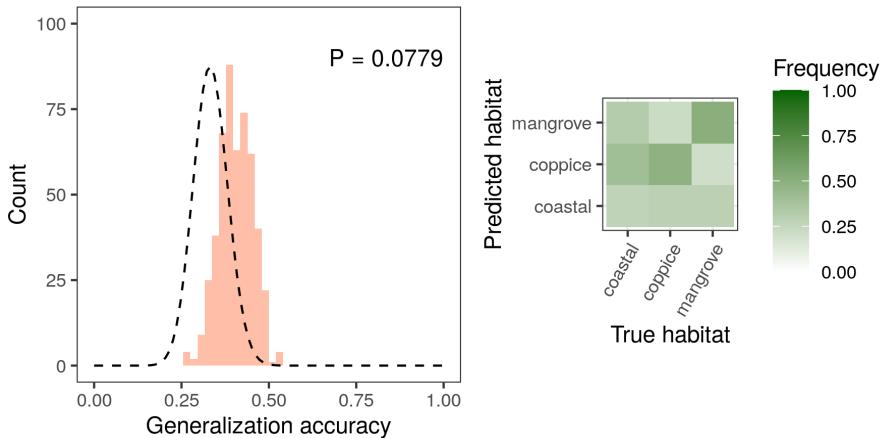


Figure S11: Archipelago-wide LDA classification accuracy based on reflectance data at 5nm intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 2.

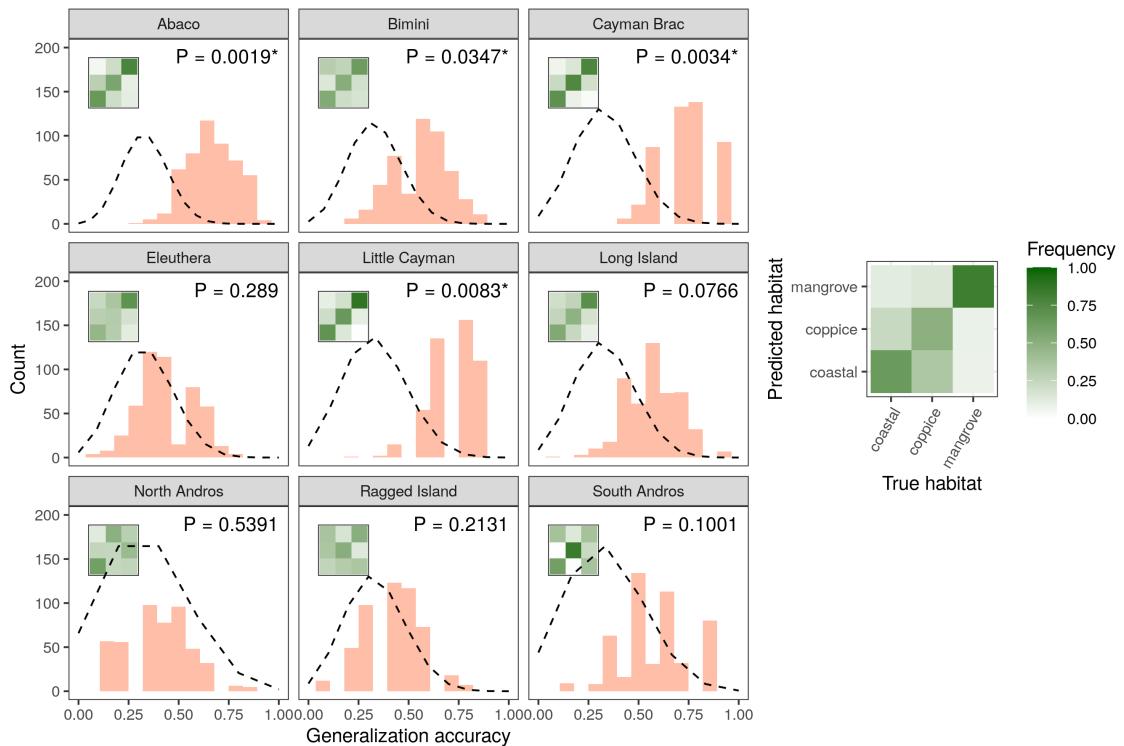


Figure S12: LDA classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

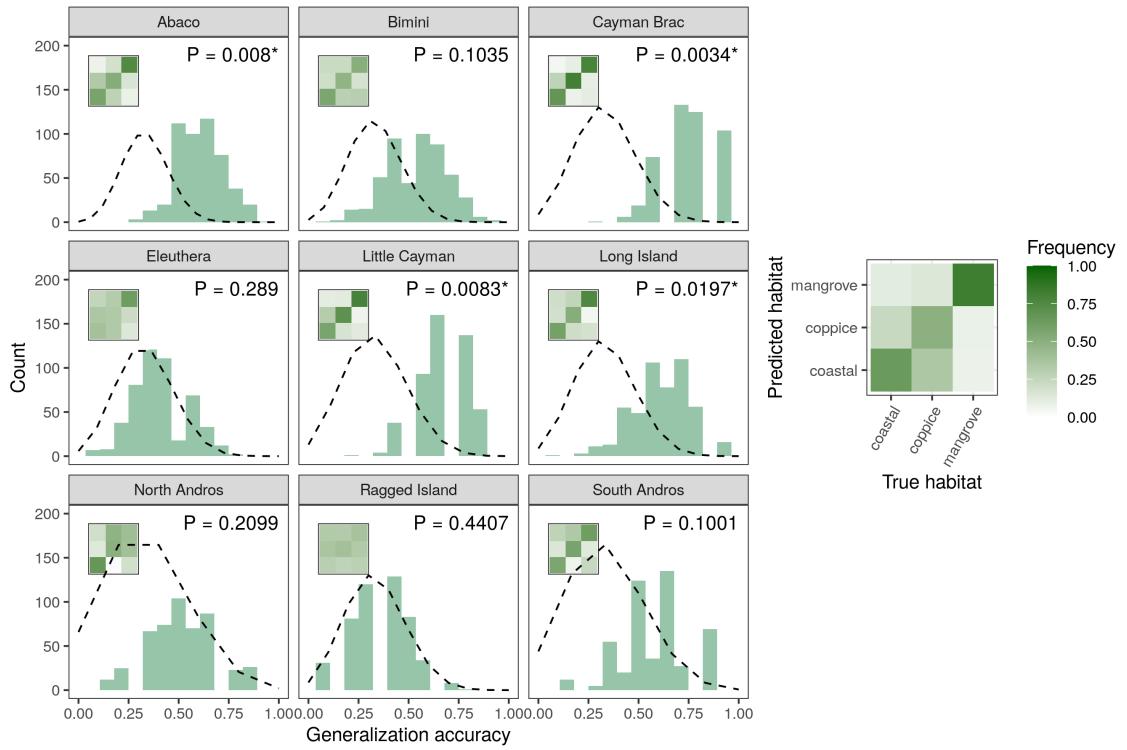


Figure S13: SVM classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

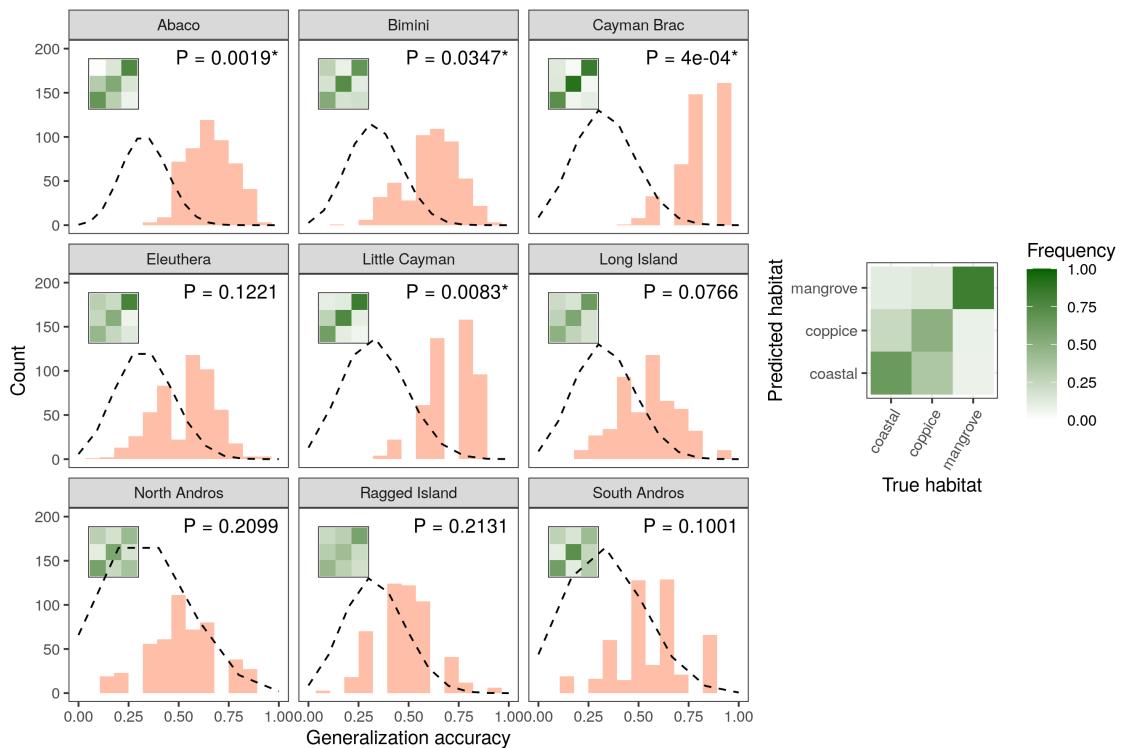


Figure S14: LDA classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

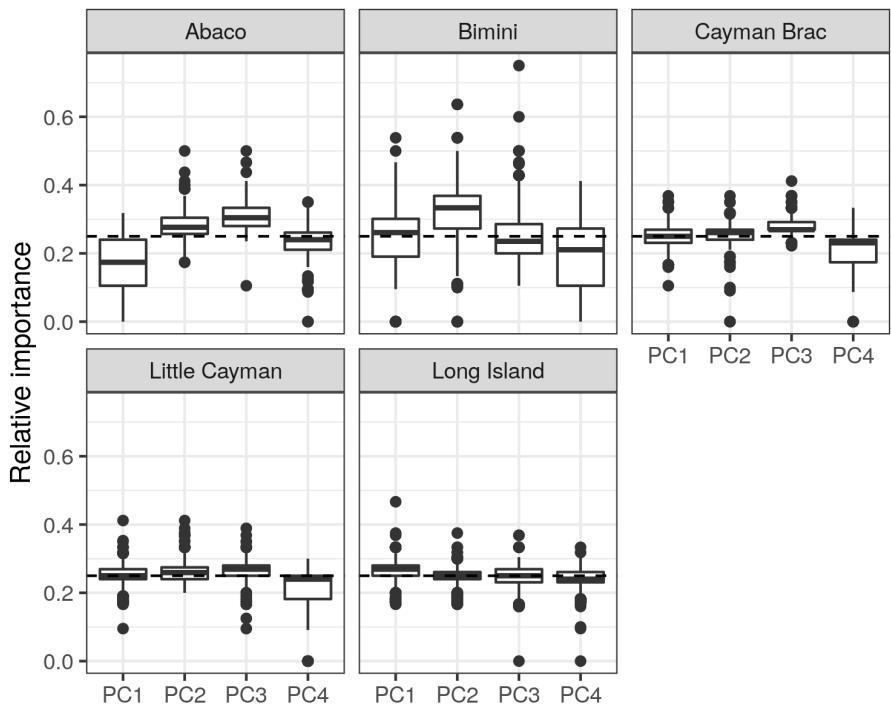


Figure S15: Sensitivity analyses of the different input variables in the within-island SVM classification on principal component data (Figure ??), with relative importance computed for every machine.

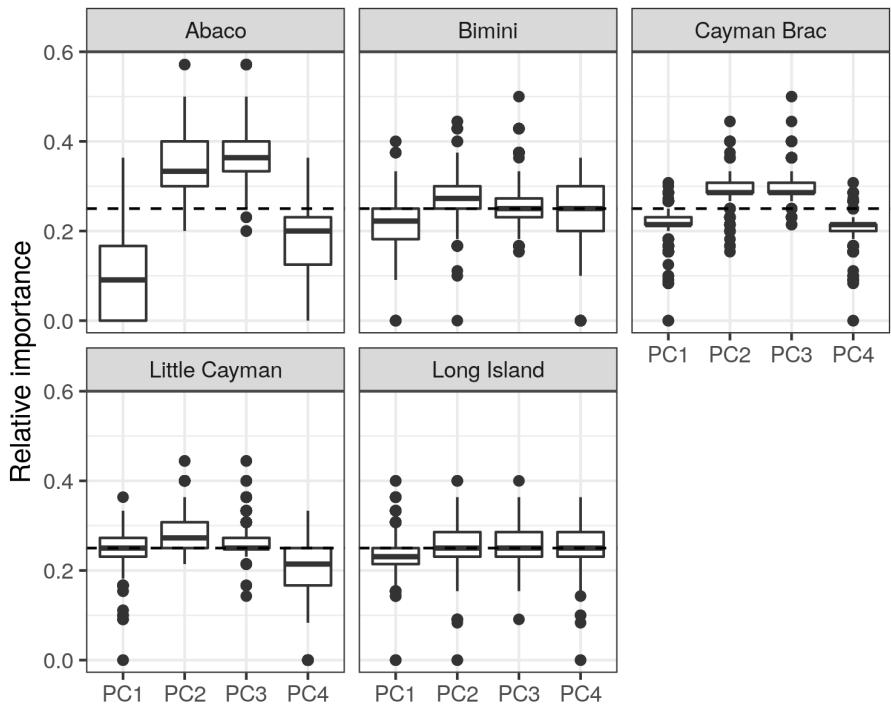


Figure S16: Sensitivity analyses of the different input variables in the within-island LDA classification on principal component data (Figure S12), with relative importance computed for every machine.

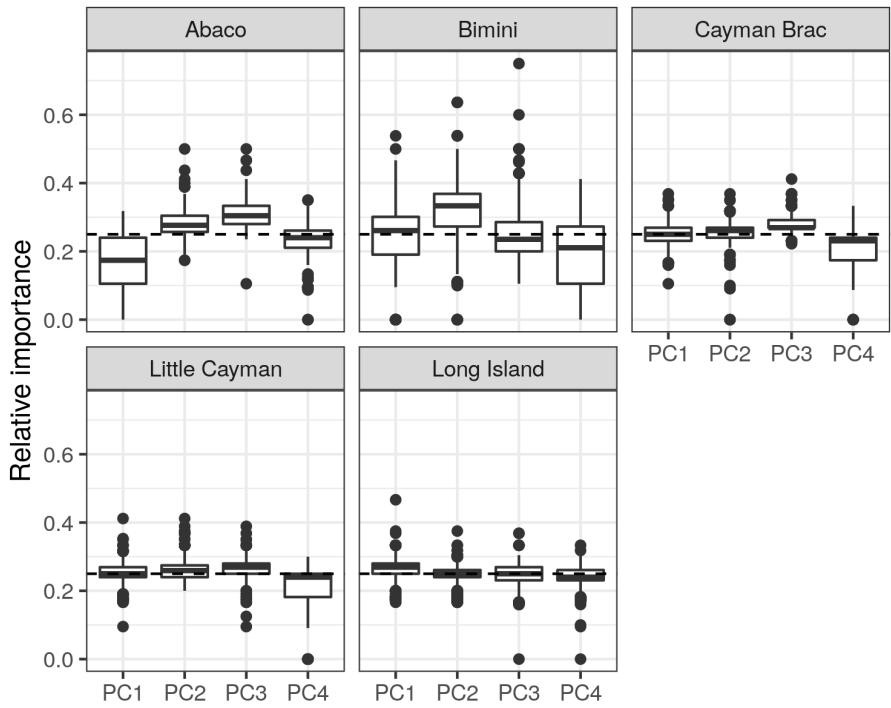


Figure S17: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance at 50nm-intervals in wavelength (Figure S13), with relative importance computed for every machine.

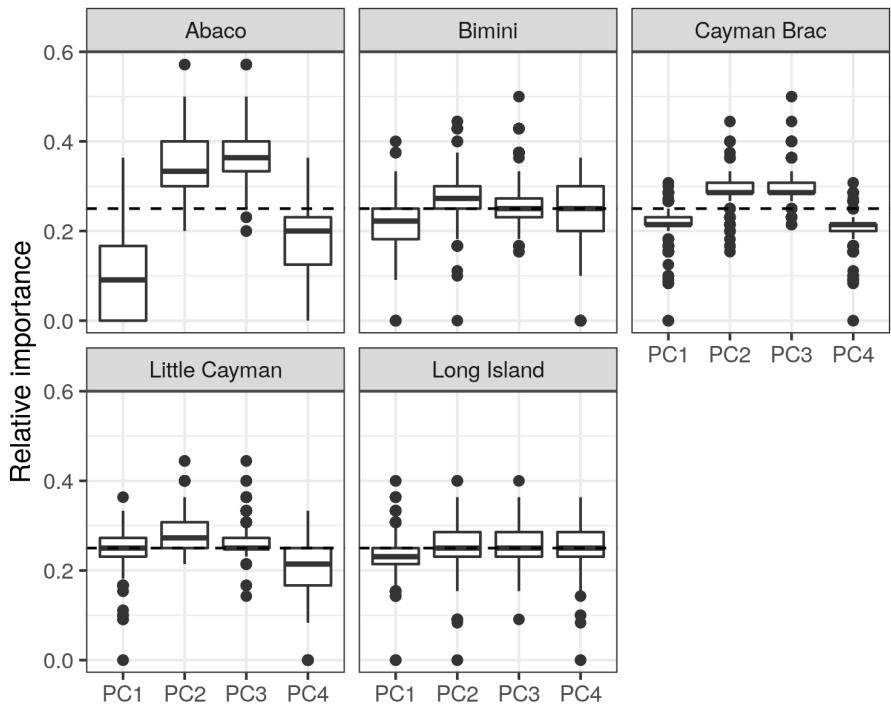


Figure S18: Sensitivity analyses of the different input variables in the archipelago-wide LDA classification on reflectance at 50nm-intervals in wavelength (Figure S14), with relative importance computed for every machine.

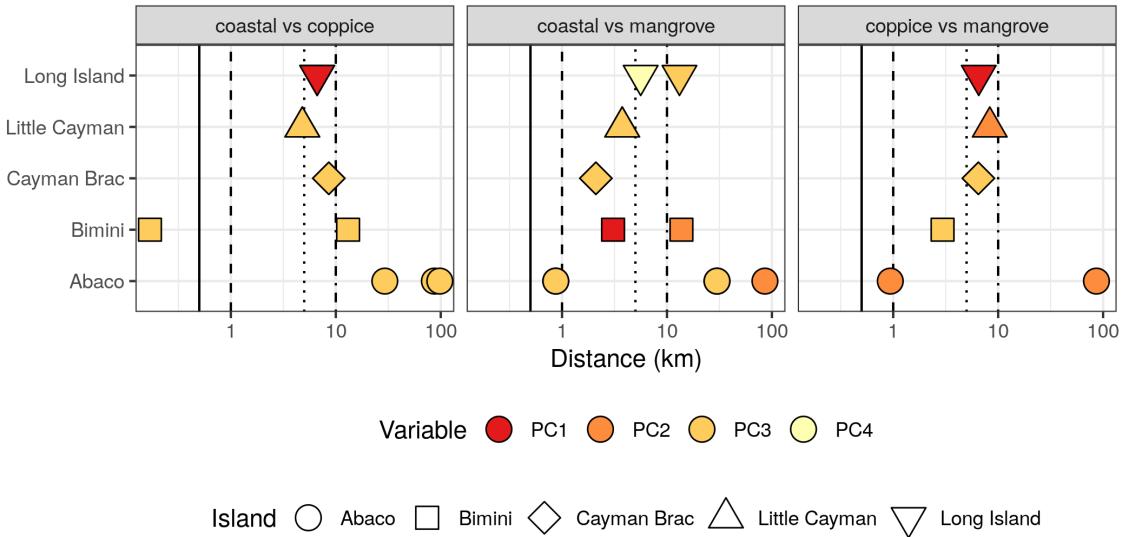


Figure S19: Spatial scale of between-habitat differences in dewlap coloration. For each variable and each pair of habitats where significant differences were detected (Figure 3), we performed multiple post hoc pairwise comparisons between the sites involved (Figure S1, Table S2), using nonparametric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

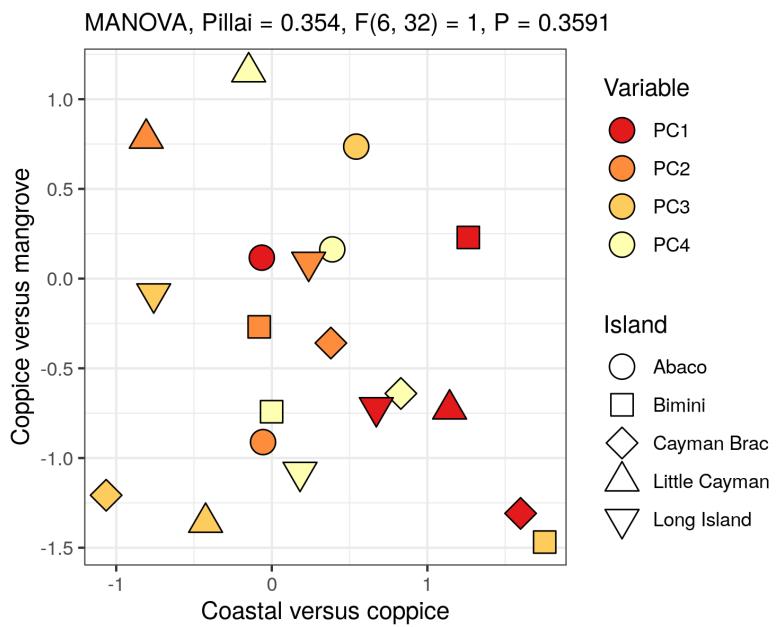


Figure S20: Test of parallel divergence between islands. Differences in habitat-means, or contrasts, are shown for two pairs of habitats for each principal component on each island, rescaled so the standard deviation of the means along each principal component is one. The contrasts represent the patterns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

## 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.  $\chi^2$ , 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	$\text{df}_{\text{LRT}}$	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