

# 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 one widespread species, *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 competition for mates, species recognition, aposematism, cooperation, etc. (Bradbury and Vehrenamp, 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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39 Halfwerk et al., 2014). This selective pressure may drive the local adaptation of communication  
40 signals.

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

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

68 Within species, studies have shown a link between variation in dewlap coloration and differ-  
69 ences in habitats or climatic conditions (Macedonia, 2001; Leal and Fleishman, 2002; Thorpe and  
70 Stenson, 2002; Thorpe, 2002; Leal and Fleishman, 2004; Vanhooydonck et al., 2009; Ng et al.,  
71 2012, 2013, 2016; Vanhooydonck et al., 2009; Driessens et al., 2017). Some studies suggest that  
72 those differences may be adaptive, and that dewlaps may have evolved to maximize detectability  
73 given local light conditions (Fleishman and Persons, 2001; Leal and Fleishman, 2002, 2004). Other  
74 studies testing this hypothesis, however, found no pattern (Fleishman et al., 2009; Ng et al., 2012;  
75 Macedonia et al., 2014).

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

83 The species *Anolis sagrei* is widespread across islands of the West Indies (Reynolds et al.,  
84 2020). It is a model organism in studies of local adaptation (Losos et al., 1994, 1997, 2001; Kolbe  
85 et al., 2012), biological invasion (Kolbe et al., 2008) and sexual selection (Tokarz, 2002; Tokarz  
86 et al., 2005; Tokarz, 2006; Driessens et al., 2014; Steffen and Guyer, 2014; Driessens et al., 2015).  
87 Between-island variation in the mainly orange-red color of its dewlap was shown to be better ex-  
88 plained by climatic variables (Driessens et al., 2017) than biotic factors such as sexual selection or  
89 predation pressure (Vanhooydonck et al., 2009; Baeckens et al., 2018). How intra-island differences  
90 in habitat may contribute to the diversity of dewlap coloration, however, remains unexplored, and  
91 may reveal new insights into the scale of local adaptation despite gene flow.

The island bank systems of the Bahamas and Cayman Islands 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 islands has been colonized independently by *A. sagrei* (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 the hypothesis that the spatial scale was too small for phenotypic divergence to build up. If this was not the case, 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 expectations and were highly variable 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.

## Methods

### Data collection

We sampled 466 lizards 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 West Indian range of *Anolis sagrei*. 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 . 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 ??). 80.3% of all pairwise distances within islands were below 50km. Additionally, there are no major barriers to dispersal (such as mountains) 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.

148    **Analysis**

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

150    **Dimensionality reduction**

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

167    **Pooled analyses**

168    In addition to within-island PCA, we performed a PCA on pooled data from the whole archipelago.  
169    Explain what wavelengths mapped onto what PCs (Table suptab:pcavariances). We used this  
170    dataset to partition the variance in dewlap coloration among islands, habitats and habitats within  
171    islands, using a two-way multivariate analysis of variance (MANOVA) with an interaction term.  
172    However, because the assumptions of parametric MANOVA were violated for all islands but Ragged  
173    Island (multivariate normality and homogeneity of covariance matrices, Tables suptab:multinorm,  
174    suptab:covariance), we used a semi-parametric MANOVA instead (R package MANOVA.RM, citation),  
175    with P-values calculated from a bootstrap procedure with 1,000 iterations. We calculated the  
176    proportion of variance explained by islands, habitats and the habitat-by-island interaction using  
177    partial effect sizes  $\eta^2$  on a MANOVA-approximation of the analysis (R package heplots).

178    **Machine learning**

179    Our data violated the multivariate analysis of variance (MANOVA) assumption of homogeneity  
180    of covariance matrices across groups for all islands but Ragged Island (see previous section, Box  
181    1949; Morrison 1988, implemented in the R package heplots, Fox et al. 2018, Table ??). We also  
182    detected within-habitat deviations from multivariate normality, primarily on Abaco, Bimini and  
183    Eleuthera (Henze-Zirkler's test, Henze and Zirkler 1990, implemented in the R package MVN,  
184    Korkmaz et al. 2014, Table ). For these reasons and to reduce the chances of false discovery, we  
185    conducted multivariate group comparisons using support vector machines (SVMs), a model-free,  
186    nonparametric supervised machine learning technique.

187    Machine learning for group comparison has become more popular in ecology and evolution in  
188    the recent years (e.g. Pigot et al. (2020)). In particular, SVMs are designed to find the best pos-  
189    sible nonlinear boundaries between labelled groups of points in multidimensional spaces, without  
190    assumptions about the distribution of the data (Cortes and Vapnik, 1995; Cristianini and Shawe-  
191    Taylor, 2000; Kim and von Oertzen, 2018). This makes them well suited to field biological data,  
192    which often violate the assumptions of classical linear modeling (Kim and von Oertzen, 2018) and  
193    can be, as in the case of coloration, inherently highly multivariate (Cuthill et al., 1999). First,  
194    a machine is trained to recognize differences between groups within a subset of the data called  
195    the training set. Significance of differences is then assessed by testing the accuracy of that fitted  
196    machine in predicting the group-labels of data points that were not included in the training, called  
197    a testing set, based solely on their multivariate coordinates. This cross-validation procedure re-  
198    sults 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 (citation) 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 (R pacakge rminer) 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 multinorm), we assessed univariate normality for each principal component (Shapiro's test, Table normality). For skewed PCs that deviated significantly from normality, we repeated the analysis using a nonparametric Kruskal-Wallis tests. We found no multivariate outliers based on the Mahalanobis distance (package). We used the cases of better fit of the GLS model relative to the OLS model as evidence for heterogeneity of variances, which were then accounted for by the GLS approach (Table anova).

256 Significant *post hoc* contrasts were assessed using Tukey's Honest Significant Difference (HSD)  
257 test whenever the assumptions of normality and homogeneity of variances was met, Dunnett's T3  
258 method when only homogeneity of variances was violated but not normality, and Nemenyi's test  
259 when normality was violated.

260

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

## 267 Spatial autocorrelation

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

## 278 Site differences

279 In this study, we were interested in the minimum spatial scale at which significant differences  
280 between habitats could be detected within islands. We performed multiple pairwise nonparametric  
281 Wilcoxon tests to compare dewlap coloration between sites with different habitat-types, for each  
282 pair of habitats and each variable where significant differences were detected with our analyses of  
283 variances.

## 284 Results

285 We tested for variation in *A. sagrei* dewlap coloration between populations living in three charac-  
286 teristic habitat types across nine islands that span the West Indian range of the brown anole (Fig  
287 1, [S1](#)). We found that most of the variation in coloration partitioned between islands (two-way  
288 semi-parametric MANOVA, modified ANOVA-type statistic (MATS) = 2009.6,  $P < 0.001$ , Fig.  
289 [S4](#), explained variance  $\eta^2 = 44.3\%$ , MANOVA approximation). Nonetheless, we did find evidence  
290 for differences in dewlap coloration between habitat-types, and those were mostly island-specific  
291 (habitat-by-island interaction term, MATS = 384.4,  $P < 0.001$ , explained variance  $\eta^2 = 11.4\%$ ),  
292 leaving a small but significant portion of the variation explained by an archipelago-wide habitat  
293 effect (MATS = 42.5,  $P = .001$ ,  $\eta^2 = 4.8\%$ ).

294

295 The small archipelago-wide effect of habitat-type was detected for PC1, PC2 and PC3 (mixed-  
296 effect ANOVA with island as a random effect, Table ??, backed up by KW), but this effect was  
297 too small for post hoc tests to find which habitats differed. Archipelago-wide differences in dewlap  
298 coloration between habitats were also detected by SVMs trained on pooled data regardless of island  
299 identity, both for PCA data and reflectance scores (Fig. ??, ??). This pattern seemed to be driven  
300 by mangrove lizards being correctly reassigned more often than predicted by chance. Sensitivity  
301 analyses on these machines suggest a relatively small role of long wavelengths (red reflectance) in  
302 driving this pattern (Fig. ??), but did not reveal strong differences between the PCs in relative  
303 importance (Fig. ??). Archipelago-wide differences were not detected by LDA classifiers at all  
304 (Fig. ??, ??). So the difference could be in something else than means.

305

306 Within islands, SVM classifiers correctly assigned individuals to their habitat of origin based  
307 solely upon dewlap coloration on five islands: Abaco, Bimini, Cayman Brac, Little Cayman, and

Long island (Fig. ??). An LDA approach yielded similar success rates (Fig. ??), 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 ??). 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. ?? and ??).

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. ??, Table , Table KW). Figure ??A 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. ??, ??), consistent with our ANOVA results (Fig. ??B). 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. ??). This pattern was not recovered for LDAs trained on reflectance scores (Fig. ??).

We did not find significant spatial autocorrelation between the sampling sites on the islands where we detected a significant habitat effect. We did, however, detect a significant positive signal of autocorrelation on Eleuthera ( $P = 0.02$ , Table ), suggesting possible color differentiation through isolation-by-distance on this island.

In contrast, differences in dewlap coloration between habitats were often detected in close geographical proximity. For example, Bimini, Cayman Brac and Little Cayman were among the smallest islands in our study (Fig. S1). Besides, for pairs of habitats where significant differences in dewlap coloration were detected along some principal components, comparisons of the actual sampling sites indicate that the most detectable differences involved sites that were mostly 5-10km

366 apart. Our most extreme case of significant differences occurred for PC3 between the beach scrub  
367 site and a coppice site, separated from each other by a few hundreds of meters at most on Bimini  
368 (Wilcoxon test, Fig. S18, Table ??).

369  
370 Patterns of differentiation were inconsistent across the five most significant islands. Contrasts  
371 in principal components between habitats, calculated on pooled data from the whole archipelago,  
372 were not similar, for any component, among islands (contrast figure, MANOVA,  $P > 0.05$ ). No  
373 pattern of variation was shared by all five significant islands, along any dimension. Some patterns  
374 did seem more common however, such as darker dewlaps among coppice lizards (Cayman Brac,  
375 Little Cayman, and Long Island, Fig. ??) or the intermediate position of coppice lizards in chro-  
376 matic color space (Cayman Brac and Long Island). In other cases, patterns of differentiation were  
377 reversed from one island to another, with more ultraviolet reflecting dewlaps in mangroves than in  
378 coastal habitat on Abaco and Cayman Brac, but the opposite on Little Cayman and Long Island.  
379 Overall, it seemed that patterns of heterogeneity of variance were often driven by higher variances  
380 in coloration within beach scrub lizards (Fig. ??, Table ). Yet other patterns were idiosyncratic,  
381 such as the combination of higher red and ultraviolet reflectance in coastal lizards on Little Cay-  
382 man, where the rule seemed to be a negative correlation between ultraviolet and red reflectance  
383 across every other island.

384

## 385 Discussion

386 **Dewlap coloration differs between habitat-types** We found that male dewlap coloration in  
387 *A. sagrei* significantly varied between habitat-types (beach scrub bush, primary coppice forest and  
388 mangrove forest) on five islands of the West Indies: Abaco, Bimini, Cayman Brac, Little Cayman  
389 and Long Island. However, the habitat-specific variation in dewlaps was not consistent between  
390 these islands. Although those results are consistent with adaptation at a very local scale, other  
391 evolutionary drivers could be at work, including phenotypic plasticity, random drift, or historical  
392 contingency including multiple colonization events. We reject this last explanation because all  
393 of the island populations in this study are strictly monophyletic, implying a single colonization  
394 event per island (van de Schoot, unpublished thesis; Driessens et al. 2017). While random drift  
395 cannot be completely ruled out, we see little evidence for a role of phenotypic isolation-by-distance  
396 (spatial autocorrelation) in explaining the differences we report. While we found a significant signal  
397 of isolation-by-distance on Eleuthera, we did not detect differences in dewlap coloration between  
398 habitats on this island.

399 **A role of phenotypic plasticity is unlikely** Phenotypic plasticity could explain differences  
400 in coloration between habitat populations because of diet differences. The yellow, orange and red  
401 coloration in anoline dewlaps are produced by pterins and carotenoids (Ortiz, 1962; Ortiz et al.,  
402 1962; Ortiz and Williams-Ashman, 1963; Ortiz and Maldonado, 1966; Macedonia et al., 2000;  
403 Steffen and McGraw, 2007, 2009). Animals lack the ability to synthesize carotenoids, and those  
404 must therefore be found in the diet, while pterins are synthesized from nucleotides (Goodwin,  
405 1984; Hill et al., 2002; Hill and McGraw, 2006). Experimental manipulation of dietary carotenoid  
406 content showed no effect on dewlap coloration in *A. sagrei* (Steffen et al., 2010) nor in *A. distichus*  
407 (Ng et al., 2013), another species with an orange-based dewlap. Plasticity due to differences in  
408 development (e.g. egg rearing conditions) is unlikely because dewlap coloration develops at sexual  
409 maturity (Ng et al., 2013). Cox et al. (2017) further found a high degree of heritability of dewlap  
410 coloration in *A. sagrei*. These studies suggest that dewlap coloration is not a plastic trait, although  
411 transgenerational plastic effects cannot be completely ruled out by these one or two-generation  
412 common garden experiments (Tariel et al., 2020). That leaves an adaptive explanation, where  
413 dewlap color could be under differential natural and/or sexual selection in these different habitats.

414 **Divergence is maintained in the face of gene flow** The small spatial scale (the median  
415 distance between island sample sites was 11.2km + mention the minimum distance at which we  
416 found differences) and the lack of geographic barriers within islands, together with the high mobility  
417 of *A. sagrei* individuals (Kamath and Losos, 2018), imply ample opportunity for extensive gene flow  
418 between the populations in this study. In agreement with this, populations from different habitats

were found to not be monophyletic based on <insert marker name here> (van de Schoot et al. unpublished thesis). While gene flow is expected to erase any differences acquired by drift without selection maintaining such polymorphism (Dieckmann and Doebeli, 1999), these divergent patterns remain, which may support an adaptive explanation. Maintenance of dewlap color divergence despite gene flow has been found in *A. distichus* in Hispaniola (Ng et al., 2012, 2016) and proposed 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). Divergence in body coloration, but not dewlap coloration, was also reported in *A. conspersus* on another small island, Grand Cayman (Macedonia, 2001). Those results suggest a high adaptive potential of the anoline dewlap.

**Sexual selection could be at play** Substantial levels of promiscuity in *A. sagrei* suggest ample opportunity for female mate choice and sexual selection (Kamath and Losos, 2018). A number of studies suggest that characteristics of the dewlap are indicators of male quality and may therefore act as a cue in mate choice, according to the "good genes" model of sexual selection (Andersson, 1994). 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 males. 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 may be important for sexual selection, for example, dewlap size correlates with bite force (Vanhooydonck et al., 2005) and sexual size dimorphism (a proxy for sexual selection; Vanhooydonck et al. 2009). Even though display frequency increases in the presence of females (Driessens et al., 2014), there seems to be no link between dewlap display frequency and mating success (Tokarz, 2002; Tokarz et al., 2005) or individual quality (Driessens et al., 2015). Our report of inconsistent divergence between islands, however, is at odds with the good-genes model of sexual selection, which would predict the evolution of dewlap coloration in the same direction across the archipelago. This is consistent with Baeckens et al. (2018), who found no link between the average island-dewlap coloration and sexual size dimorphism in *A. sagrei* across the West Indies. If sexual selection is at play in our system, it more likely involves divergent female preferences that are not linked to male quality but rather to components of the environment, and may well be arbitrary (Fisherian sexual selection; Andersson 1994).

**Dewlap coloration could be locally adapted** Presently, we do not know the adaptive drivers of dewlap color divergence observed in this study. Some degree of parallel evolution is usually a good indicator for an adaptive process (Losos, 2011), and convergent patterns of dewlap color evolution in similar environments across islands and species have been documented (Thorpe and Stenson, 2002; Thorpe, 2002). Here, we found evidence for a few consistencies in within-island divergence across the nine islands studied (show some archipelago-wide results?); dewlap brightness was lower in the primary coppice habitat on three islands, and the primary coppice habitat was intermediate in coloration between mangrove and beach scrub on two islands. Those patterns could reflect adaptation to components of the habitat (Endler and McLellan, 1988). However, it is not clear what those components of the habitat might be. Previous studies have found that dewlap coloration maximizes detectability given the light conditions in the local habitat, primarily through UV contrast – with UV-brighter dewlaps in UV-dark, mesic habitats and UV-darker dewlaps in UV-bright, xeric habitats – in *A. cristatellus* and *A. cooki* on Puerto Rico (Leal and Fleishman, 2002, 2004). We found no such pattern in *A. sagrei*, where instead, we found the darkest dewlaps in the darkest, 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

475 dewlap coloration but no evidence for higher detectability (Fleishman et al., 2009; Ng et al., 2012).  
476 Habitats on different islands may also differ in other aspects than light conditions such as densities  
477 of predators or other anole species, which have been shown to affect among-island dewlap diversity  
478 (Vanhooydonck et al., 2009; Baeckens et al., 2018). In particular, Baeckens et al. (2018) recently  
479 showed that dewlaps with spotted patterns occurred more often in *A. sagrei* on islands with more  
480 coexisting species of anoles.

481 **Implications in the context of speciation** Local adaptation can be a precursor to ecological  
482 speciation, a process that may have given rise to the adaptive radiation of *Anolis* lizards (Harmon  
483 et al., 2003; Gavrilets and Losos, 2009). Ecologically-mediated divergence of a sexual signal may  
484 be a potent path to the evolution of reproductive isolation through divergent sexual selection  
485 (Reynolds and Fitzpatrick, 2007; Servedio et al., 2011). Evidence suggests that dewlap coloration  
486 could take this role in anoles (Ng and Glor, 2011; Lambert et al., 2013; Geneva et al., 2015; Ng et al.,  
487 2017), or at least that it is frequently involved in species recognition (Williams, 1969; Williams and  
488 Rand, 1977; Losos, 1985; Macedonia and Stamps, 1994; Fleishman, 2000; Macedonia et al., 2013;  
489 Ingram et al., 2016; Baeckens et al., 2018). Although this signal is not detected at the phylogenetic  
490 scale of the whole genus (Nicholson et al., 2007; Harrison and Poe, 2012; Ingram et al., 2016),  
491 sexual signals are often evolutionarily very labile (Kraaijeveld et al., 2011), and the anole dewlap  
492 in particular is capable of rapid macroevolution; for example, *A. conspersus* on Grand Cayman  
493 evolved a UV-blue dewlap from an ancestral orange dewlap in 2 to 3 million years (Macedonia,  
494 2001). We present evidence of multiple cases of potentially adaptive maintenance of habitat-  
495 associated dewlap divergence over small geographical scale in *A. sagrei* across the West Indies.  
496 While these populations do not appear to be in the process of speciation, our results strongly  
497 suggest that the anoline dewlap has enough micro-scale, local adaptive potential to participate in  
498 the build-up of reproductive isolation, should it be recruited for assortative mating.

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## Figures

507

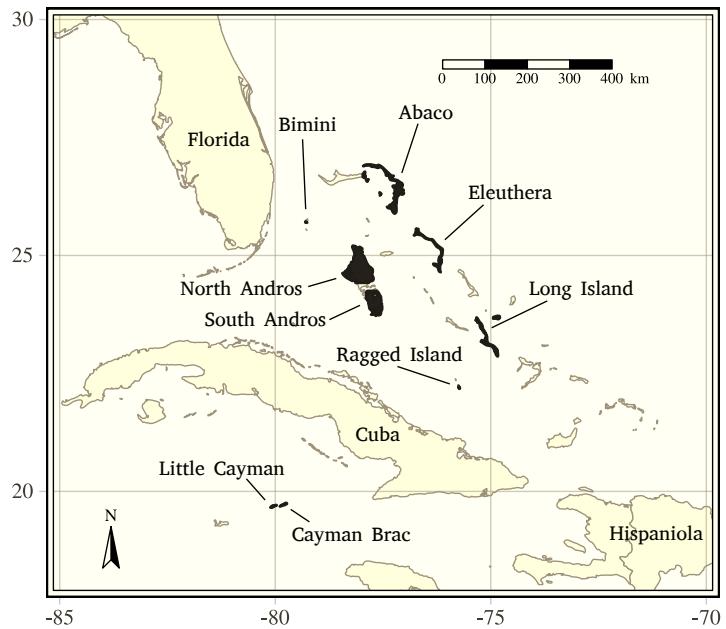


Figure 1: Map of the West Indies with sampled islands highlighted in black.

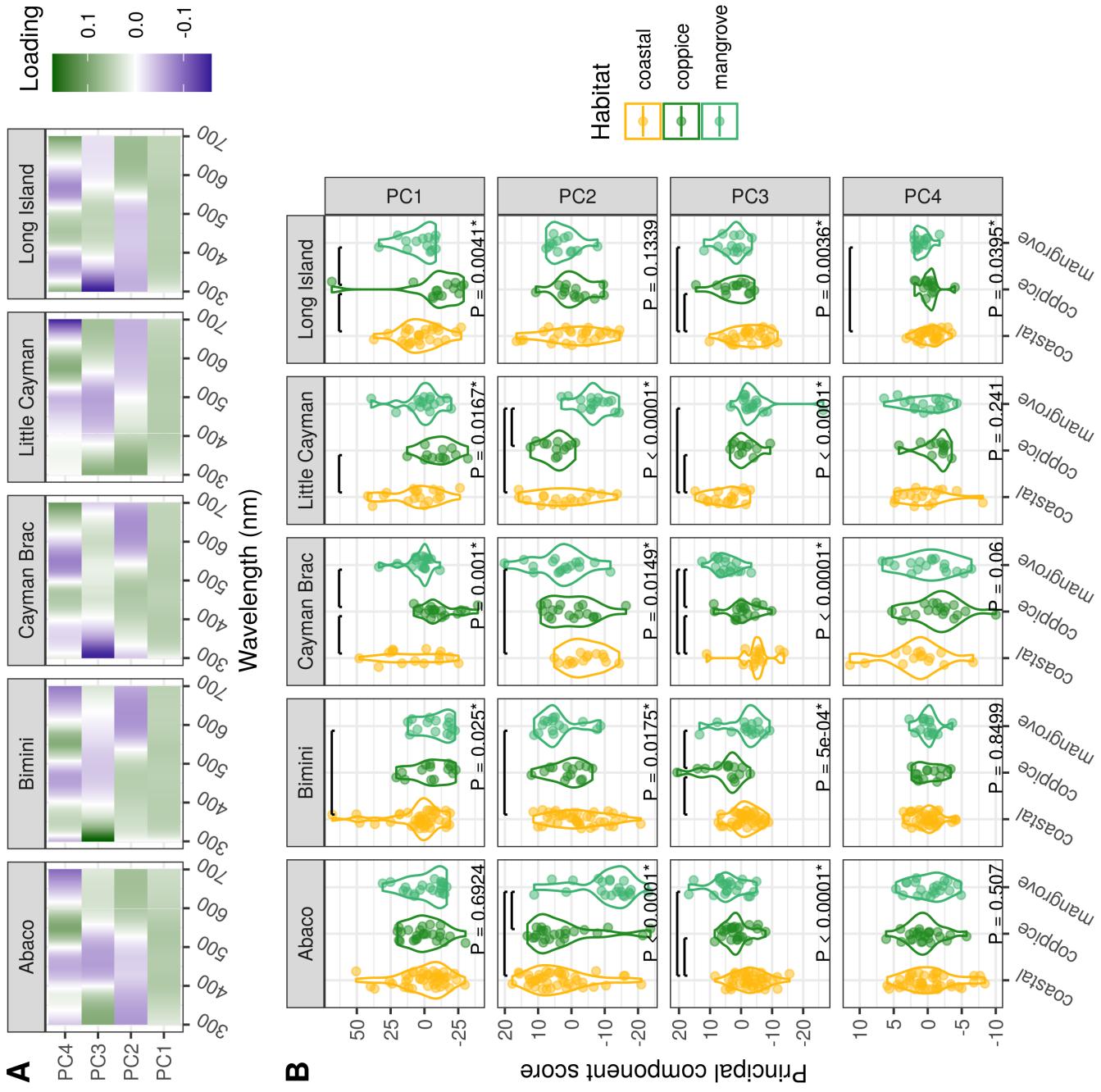


Figure 2: Dewlap color variation between habitat-types across the most significant islands. (A) Mapping of reflectance at various wavelengths onto the principal components (loadings from the PCA rotation matrix). (B) Distribution of PC scores between habitats along the first four PCs on each island where 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. \*,  $P < 0.05$ .

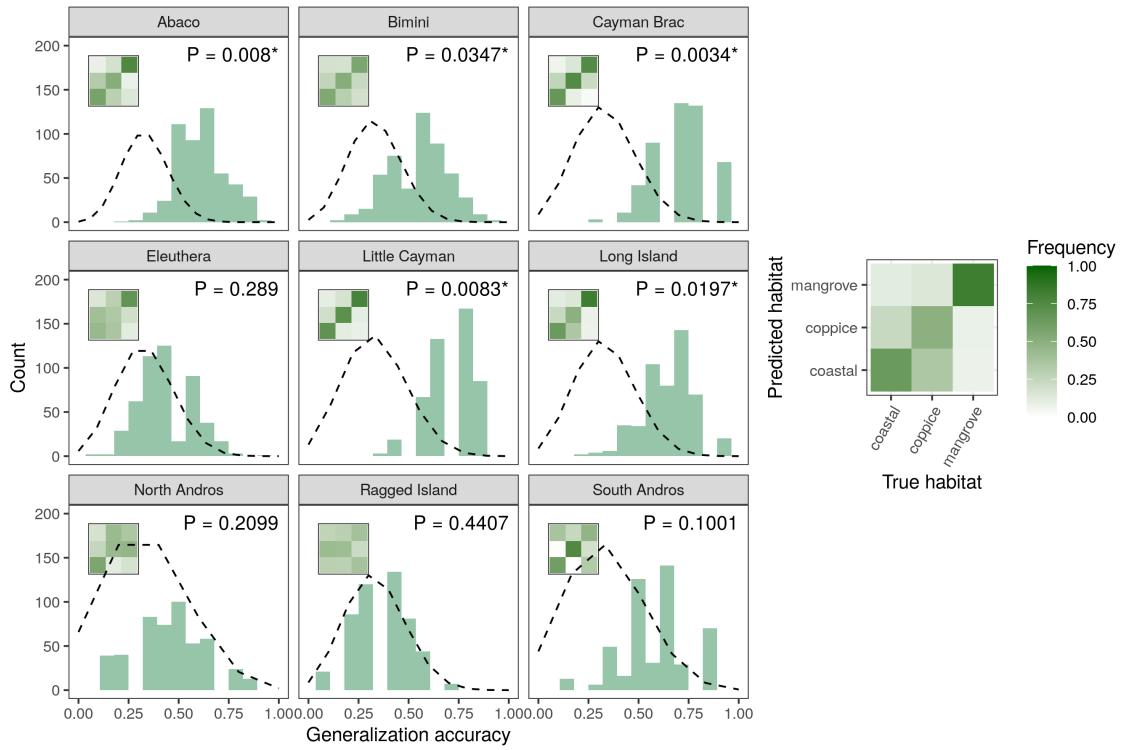


Figure 3: SVM classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The dashed line is the density of a corresponding null binomial distribution, which would be expected under 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. \*,  $P < 0.05$ .

Table 1:

nesting	variable	best_fit	df_model	AICc	dAICc	AICcw	df_LRT	loglik	lratio	pvalue	s
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	*

508 **Tables**

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## Supplementary Figures

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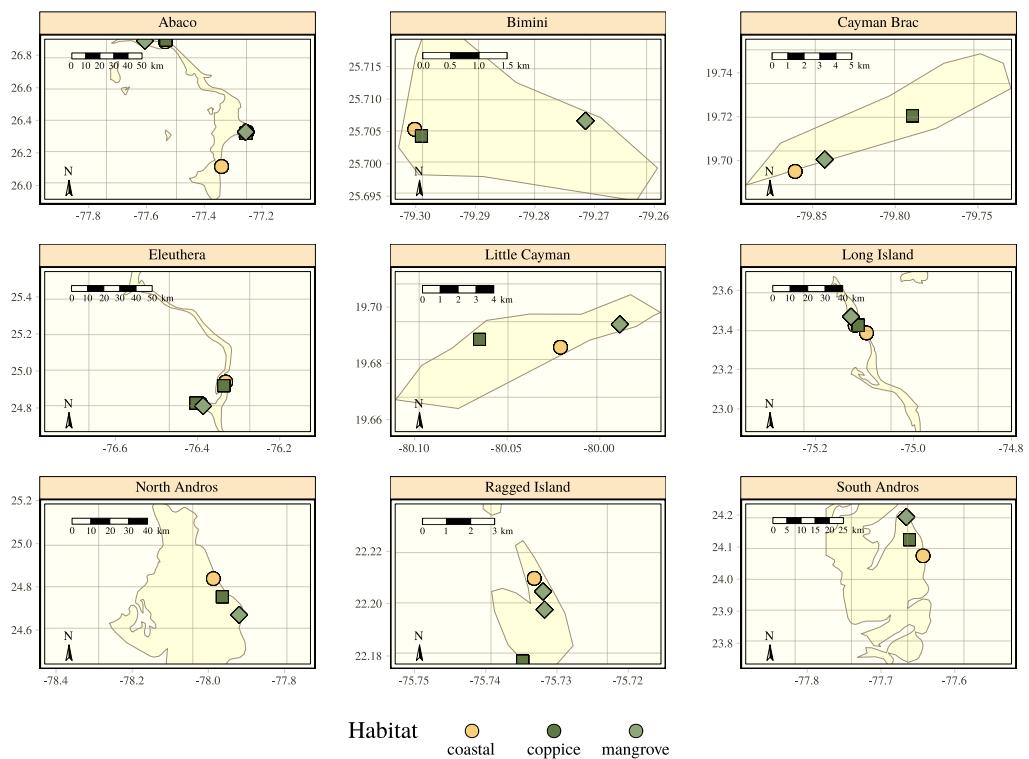


Figure S1: Map of the sampling sites and corresponding habitats across nine islands of the West Indies.

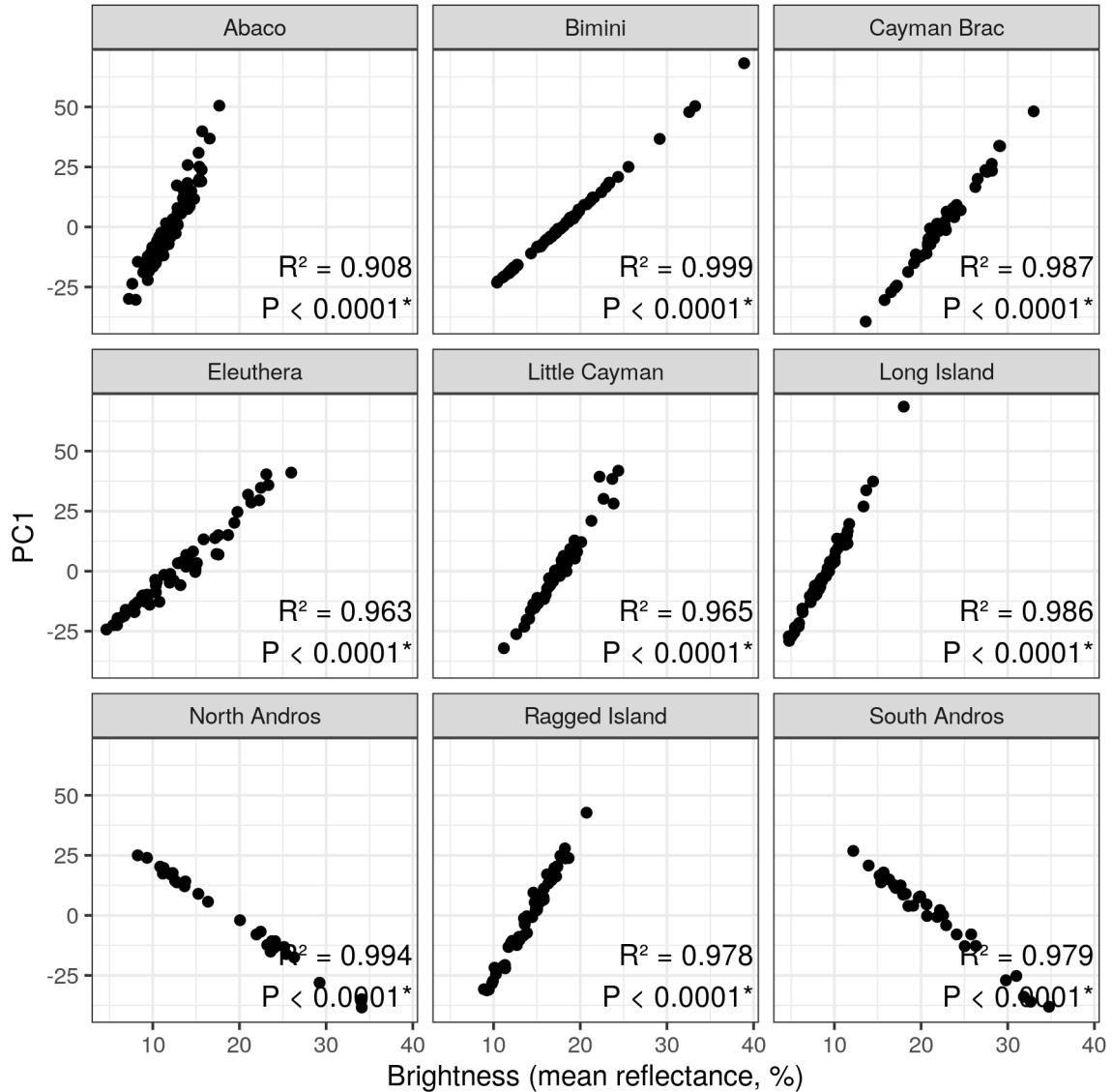


Figure S2: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score for each island. Pearson's squared correlation coefficients are reported. \*,  $P < 0.05$ .

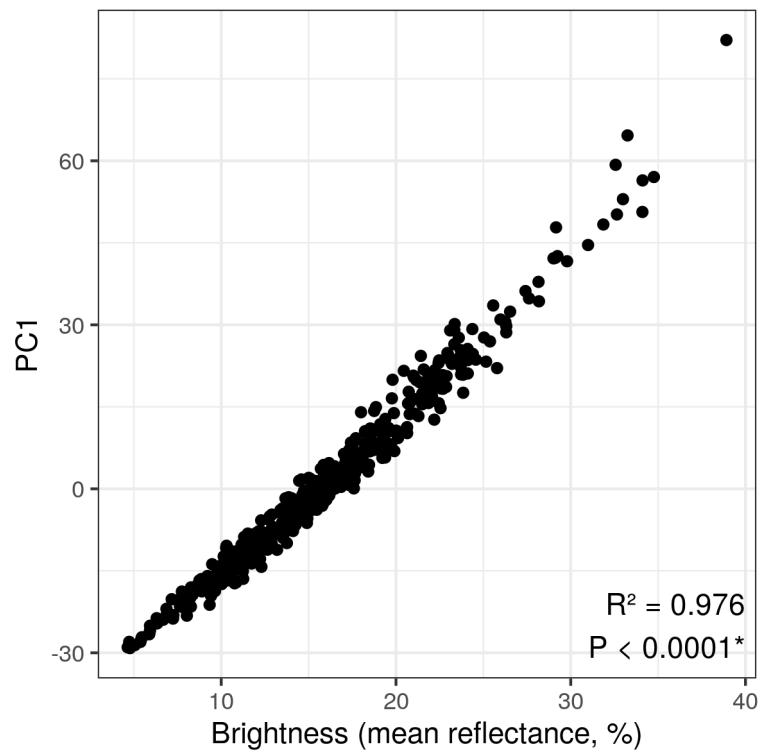
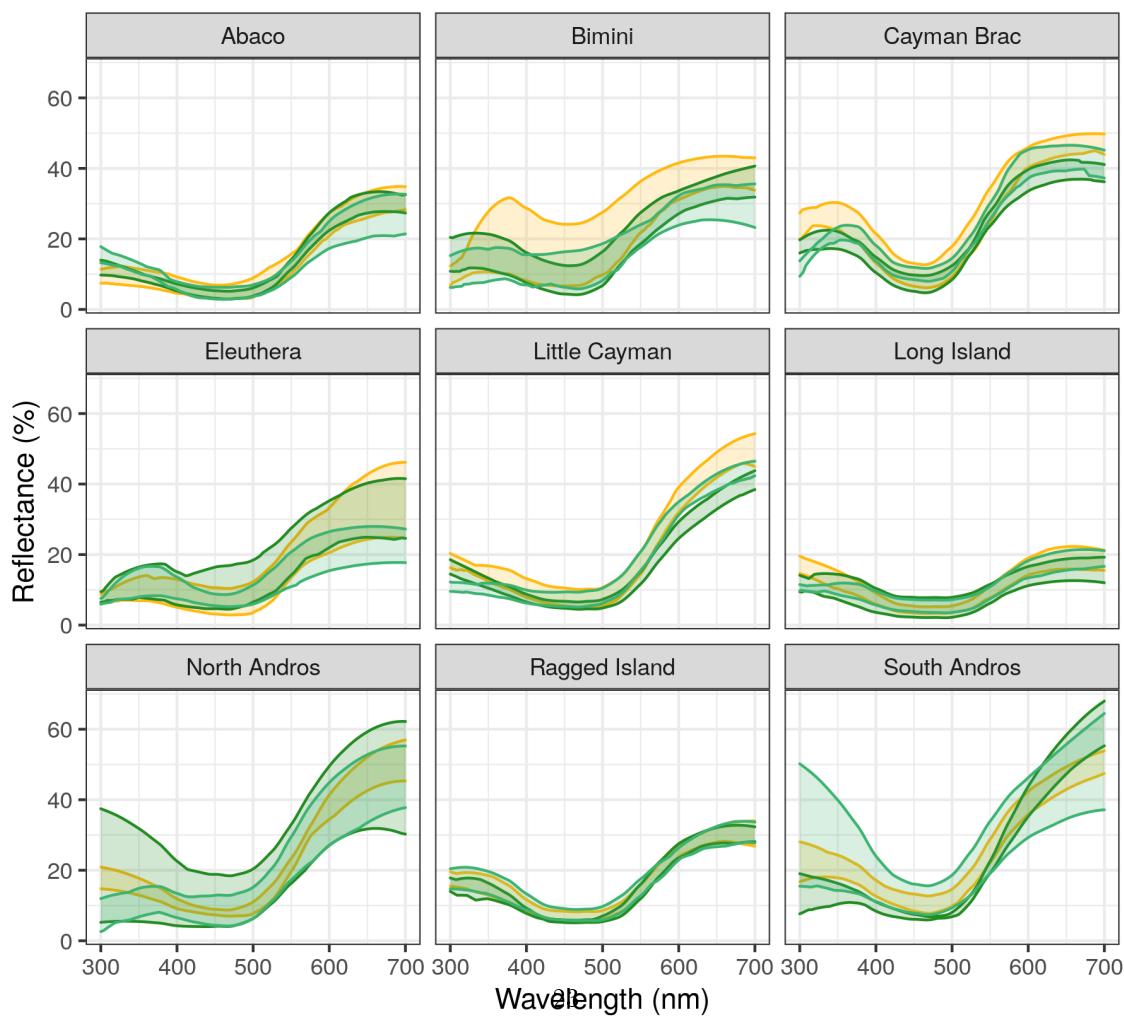


Figure S3: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across the whole archipelago. Pearson's squared correlation coefficient is reported. \*, P < 0.05.



Habitat: █ coastal, █ savanna, █ mangrove

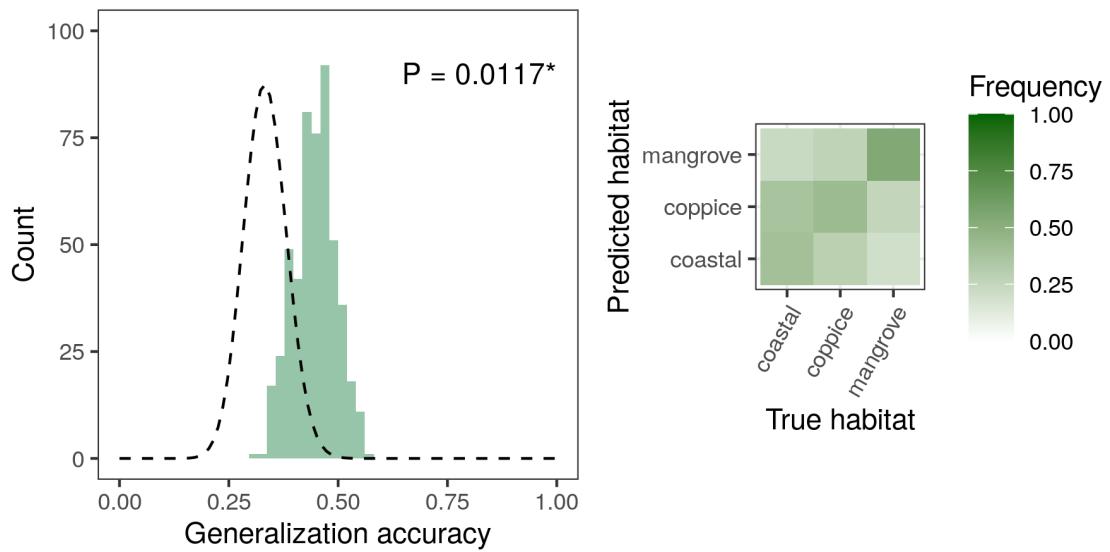


Figure S5: Archipelago-wide SVM classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 3.

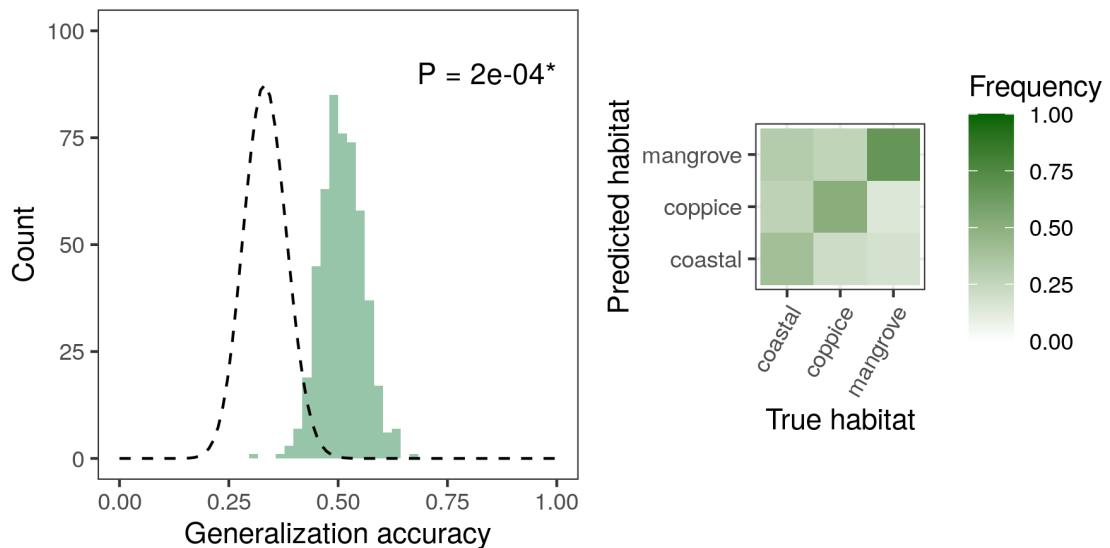


Figure S6: Archipelago-wide SVM classification accuracy based on reflectance data at 50nm-intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 3.

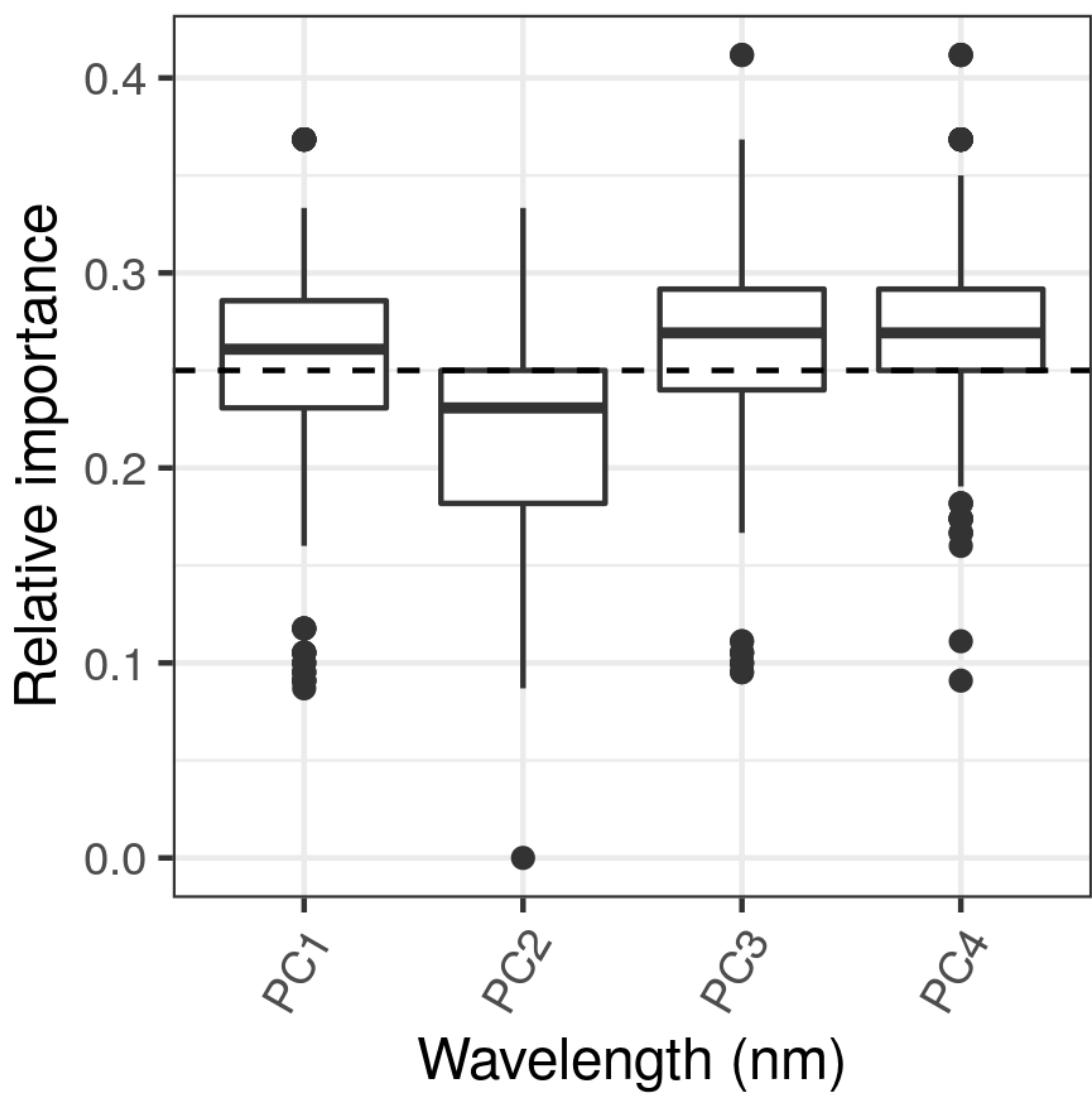


Figure S7: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on principal component data (Figure S5), with relative importance computed for every machine.

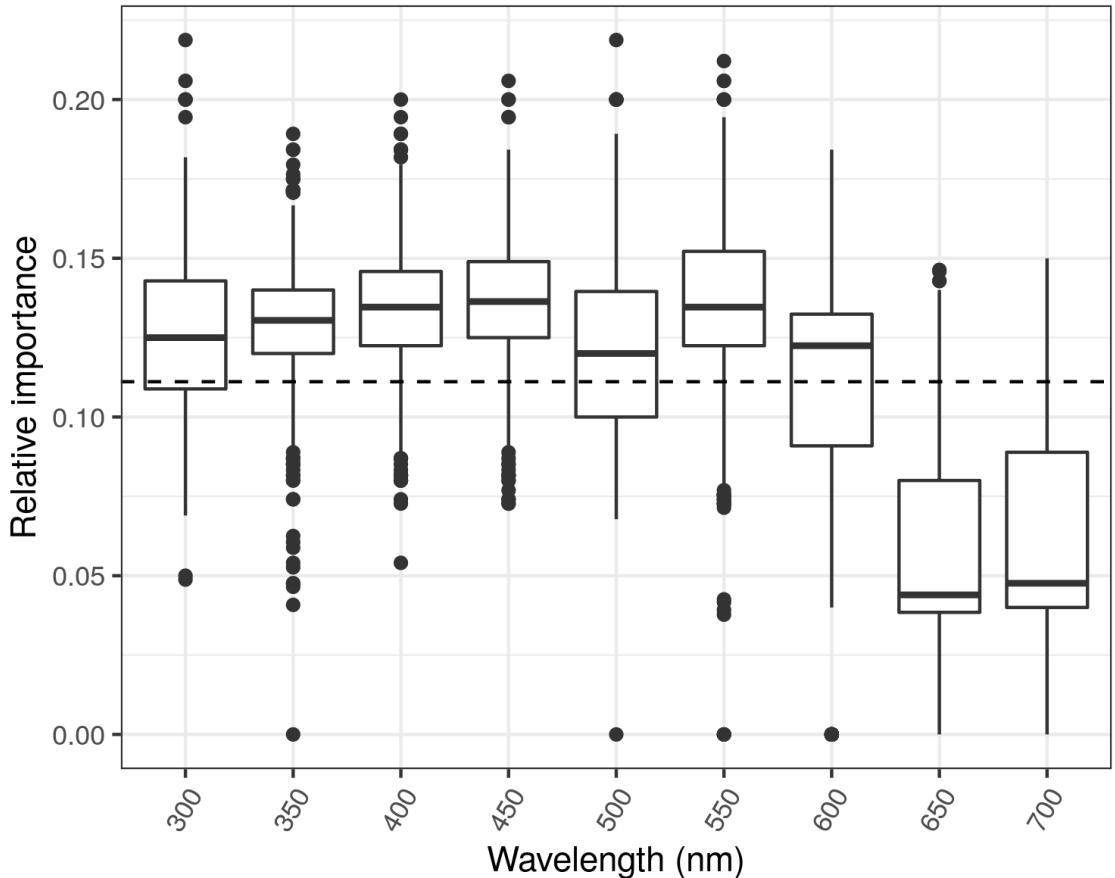


Figure S8: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance data at 50nm-intervals in wavelength (Figure S6), with relative importance computed for every machine.

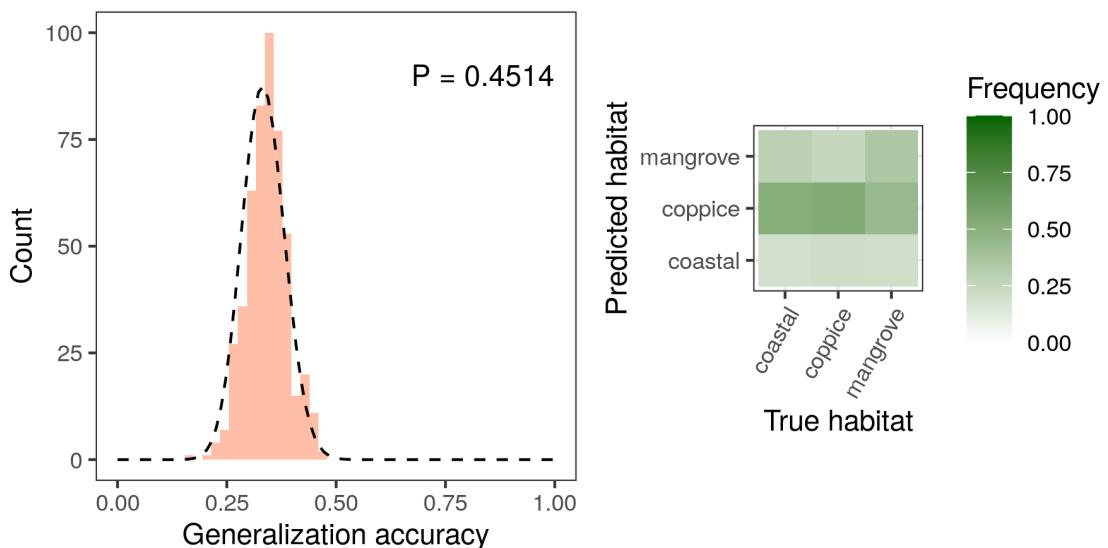


Figure S9: Archipelago-wide LDA classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 3.

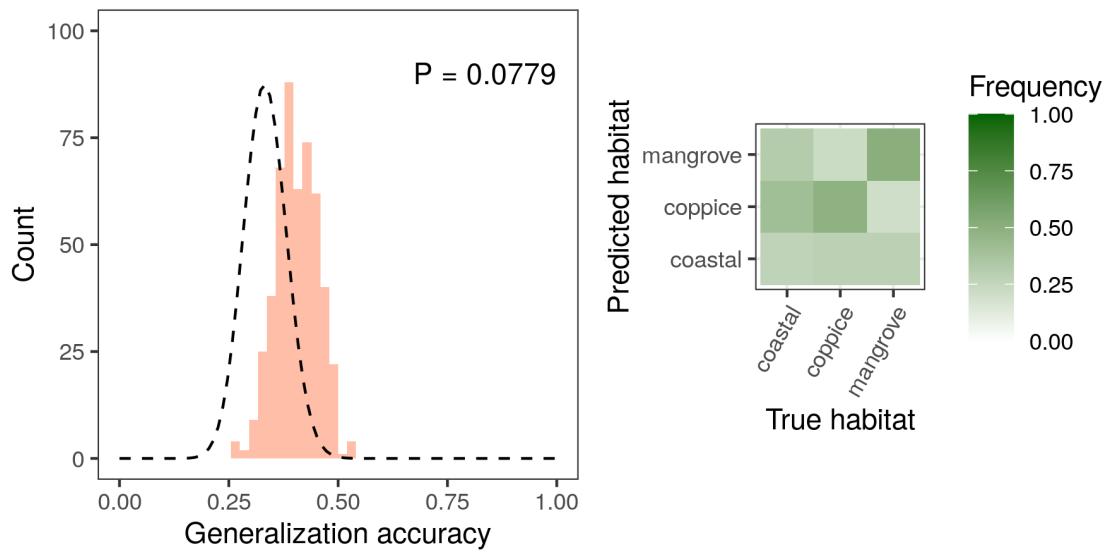


Figure S10: Archipelago-wide LDA classification accuracy based on reflectance data at 50nm intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 3.

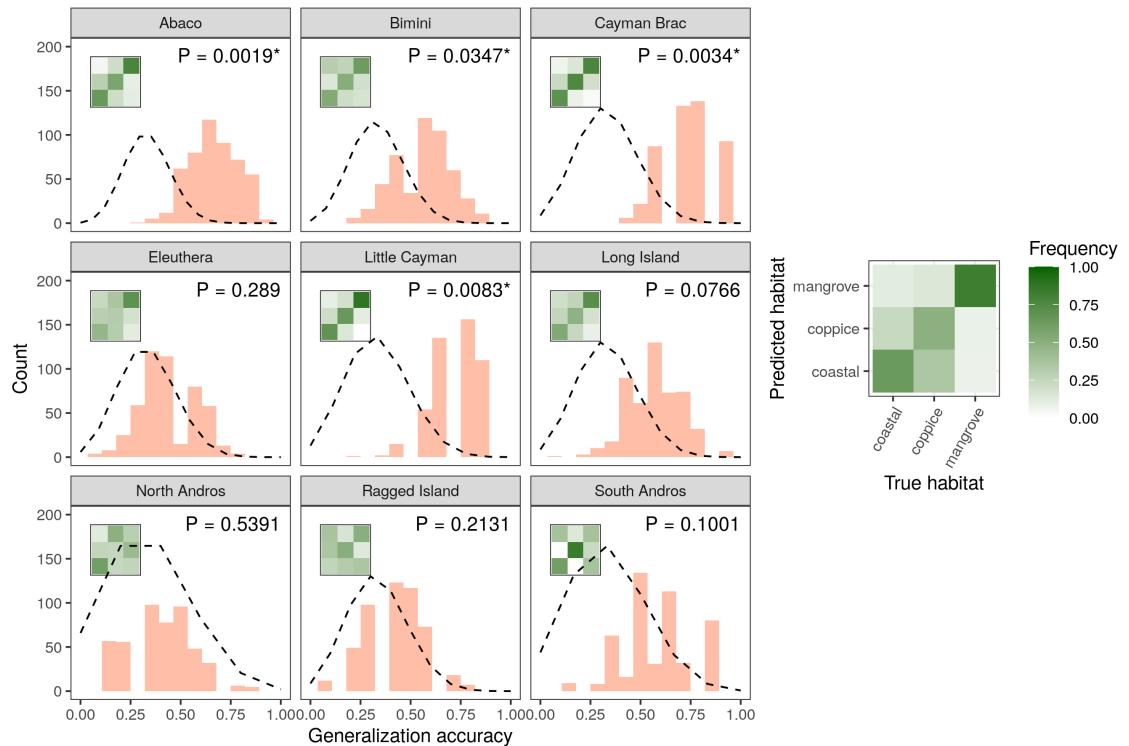


Figure S11: LDA classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 3.

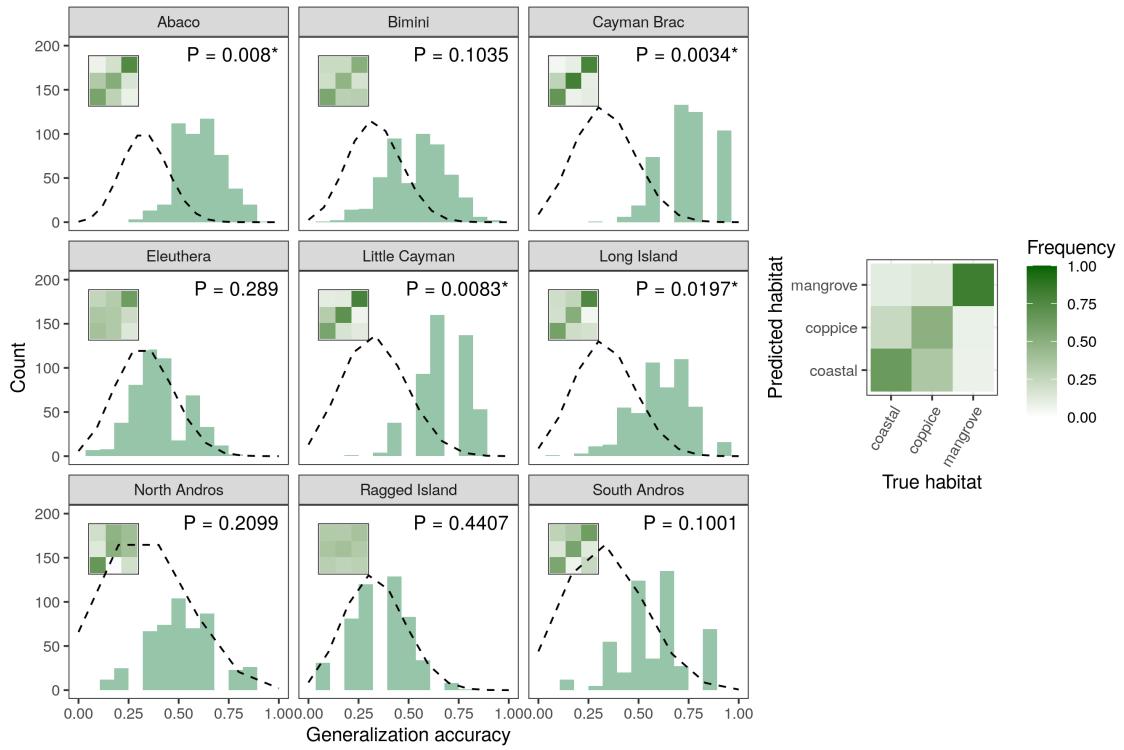


Figure S12: SVM classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 3.

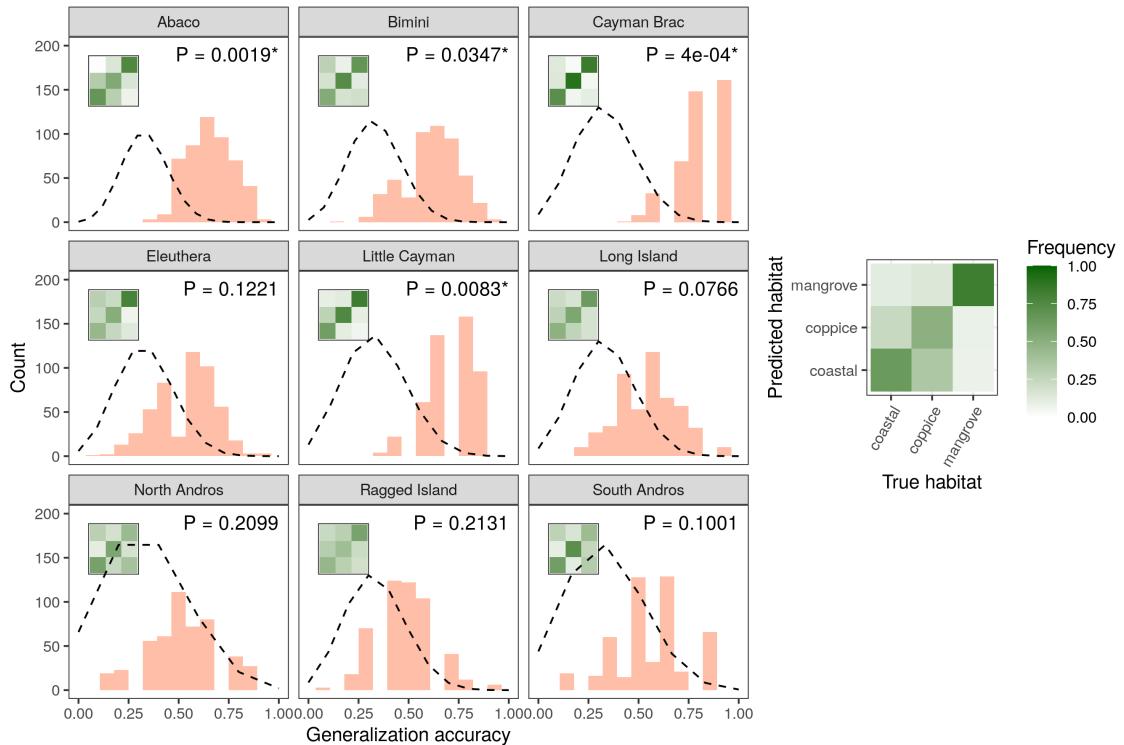


Figure S13: LDA classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 3.

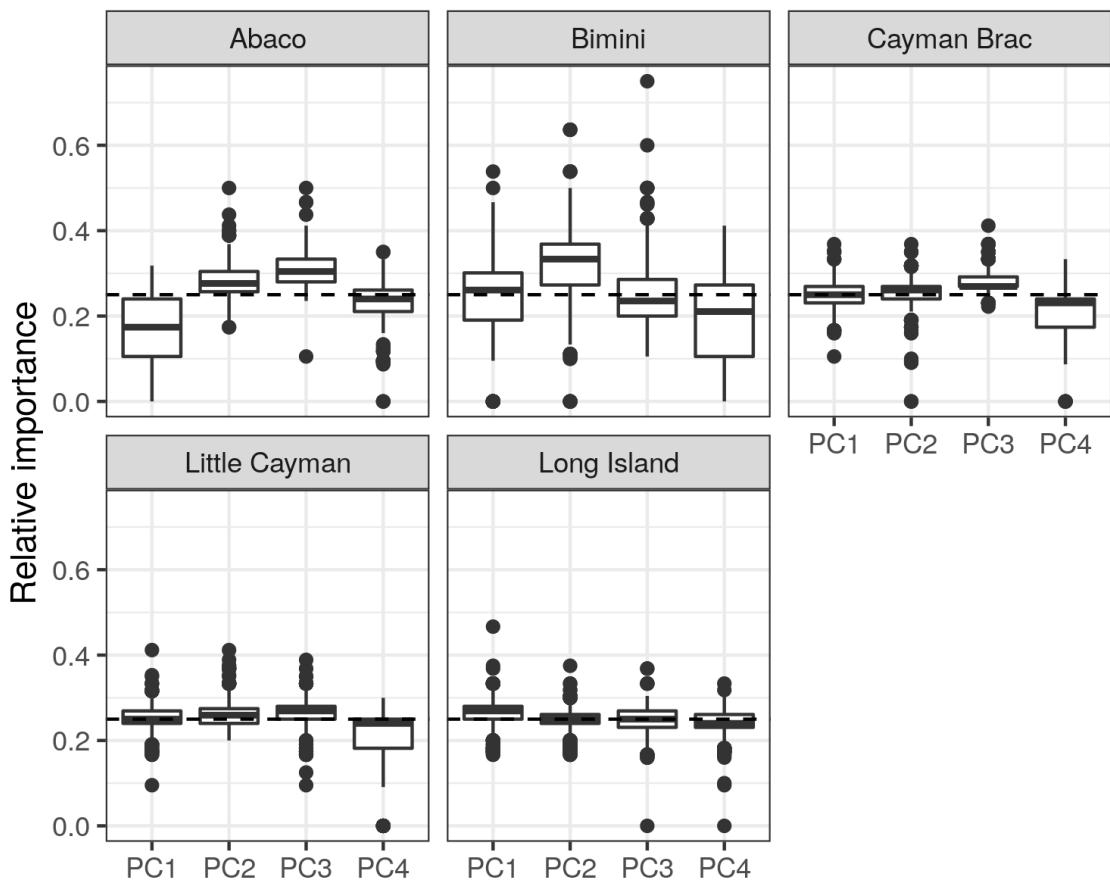


Figure S14: Sensitivity analyses of the different input variables in the within-island SVM classification on principal component data (Figure ??), with relative importance computed for every machine.

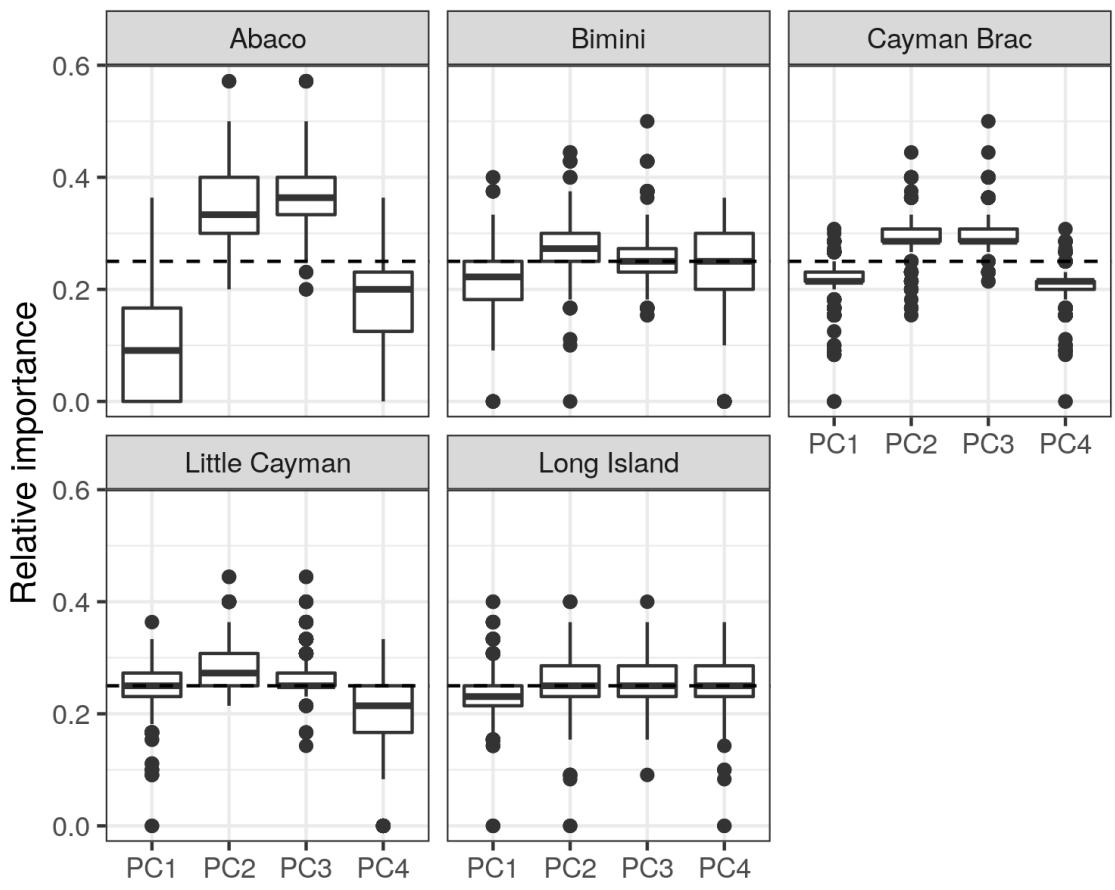


Figure S15: Sensitivity analyses of the different input variables in the within-island LDA classification on principal component data (Figure S11), with relative importance computed for every machine.

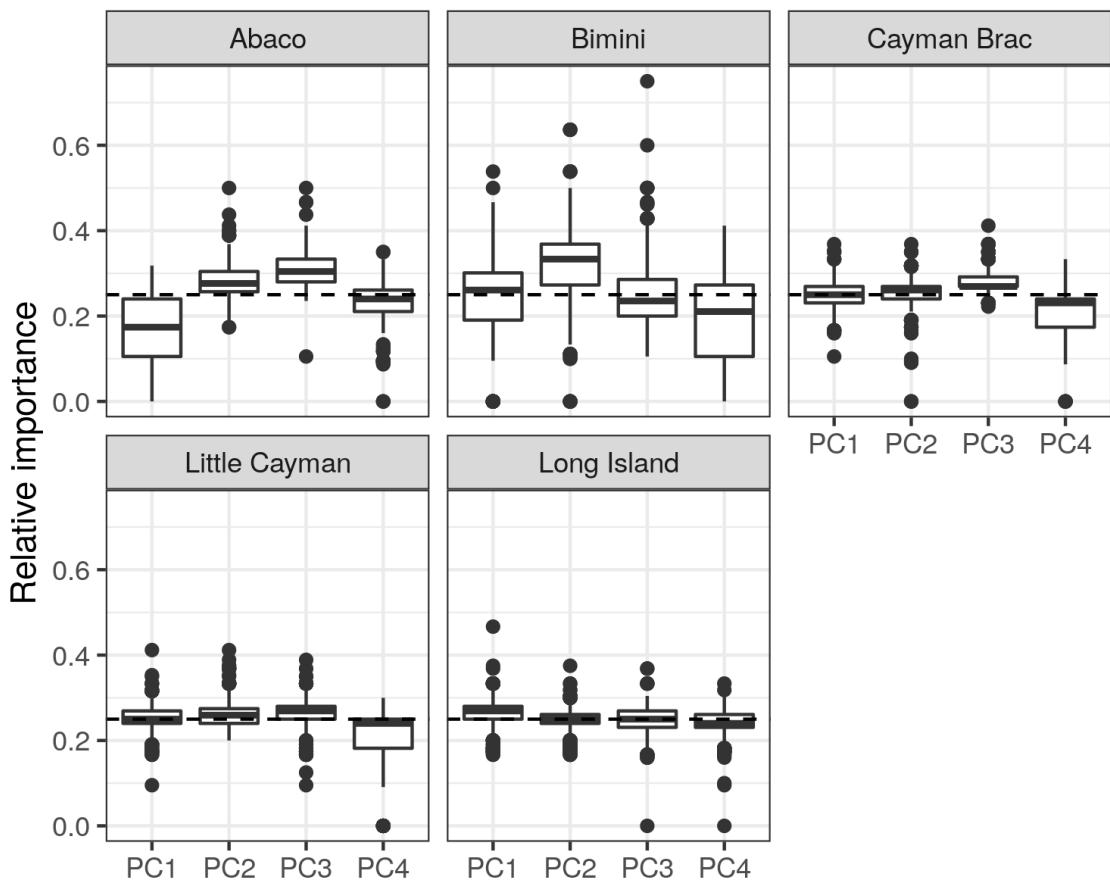


Figure S16: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance at 50nm-intervals in wavelength (Figure S12), with relative importance computed for every machine.

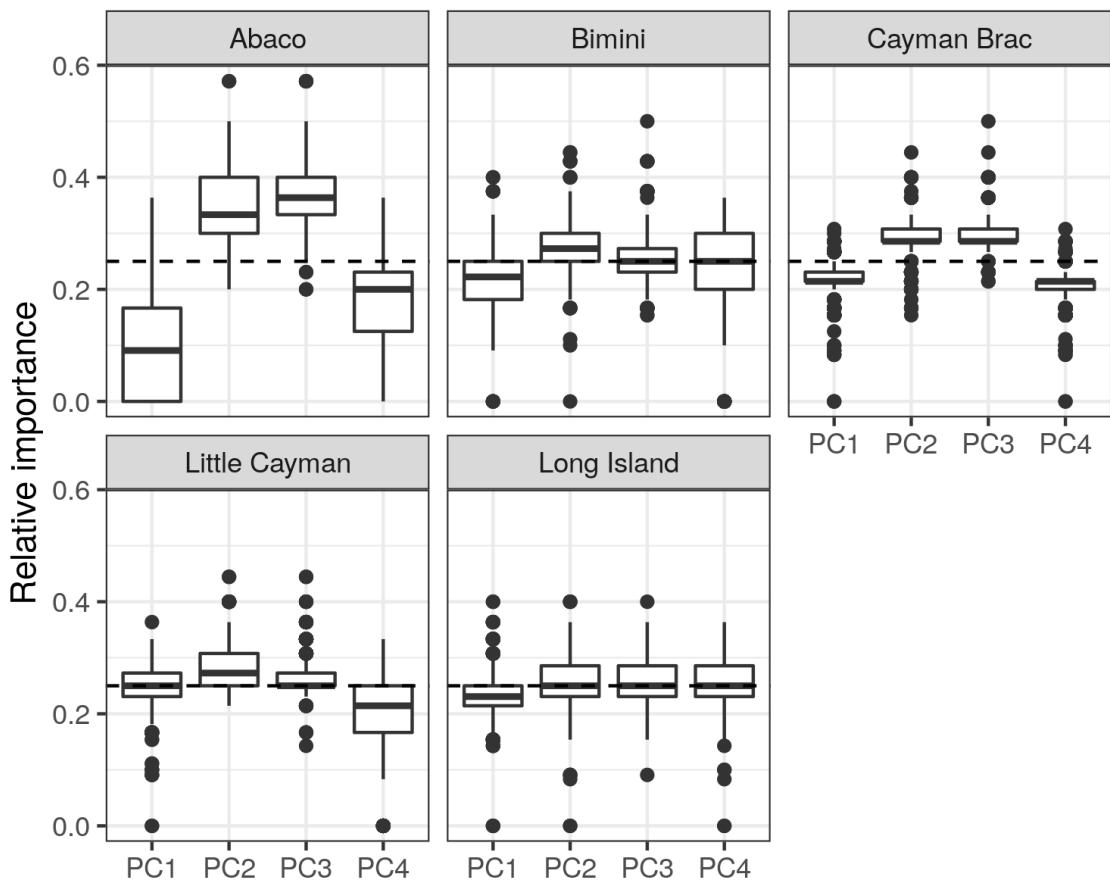


Figure S17: Sensitivity analyses of the different input variables in the archipelago-wide LDA classification on reflectance at 50nm-intervals in wavelength (Figure S13), with relative importance computed for every machine.

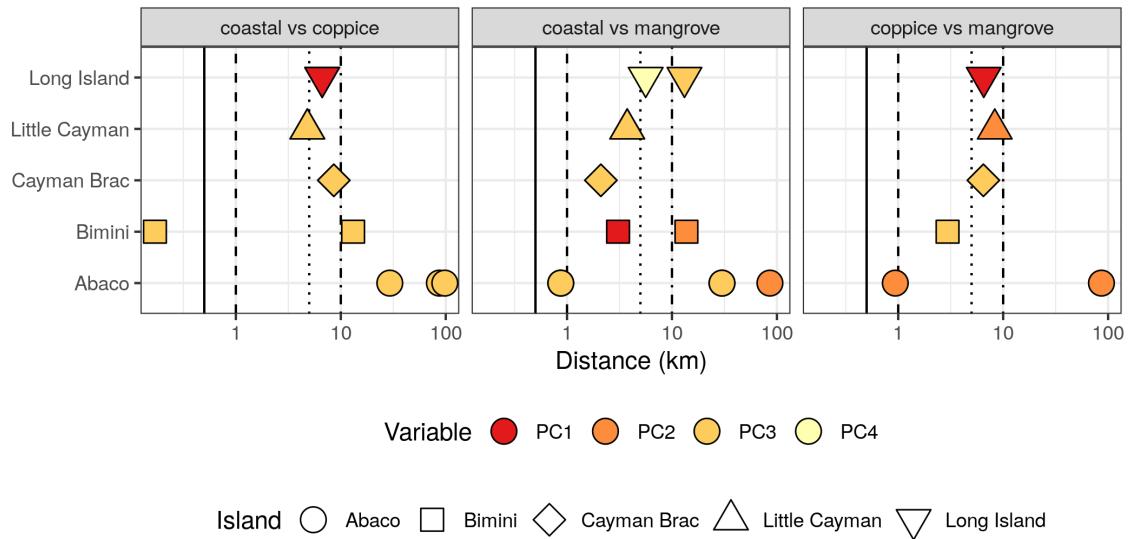


Figure S18: Spatial scale of between-habitat differences in dewlap coloration. For each variable and each pair of habitats where significant differences were detected (Figure 2), we performed multiple post hoc pairwise comparisons between the sites involved (Figure S1, Table ), using nonparametric Wilcoxon-Mann-Whitney 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).

MANOVA, Pillai = 0.354, F(6, 32) = 1, P = 0.3591

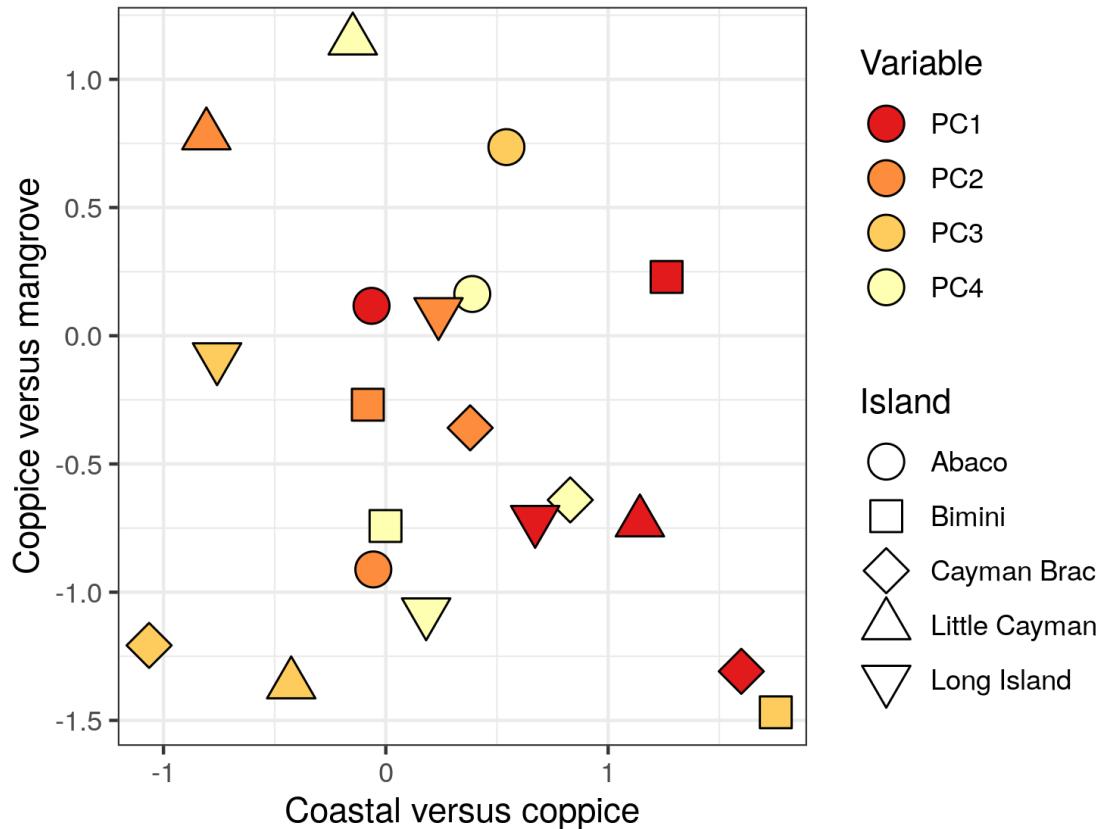


Figure S19: Test of parallel divergence between islands. Differences in habitat-means, or contrasts, are shown for two pairs of habitats for each principal component on each island, rescaled so the standard deviation of the means along each principal component is one. The contrasts represent the patterns 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.

Table S1:

	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

## Supplementary Tables

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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:

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: Results of 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	pvalue	signif
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: Results of 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	chisq	df	pvalue	signif
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: Univariate Shapiro-Wilk's tests of normality performed on each island where significant differences were detected by SVM classification, in each habitat where deviations from multivariate normality were detected (Table ). W, test statistic. \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001.

island	habitat	variable	W	pvalue	signif
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: Results of ANOVAs performed on each principal component across the whole archipelago.  
Legend is the same as for Table .

variable	best_fit	df_model	AICc	dAICc	AICcw	df_LRT	loglik	lratio	pvalue	signif
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	accu	n	ptest	ntest	pvalue	
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 (Table ).

island	variable	chisq	df	pvalue	signif
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. \*,  $P < 0.05$ .

island	robs	pvalue	nsites	
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	