

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, for example, competition for mates, species recognition, aposematism, and cooperation (Bradbury and Vehrencamp, 2011). A primary evolutionary factor shaping communication signals is the sensory system and behavior of their recipient(s) (the sensory drive hypothesis; Endler and McLellan 1988; Endler 1992, 1998). Over the past decades, scientists have established that signals evolve in an ecological context and are dependent on environmental conditions (Endler, 1992, 1993a,b). Just as different habitats may favor different combinations of eco-morphological traits to maximize performance and fitness (Arnold, 1983), they may also shape different forms of a signal, so as to maximize its transmission and detection (e.g. Seehausen 1997), or reduce its detection by unintended recipients such as predators (Endler, 1984, 1990, 1991;

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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 Shoot 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.

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 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) 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 ([R Core Team, 2019](#)).

150 Dimensionality reduction

151 Reflectance curves were smoothed using the R package [pavo](#) ([Maia et al., 2013](#)) as well as custom
152 R functions, down to one reflectance value at each nanometer in wavelength from 300 to 700nm.
153 Because neighboring wavelengths are highly collinear in reflectance, we reduced the dimensionality
154 of the data using principal component analysis (PCA), as per [Cuthill et al. \(1999\)](#) and [Leal and](#)
155 [Fleishman \(2002\)](#). We performed PCA on each island separately and systematically retained the
156 first four principal components (PC), which together always explained more than 88.8% of the
157 variance across islands (Table [S3](#)). PC1 explained between 40 and 56% of the variance across
158 islands; PC2 explained 17.4–27.9%; PC3 12.7–17.6% and PC4 4.3–10.5%. The first four PCs
159 explained similar proportions of variance when calculated for all islands together (Table [S3](#)). PCs
160 need not represent the same wavelengths across islands because they are fitted on different datasets.
161 Nevertheless, PC1 was very collinear with brightness for all islands (Figure [S2](#), Table [S4](#)). PC2
162 correlated highly with the red and ultraviolet ends of the spectrum, which were inversely correlated
163 with each other (Fig. [3A](#)). Higher PCs corresponded to various combinations of wavelengths.
164 Because PC1 correlated uniformly with all wavelengths across the spectrum we considered PC2
165 onwards to capture the chromatic dimensions of color space, i.e. the relative contributions of the
166 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 The first four principal components explained 91.3% of the variance (Table [S3](#)). Again PC1 strongly
170 correlated with brightness (Fig. [S3](#), Table [S4](#)). PC2 was positively correlated to short wavelengths
171 (ultraviolet to blue) and negatively correlated to long wavelengths (green to red, Fig. [S4B](#)). PC3
172 was strongly negatively correlated with UV reflectance and positively correlated with blue-green.
173 PC4 was made of a mosaic of wavelengths, correlating positively with blue and red but negatively
174 with ultraviolet and yellow.

175 We used this dataset to partition the variance in dewlap coloration among islands, habitats
176 and habitats within islands, using a two-way multivariate analysis of variance (MANOVA) with
177 an interaction term. However, because the assumptions of parametric MANOVA were violated
178 for all islands but Ragged Island (multivariate normality, Henze-Zirkler's test, [Henze and Zirkler](#)
179 [1990](#), R package [MVN](#), [Korkmaz et al. 2014](#), Table [S5](#); and homogeneity of covariance matrices,
180 Box's M-test, [Box 1949](#); [Morrison 1988](#), R package [heplots](#), [Fox et al. 2018](#), Table [S6](#)), we used
181 a semi-parametric MANOVA instead (R package [MANOVA.RM](#), [Friedrich et al. 2018](#)), with P-
182 values calculated from a bootstrap procedure with 1,000 iterations. We calculated the proportion
183 of variance explained by islands, habitats and the habitat-by-island interaction using partial effect
184 sizes η^2 on a MANOVA-approximation of the analysis (R package [heplots](#), [Fox et al. 2018](#)).

186 Machine learning

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

190 Machine learning for group comparison has become more popular in ecology and evolution in
191 the recent years (e.g. [Pigot et al. 2020](#)). In particular, SVMs are designed to find the best pos-
192 sible nonlinear boundaries between labelled groups of points in multidimensional spaces, without
193 assumptions about the distribution of the data ([Cortes and Vapnik, 1995](#); [Cristianini and Shawe-](#)
194 [Taylor, 2000](#); [Kim and von Oertzen, 2018](#)). This makes them well suited to field biological data,
195 which often violate the assumptions of classical linear modeling ([Kim and von Oertzen, 2018](#)) and
196 can be, as in the case of coloration, inherently highly multivariate ([Cuthill et al., 1999](#)). First,
197 a machine is trained to recognize differences between groups within a subset of the data called
198 the training set. Significance of differences is then assessed by testing the accuracy of that fitted
199 machine in predicting the group-labels of data points that were not included in the training, called

a testing set, based solely on their multivariate coordinates. This cross-validation procedure results in a proportion of correctly classified points, or generalization accuracy score, which can be compared to that expected under random guessing using a binomial test.

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

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

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

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

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

Univariate analyses

For each island where significant differences in multivariate dewlap coloration were detected between habitats, we used multiple univariate analyses of variance (ANOVA) to identify which variables were responsible for the observed differences. We constructed our ANOVA models in two steps, as per Zuur (2009). In a first step, we accounted for heterogeneity of variances across groups by systematically comparing the goodness-of-fit of an ANOVA model estimated with ordinary least squares (OLS) with that of a model estimated with generalized least squares (GLS), which allowed one estimate of residual variance per habitat (using the R package nlme, Pinheiro and Bates 2000; Pinheiro et al. 2020). Both models were fitted with restricted maximum likelihood (REML). Goodness-of-fit was estimated using Akaike's Information Criterion corrected for small sample sizes (AICc, R package MuMIn, Bartoń 2019), and the estimation method yielding the lowest AICc was retained. In a second step, we re-fitted the retained model with maximum likelihood (ML) to test for the effect of habitat-type using likelihood ratio tests (LRT) between a model including a habitat-term and a null model lacking the habitat-term.

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

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

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

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

272 Spatial autocorrelation

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

283 Site differences

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

290 Results

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

300
301 The small archipelago-wide effect of habitat-type was detected for PC1, PC2 and PC3 (mixed-
302 effect ANOVA with island as a random effect, Table S8), but this effect was too small for post hoc
303 tests to find which habitats differed. Archipelago-wide differences in dewlap coloration between
304 habitats were also detected by SVMs trained on pooled data regardless of island identity, both for
305 PCA data and reflectance scores (Fig. S6, S7). This pattern seemed to be driven by mangrove
306 lizards being correctly reassigned more often than predicted by chance. Sensitivity analyses on
307 these machines suggest a relatively small role of long wavelengths (red reflectance) in driving this
308 pattern (Fig. S9), but did not reveal strong differences between the PCs in relative importance
309 (Fig. S8). Archipelago-wide differences were not detected by LDA classifiers at all (Fig. S10, S11).

So the difference could be in something else than means. 310

Within islands, SVM classifiers correctly assigned individuals to their habitat of origin based 311
solely upon dewlap coloration on five islands: Abaco, Bimini, Cayman Brac, Little Cayman, and 312
Long island (Fig. 2). An LDA approach yielded similar success rates (Fig. S12), suggesting robust 313
differences between these populations. Of the five islands, Little Cayman was the best discrimi- 314
nated with a mean SVM generalization success of 73.4% (Table S9). The results of the classification 315
analyses on PCA data were very similar to results from SVMs and LDAs trained on reflectance 316
values at 50nm-spaced wavelengths from 300 to 700nm (Fig. S13 and S14). 317

Differentiation in dewlap coloration occurred in multiple dimensions of color space. Moreover, 318
the differences in dewlaps between habitats were not always consistent between islands, thus, we 319
will discuss the habitat-specific variation in dewlap coloration for each island where significant 320
differences were detected in turn (Fig. 3, Tables 1, S10). Figure 3A provides a key to map principal 321
component scores to the underlying wavelengths. 322

On Abaco, dewlaps did not differ in PC1, which represents brightness. Mangrove lizards had 323
significantly lower PC2 scores, corresponding to higher ultraviolet reflectance and lower red re- 324
flectance. Coastal beach scrub lizards had lower scores on PC3, corresponding to lower ultraviolet 325
reflectance and higher blue reflectance. 326

On Bimini, coastal beach scrub lizards had significantly brighter dewlaps than lizards from 327
mangroves (PC1), but mangrove lizards had higher PC2 scores than beach scrub lizards, indicat- 328
ing higher violet and blue reflectance, and lower red reflectance. Lizards from primary coppice had 329
higher PC3 scores overall, which correlated very positively with ultraviolet reflectance. 330

On Cayman Brac, coppice-lizard dewlaps were significantly less bright than lizards from the 331
other habitats. Coastal beach scrub lizards had dewlaps that scored low on PC2, corresponding 332
to lower violet-blue and more red, while the mangrove lizards exhibited the opposite: relatively 333
higher levels of violet-blue and less red. In PC3 space we found that dewlaps from lizards in the 334
coastal habitat had high ultraviolet reflectance, coppice lizards had intermediate levels, and man- 335
grove lizards had relatively low levels. 336

On Little Cayman, the dewlaps of coppice lizards were significantly darker (PC1) than coastal- 337
lizards. Mangrove lizards had less ultraviolet and redder dewlaps (PC2). The dewlaps of the 338
coastal beach scrub lizards had higher levels of red and ultraviolet reflectance and less blue re- 339
flectance than the dewlaps of the other habitat-populations (PC3). 340

On Long Island, lizards from the coppice habitat had darker dewlaps than lizards from the other 341
habitats (PC1). Coastal lizards had relatively more ultraviolet and less blue-green reflectance in 342
their dewlaps (PC3). These coastal-habitat lizards also scored lower on PC4, corresponding to 343
slightly more violet and green-yellow dewlaps, and less blue dewlaps, than the mangrove lizards 344
on the island. 345

Sensitivity analyses on classifiers suggested an overall higher relative importance for PC2 and 346
PC3 in determining between-group differences on Abaco, both in SVM and LDA classifiers (Fig. 347
S15, S16), consistent with our ANOVA results (Fig. 3B). There was no strong signal of differences 348
in relative importance among principal components on the other islands. Sensitivity analyses of 349
SVMs trained on reflectance scores rather than principal components revealed, however, a consis- 350
tently higher importance of ultraviolet reflectance in between-group differences on all islands (Fig. 351
S17). This pattern was not recovered for LDAs trained on reflectance scores (Fig. S18). 352

We did not find significant spatial autocorrelation between the sampling sites on the islands 353
where we detected a significant habitat effect. We did, however, detect a significant positive sig- 354
nal of autocorrelation on Eleuthera (Table S11), suggesting possible color differentiation through 355
isolation-by-distance on this island. 356

In contrast, differences in dewlap coloration between habitats were often detected in close ge- 357

368 ographical proximity. For example, Bimini, Cayman Brac and Little Cayman were among the
369 smallest islands in our study (Fig. S1). Besides, for pairs of habitats where significant differences
370 in dewlap coloration were detected along some principal components, comparisons of the actual
371 sampling sites indicate that the most detectable differences involved sites that were mostly 5-10km
372 apart. Our most extreme case of significant differences occurred for PC3 between the beach scrub
373 site and a coppice site, separated from each other by a few hundreds of meters at most on Bimini
374 (multiple pairwise Wilcoxon-Mann-Whitney tests, Fig. S19).

375

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

390

391 Discussion

392 **Dewlap coloration differs between habitat-types** We found that male dewlap coloration in
393 *A. sagrei* significantly varied between habitat-types (beach scrub bush, primary coppice forest and
394 mangrove forest) on five islands of the West Indies: Abaco, Bimini, Cayman Brac, Little Cayman
395 and Long Island. However, the habitat-specific variation in dewlaps was not consistent between
396 these islands. Although those results are consistent with adaptation at a very local scale, other
397 evolutionary drivers could be at work, including phenotypic plasticity, random drift, or historical
398 contingency, including multiple colonization events. We reject this last explanation because all
399 of the island populations in this study are strictly monophyletic, implying a single colonization
400 event per island (van de Schoot, unpublished thesis; Driessens et al. 2017; Reynolds et al. 2020).
401 While random drift cannot be completely ruled out, we see little evidence for a role of phenotypic
402 isolation-by-distance (spatial autocorrelation) in explaining the differences we report. We did
403 detect a significant signal of isolation-by-distance on Eleuthera, but we did not find differences in
404 dewlap coloration between habitats on this island.

405 **Divergence is maintained in the face of gene flow** We detected differences between habitats
406 at relatively small spatial scales, most of the time between sites 5-10km apart, and sometimes a
407 few hundred meters away. This, together with the lack of geographic barriers within islands and
408 the high mobility of *A. sagrei* individuals (Kamath and Losos, 2018), imply ample opportunity
409 for extensive gene flow between our study populations. In agreement with this, populations from
410 different habitats were found to not be monophyletic based on <insert marker name here> (van
411 de Schoot et al. unpublished thesis). The maintenance of phenotypic differences at such a small
412 spatial scales, in the face of gene flow, may support an adaptive explanation (Richardson et al.,
413 2014).

414

415 The persistence of dewlap color divergence despite gene flow has been documented in multiple
416 species of anoles, sometimes in relation to environmental conditions. Ng et al. (2012) and Ng
417 et al. (2016) found divergent dewlap coloration in the face of gene flow between subspecies of *A.*
418 *distichus* across Hispaniola, and proposed this as a mechanism of reproductive isolation in the
419 early stages of speciation (Ng and Glor, 2011; Lambert et al., 2013; Ng et al., 2017). Stapley et al.
420 (2011) found that dewlap color polymorphism was maintained in the absence of genetic structure
421 between populations of *A. apletophallus* from Panama. Thorpe and Stenson (2002) found that
422 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, divergence in body coloration, but not dewlap coloration, was also reported in *A. conspersus* on another small island, Grand Cayman (Macedonia, 2001). Altogether, our results align with previous studies suggesting a generally high adaptive potential of dewlap coloration in anoles over small distances, which could apply to *A. sagrei*.

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 in other species (Thorpe and Stenson, 2002; Thorpe, 2002). Here, however, we found little consistency in between-habitat divergence across the nine islands studied; except maybe for dewlap brightness being lower in the primary coppice habitat on three islands, and the primary coppice habitat being 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), but it is not clear what those components of the habitat might be. Although those patterns could reflect adaptation to components of the habitat (Endler and McLellan, 1988), it is not clear what those components might be. Alternatively, neutral processes could produce patterns similar to local adaptation, such that this possibility cannot be completely ruled out (Miles et al., 2019) (full subsection for this?).

Previous studies 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, we found no such patterns 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 dewlap coloration but no evidence for higher detectability (Fleishman et al., 2009; Ng et al., 2012). Habitats on different islands may also differ in other aspects than light conditions such as densities of predators or other anole species, which have been shown to affect among-island dewlap diversity (Vanhooydonck et al., 2009; Baekens et al., 2018). In particular, Baekens et al. (2018) recently showed that dewlaps with spotted patterns occurred more often in *A. sagrei* on islands with more coexisting species of anoles. Therefore, dewlap coloration in *A. sagrei* may be under the influence of a mosaic of interacting local environmental factors, which need not be the same across islands.

A role of phenotypic plasticity is unlikely Phenotypic plasticity could explain differences in coloration between habitat populations because of diet differences. The yellow, orange and red coloration in anoline dewlaps are produced by pterins and carotenoids (Ortiz, 1962; Ortiz et al., 1962; Ortiz and Williams-Ashman, 1963; Ortiz and Maldonado, 1966; Macedonia et al., 2000; Steffen and McGraw, 2007, 2009). Animals lack the ability to synthesize carotenoids, and those must therefore be found in the diet, while pterins are synthesized from nucleotides (Goodwin, 1984; Hill et al., 2002; Hill and McGraw, 2006). However, experimental manipulation of dietary carotenoid content showed no effect on dewlap coloration in *A. sagrei* (Steffen et al., 2010) nor in *A. distichus* (Ng et al., 2013), which also has an orange-based dewlap. This makes a plastic response to differences in diet across habitats unlikely. Developmental plasticity (e.g. due to differences egg rearing conditions) is also unlikely because dewlap coloration develops at sexual maturity in anoles (Ng et al., 2013). The differences we observed could therefore be heritable. This hypothesis is further supported by Cox et al. (2017), who found a high degree of heritability of dewlap coloration in *A. sagrei*. Although most studies used one or two-generation common garden experiments and thus could not rule out transgenerational plastic effects (Tariel et al., 2020), dewlap coloration generally seems to not be a plastic trait. This further reinforces an adaptive explanation, where dewlap color could be under differential natural and/or sexual selection in these different habitats.

477 Sexual selection could be at play Substantial levels of promiscuity in *A. sagrei* suggest ample
478 opportunity for female mate choice and sexual selection (Kamath and Losos, 2018). A number of
479 studies have suggested that characteristics of the dewlap are indicators of male quality and may
480 therefore act as a cue in mate choice, according to the "good genes" model of sexual selection
481 (Andersson, 1994). Cook et al. (2013) found lower orange reflectance in dewlaps with heavily
482 parasitized *A. brevirostris*, suggesting a trade-off in carotenoid use between the immune response
483 and pigment deposition. Steffen and Guyer (2014) found that lower UV and orange-red reflectance
484 predict contest-winning success between males. Driessens et al. (2015) further found that more
485 yellow and red dewlaps (relative to UV) predict better body condition, and that higher yellow and
486 UV reflectance at the margin of the dewlap predict higher hematocrit (the concentration of red
487 blood cells), indicating a better health.

488
489 Other aspects of the dewlap may be important for sexual selection. For example, dewlap
490 size in *A. sagrei* was found to correlate with bite force (Vanhooydonck et al., 2005) and sexual
491 size dimorphism (a proxy for sexual selection; Vanhooydonck et al. 2009). Even though display
492 frequency increases in the presence of females (Driessens et al., 2014), no link was found between
493 dewlap display frequency and mating success (Tokarz, 2002; Tokarz et al., 2005) or individual
494 quality (Driessens et al., 2015). Our report of inconsistent divergence between islands, however, is
495 at odds with the good-genes model of sexual selection, which would predict the evolution of dewlap
496 coloration in the same direction across the archipelago. This is in agreement with Baeckens et al.
497 (2018), who found no link between the average island-dewlap coloration and sexual size dimorphism
498 in *A. sagrei* across the West Indies. If sexual selection is at play in our system, it more likely involves
499 divergent female preferences that are not linked to male quality but rather to components of the
500 environment, or may well be arbitrary (Fisherian sexual selection; Andersson 1994).

501 Implications in the context of speciation Local adaptation can be a precursor to ecological
502 speciation, a process that may have given rise to the adaptive radiation of *Anolis* lizards (Harmon
503 et al., 2003; Gavrilets and Losos, 2009). Ecologically-mediated divergence of a sexual signal may
504 be a potent path to the evolution of reproductive isolation through divergent sexual selection
505 (Reynolds and Fitzpatrick, 2007; Servedio et al., 2011). Evidence suggests that dewlap coloration
506 could take this role in anoles (Ng and Glor, 2011; Lambert et al., 2013; Geneva et al., 2015; Ng et al.,
507 2017), or at least that it is frequently involved in species recognition (Williams, 1969; Williams and
508 Rand, 1977; Losos, 1985; Macedonia and Stamps, 1994; Fleishman, 2000; Macedonia et al., 2013;
509 Ingram et al., 2016; Baeckens et al., 2018). Although this signal is not detected at the phylogenetic
510 scale of the whole genus (Nicholson et al., 2007; Harrison and Poe, 2012; Ingram et al., 2016),
511 sexual signals are often evolutionarily very labile (Kraaijeveld et al., 2011), and the anole dewlap
512 in particular is capable of rapid macroevolution; for example, *A. conspersus* on Grand Cayman
513 evolved a UV-blue dewlap from an ancestral orange dewlap in 2 to 3 million years (Macedonia,
514 2001). We present evidence of multiple cases of potentially adaptive maintenance of habitat-
515 associated dewlap divergence over small geographical scale in *A. sagrei* across the West Indies.
516 While these populations do not appear to be in the process of speciation, our results strongly
517 suggest that the anoline dewlap has enough micro-scale, local adaptive potential to participate in
518 the build-up of reproductive isolation, should it be recruited for assortative mating.

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Figures

527

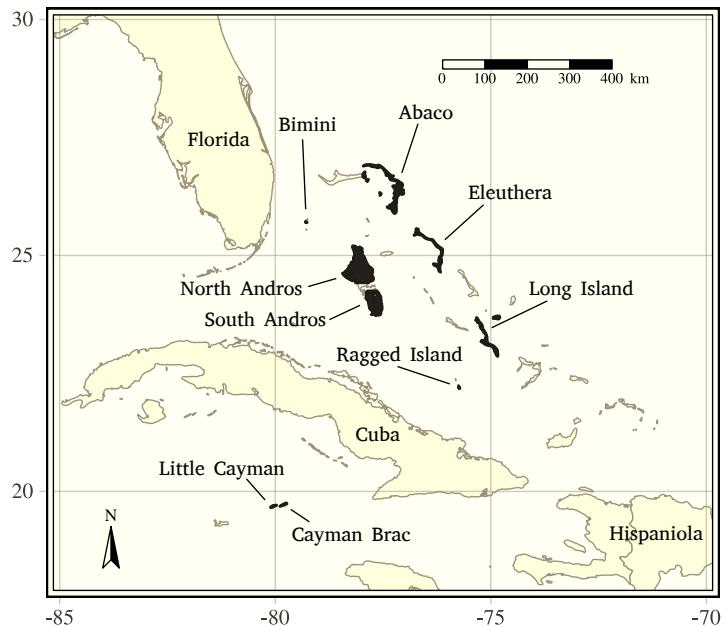


Figure 1: Map of the West Indies with sampled islands highlighted in black.

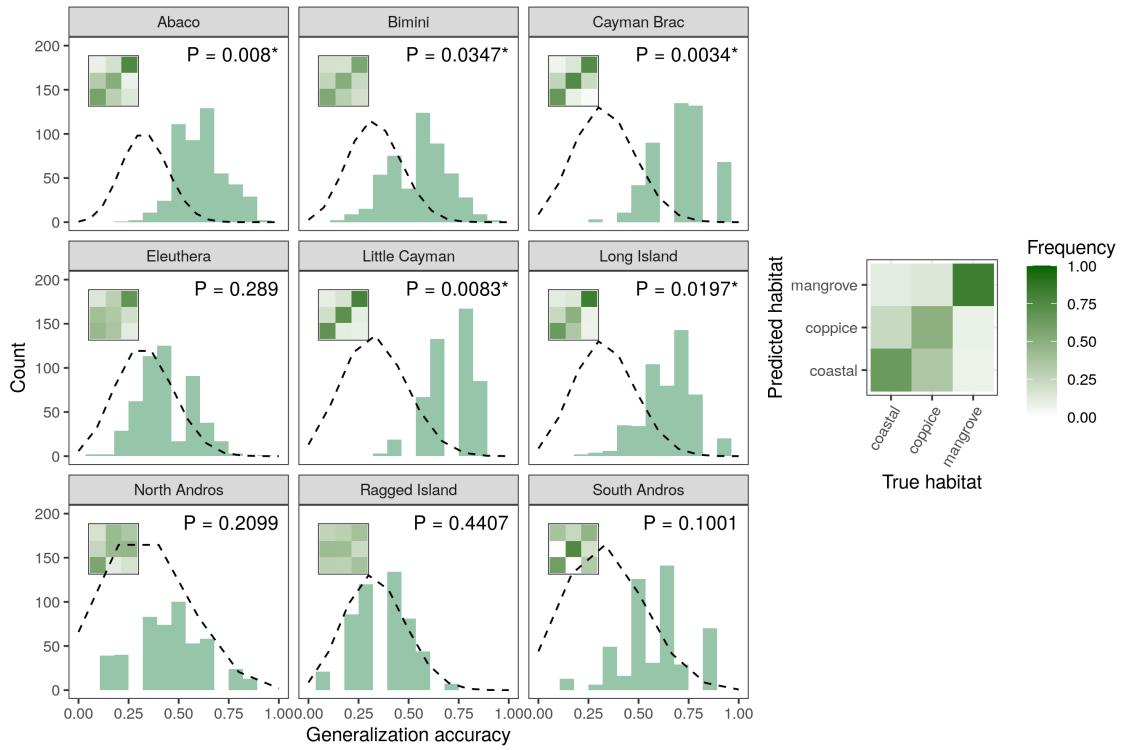


Figure 2: SVM classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The dashed line is the density of a corresponding null binomial distribution, which would be expected under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

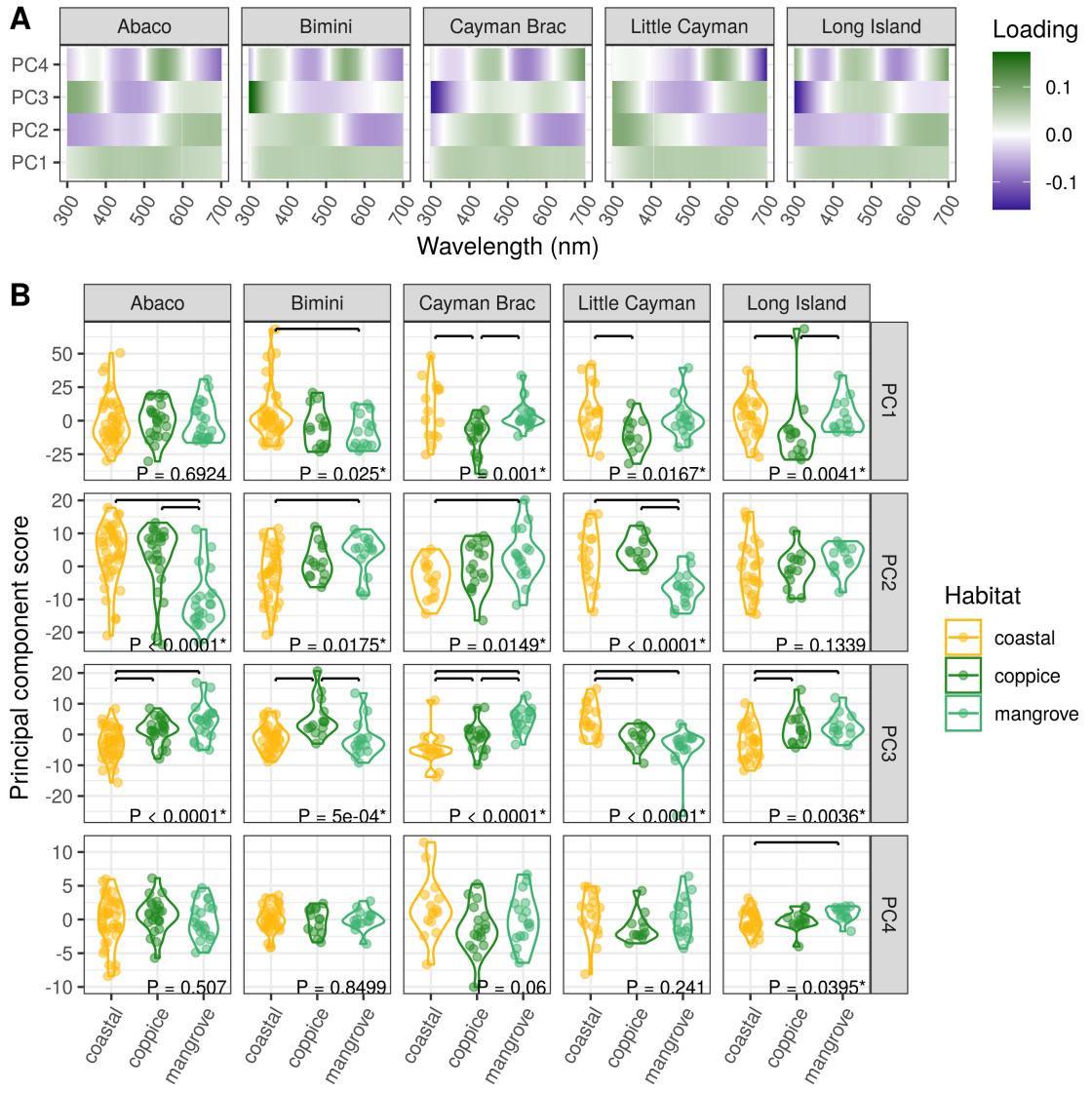


Figure 3: Dewlap color variation between habitat-types across the most significant islands. (A) Mapping of reflectance at various wavelengths onto the principal components (loadings from the PCA rotation matrix). (B) Distribution of PC scores between habitats along the first four PCs on each island where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

528 Tables

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

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

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Supplementary Figures

815

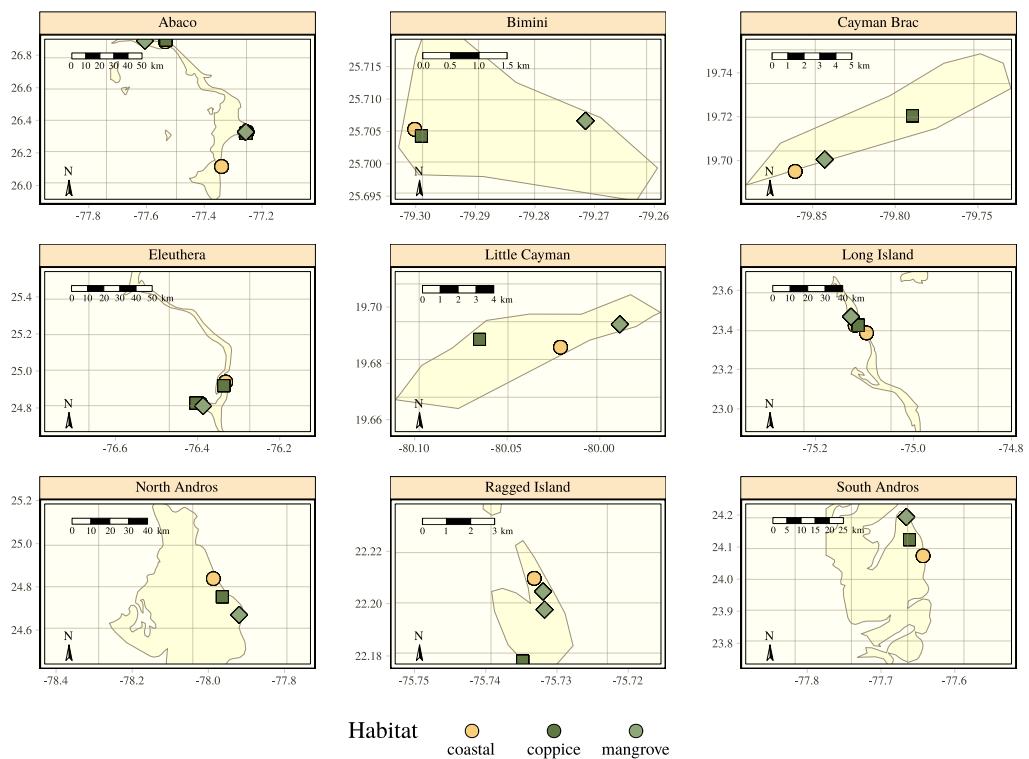


Figure S1: Map of the sampling sites and corresponding habitats across nine islands of the West Indies.

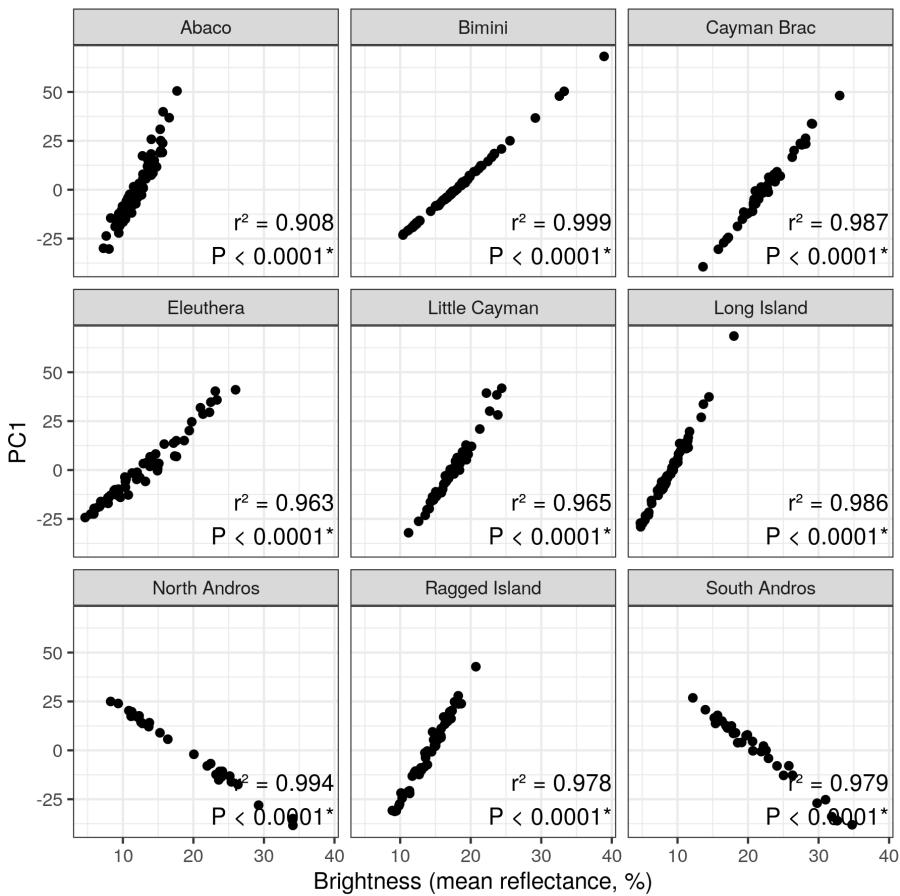


Figure S2: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score for each island. Pearson's squared correlation coefficients are reported. *, $P < 0.05$.

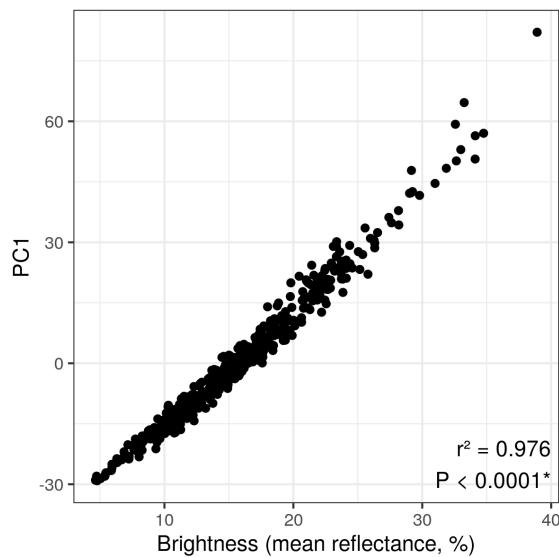


Figure S3: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across the whole archipelago. Pearson's squared correlation coefficient is reported. *, $P < 0.05$.

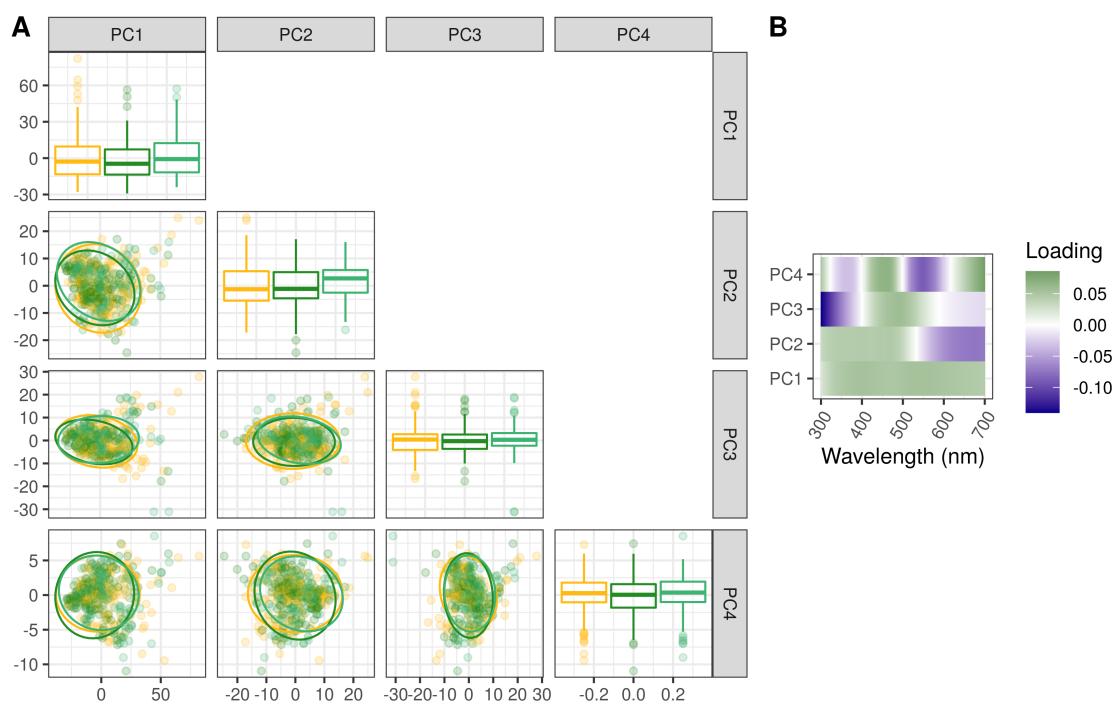


Figure S4: (A) Principal component scores and 5% confidence ellipses across habitats for the whole archipelago. The principal component analysis was performed on reflectance data from all islands pooled together. (B) PCA rotation matrix showing the loadings of each wavelength from 300 to 700nm onto the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

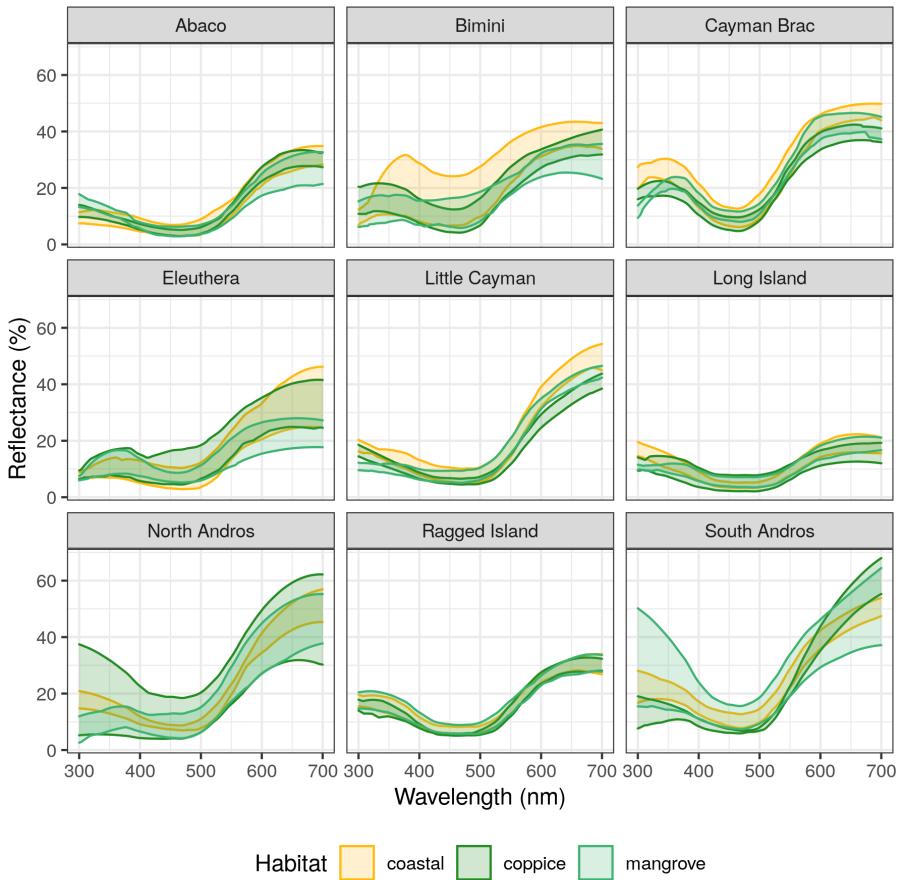


Figure S5: 5-95th percentile range of lizard dewlap reflectance values (in % of incoming light) across wavelengths for each island and each habitat.

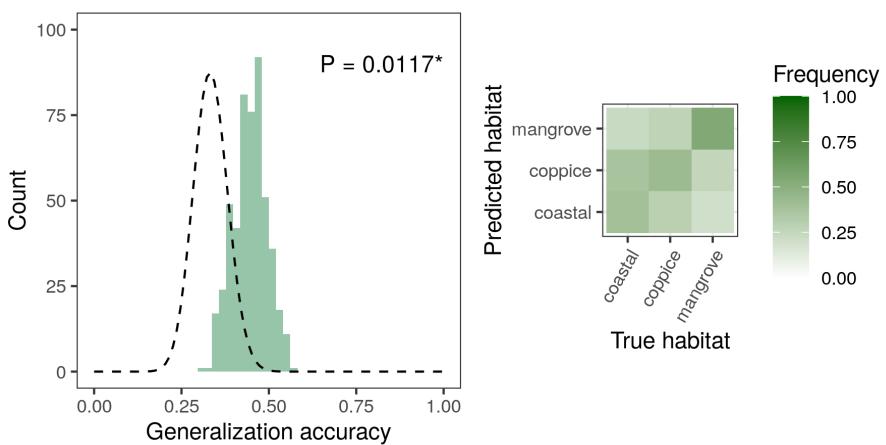


Figure S6: Archipelago-wide SVM classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure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

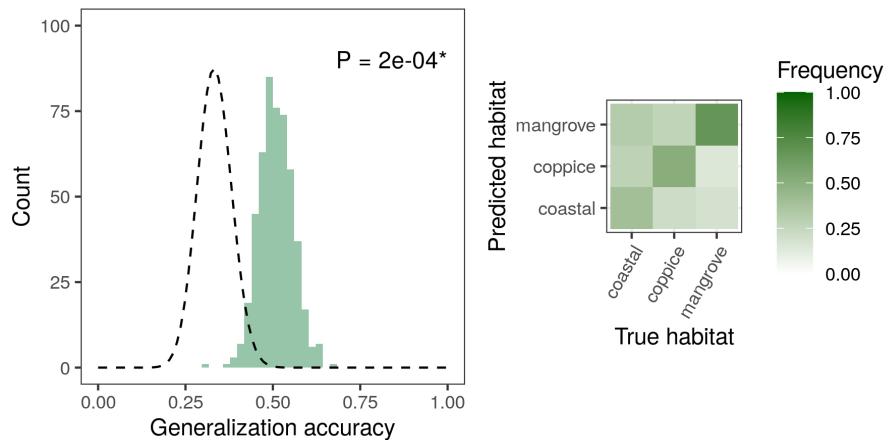


Figure S7: Archipelago-wide SVM classification accuracy based on reflectance data at 50nm-intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation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

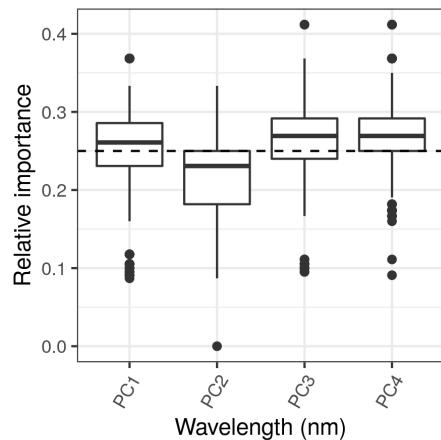


Figure S8: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on principal component data (Figure S6), with relative importance computed for every machine.

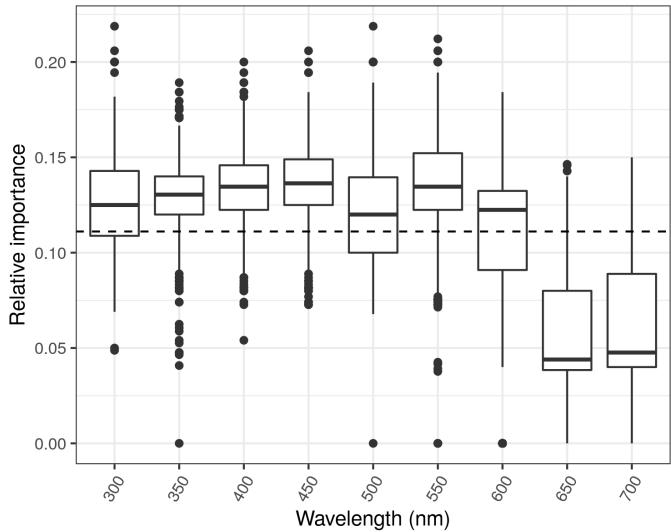


Figure S9: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance data at 50nm-intervals in wavelength (Figure S7), with relative importance computed for every machine.

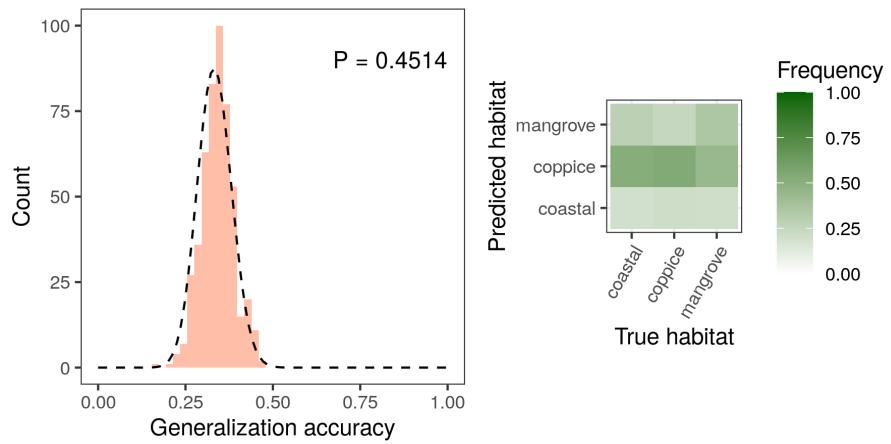


Figure S10: Archipelago-wide LDA classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure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

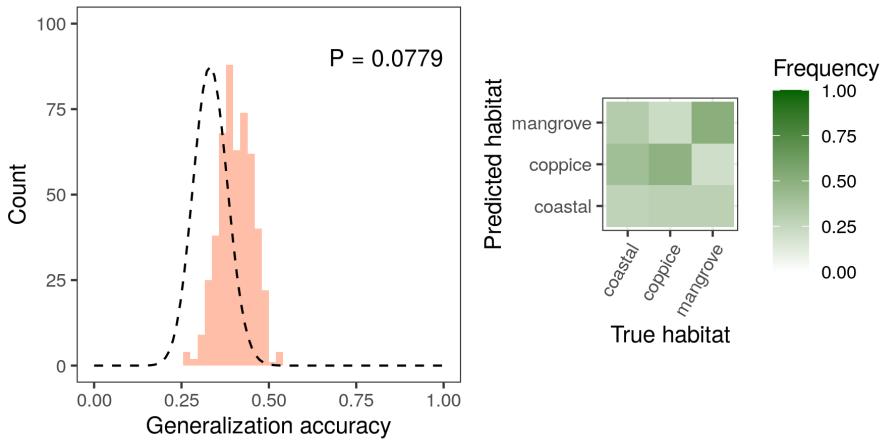


Figure S11: Archipelago-wide LDA classification accuracy based on reflectance data at 5nm intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation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

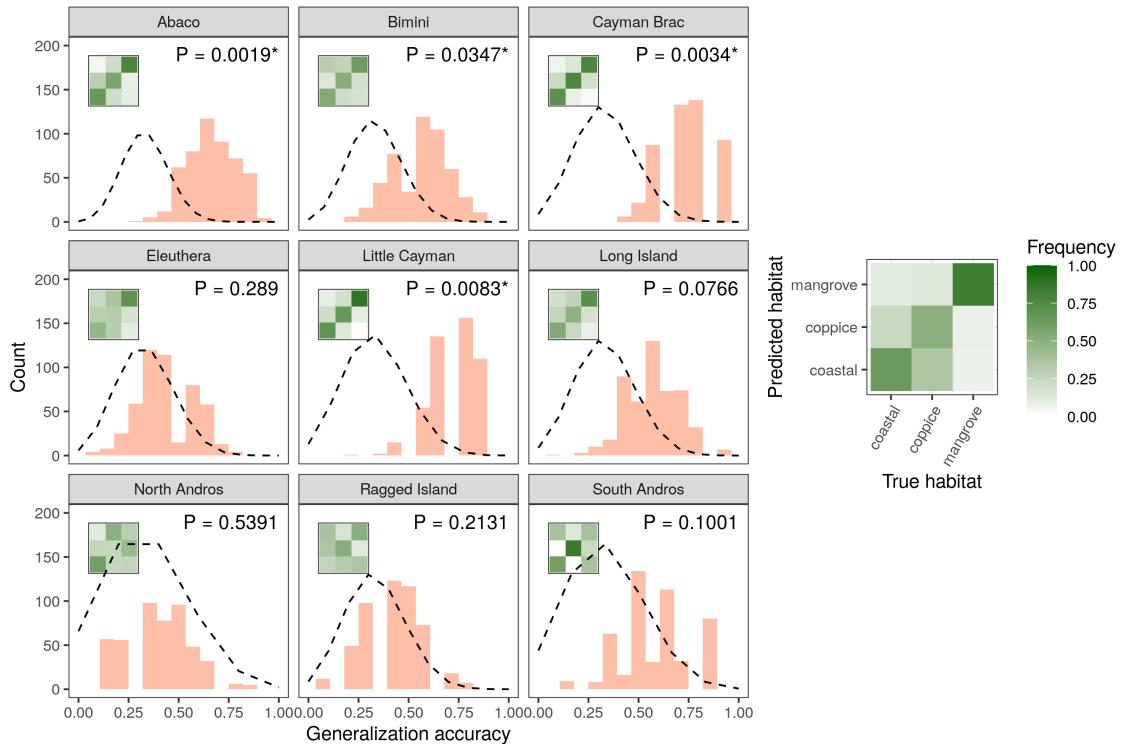


Figure S12: LDA classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

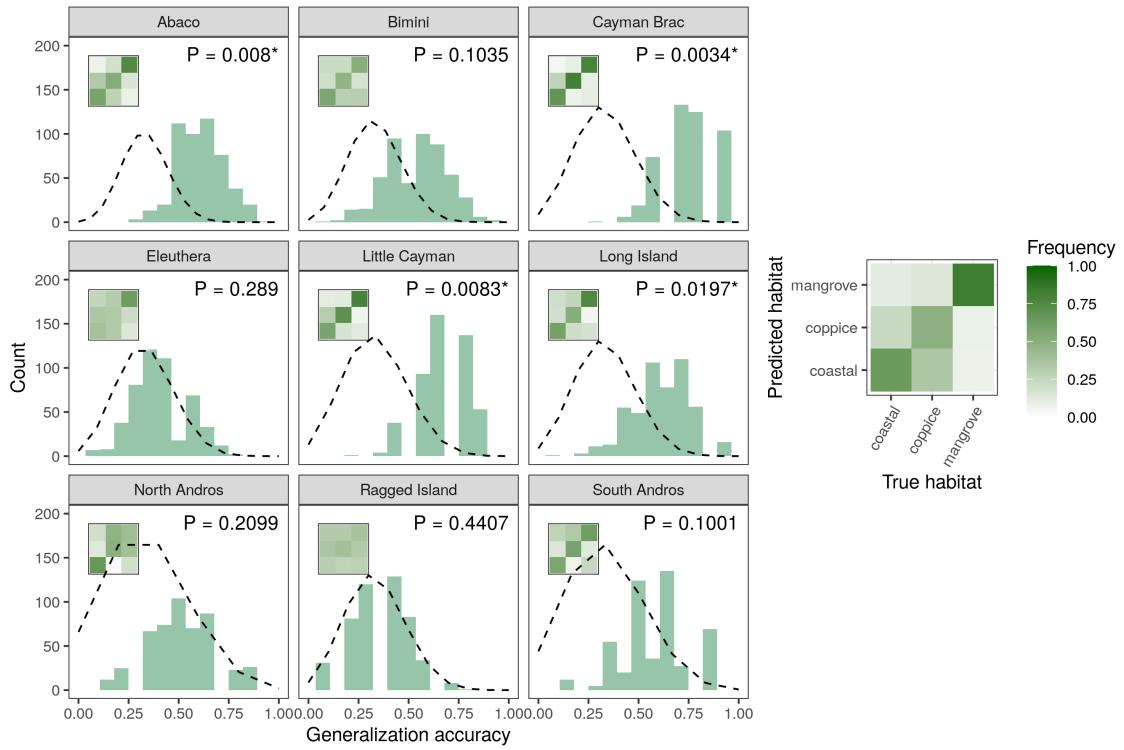


Figure S13: SVM classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

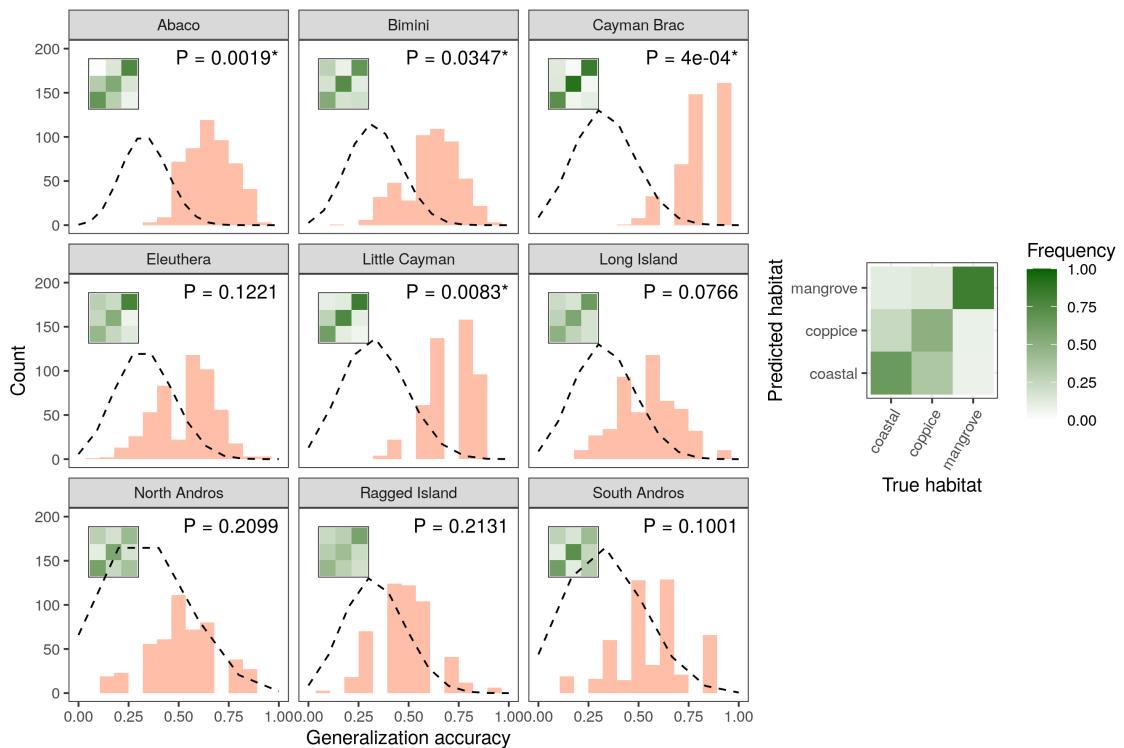


Figure S14: LDA classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

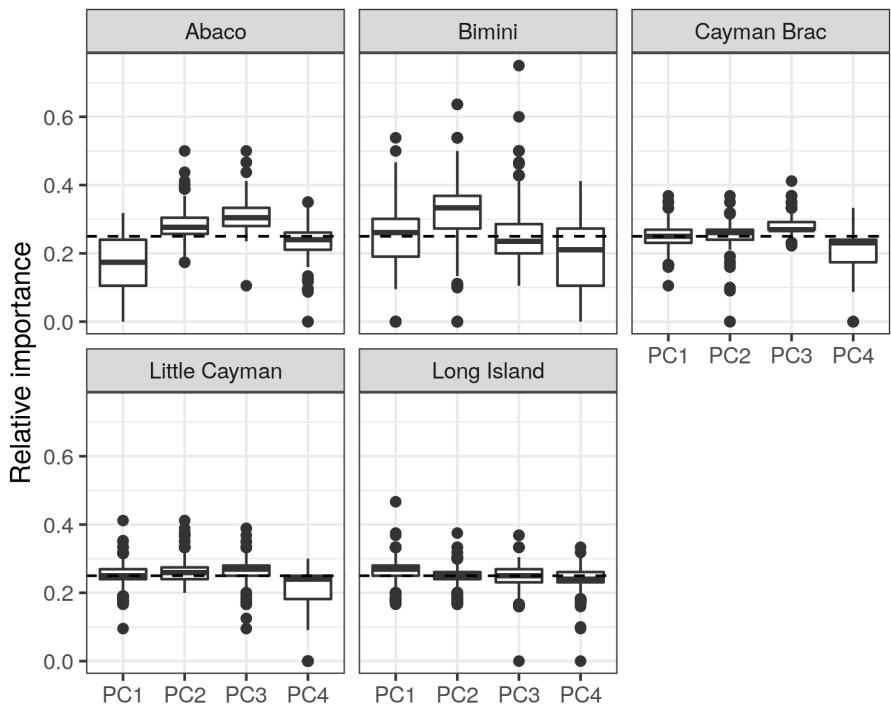


Figure S15: Sensitivity analyses of the different input variables in the within-island SVM classification on principal component data (Figure ??), with relative importance computed for every machine.

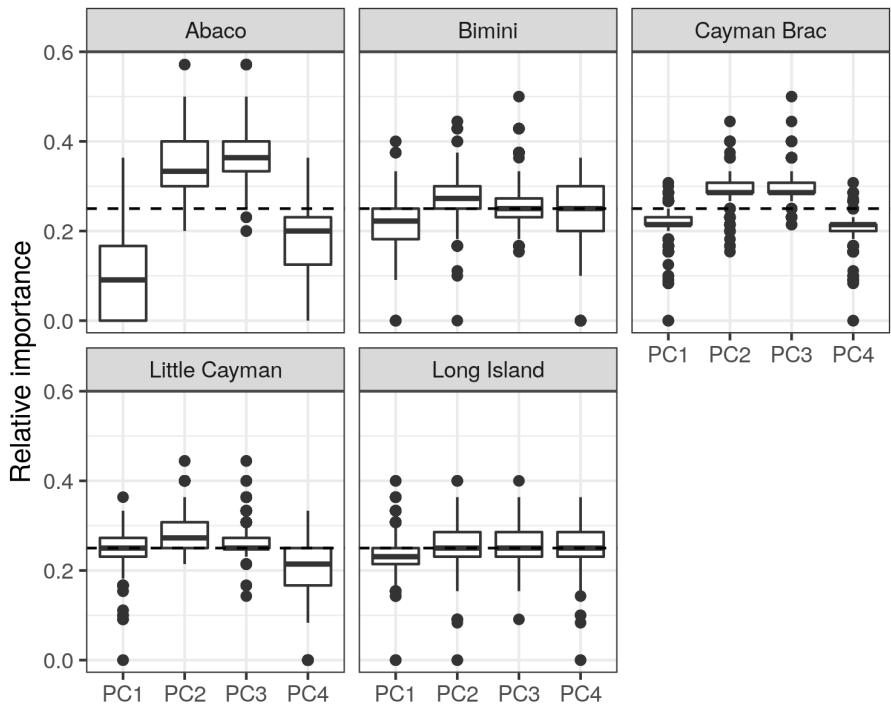


Figure S16: Sensitivity analyses of the different input variables in the within-island LDA classification on principal component data (Figure S12), with relative importance computed for every machine.

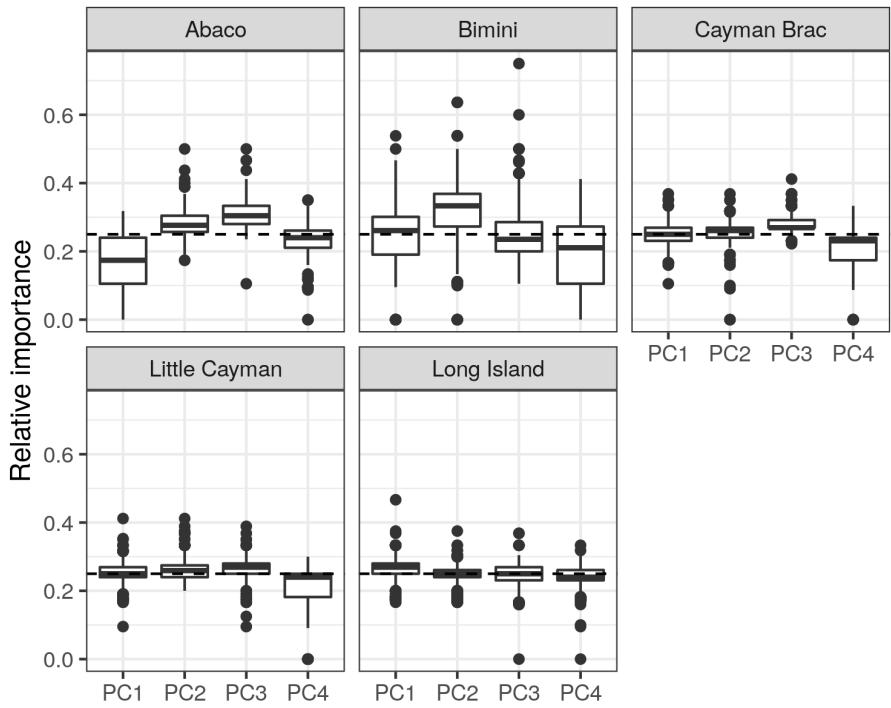


Figure S17: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance at 50nm-intervals in wavelength (Figure S13), with relative importance computed for every machine.

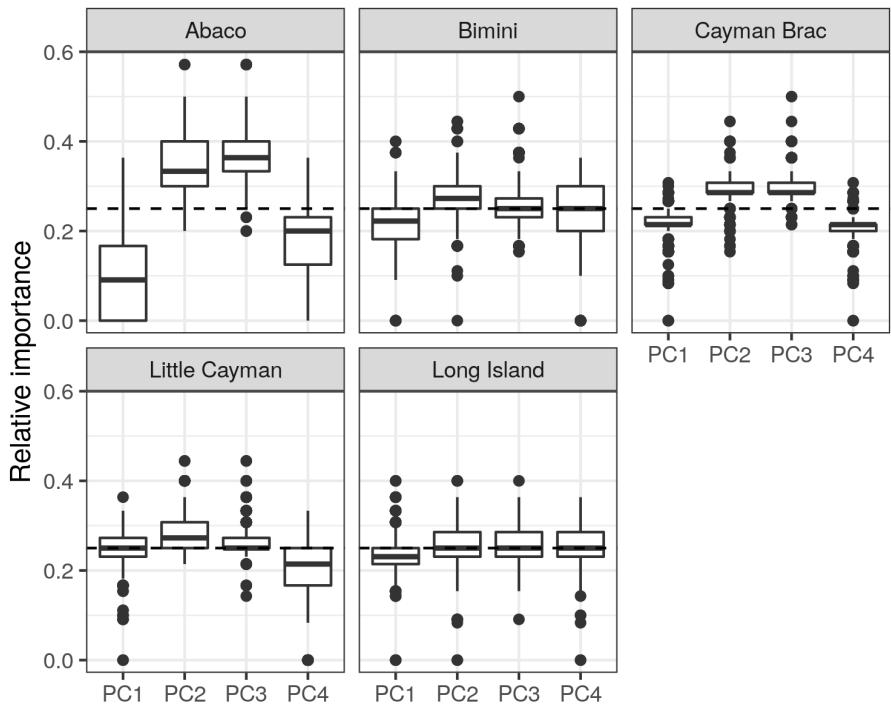


Figure S18: Sensitivity analyses of the different input variables in the archipelago-wide LDA classification on reflectance at 50nm-intervals in wavelength (Figure S14), with relative importance computed for every machine.

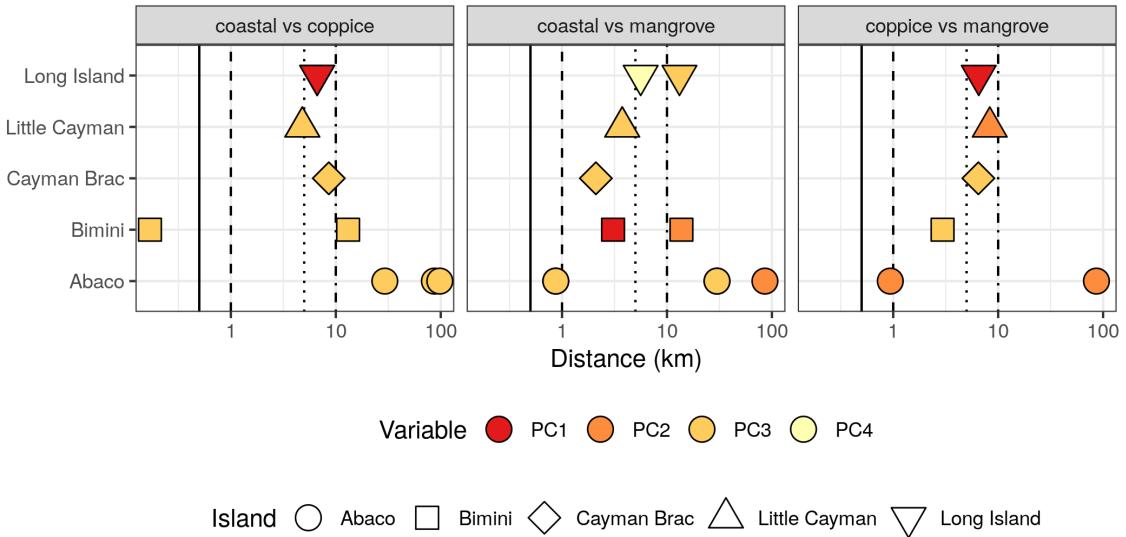


Figure S19: Spatial scale of between-habitat differences in dewlap coloration. For each variable and each pair of habitats where significant differences were detected (Figure 3), we performed multiple post hoc pairwise comparisons between the sites involved (Figure S1, Table S2), using nonparametric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

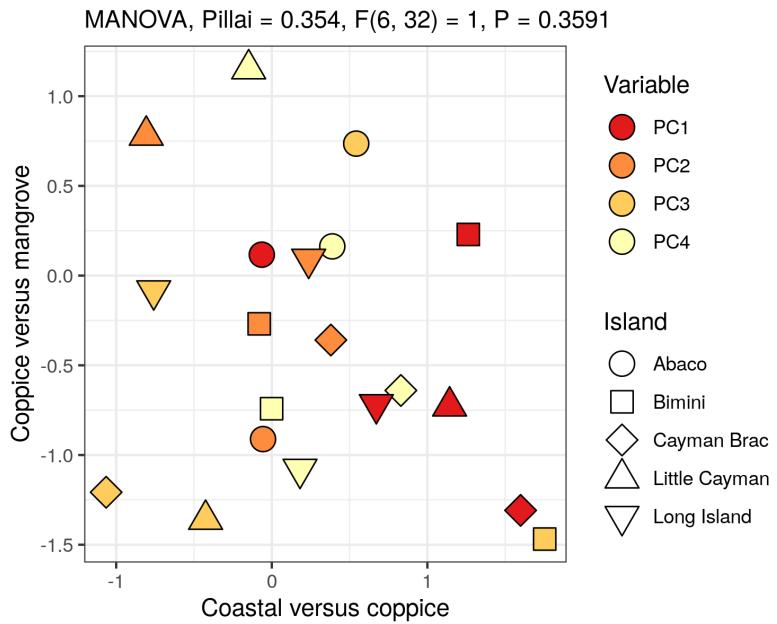


Figure S20: Test of parallel divergence between islands. Differences in habitat-means, or contrasts, are shown for two pairs of habitats for each principal component on each island, rescaled so the standard deviation of the means along each principal component is one. The contrasts represent the patterns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

⁸¹⁶ Supplementary Tables

Table S1: Number of lizards sampled in each habitat on each island.

	coastal	coppice	mangrove
Abaco	41	24	21
Bimini	38	14	15
Cayman Brac	15	18	17
Eleuthera	22	25	9
Little Cayman	17	12	16
Long Island	26	14	13
North Andros	9	9	10
Ragged Island	18	15	17
South Andros	10	9	12

Table S2: Locations of the sampling sites across islands, with mean principal component scores per site.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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