

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 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 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 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 ??). 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 S3). 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 S3). 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 S4). 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 η^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 S5). 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 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 (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 [S8](#)), but this effect was too small for post hoc
297 tests to find which habitats differed. Archipelago-wide differences in dewlap coloration between
298 habitats were also detected by SVMs trained on pooled data regardless of island identity, both for
299 PCA data and reflectance scores (Fig. [S5](#), [S6](#)). This pattern seemed to be driven by mangrove
300 lizards being correctly reassigned more often than predicted by chance. Sensitivity analyses on
301 these machines suggest a relatively small role of long wavelengths (red reflectance) in driving this
302 pattern (Fig. [S8](#)), but did not reveal strong differences between the PCs in relative importance
303 (Fig. [S7](#)). Archipelago-wide differences were not detected by LDA classifiers at all (Fig. [S9](#), [S10](#)).
304 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. 2). An LDA approach yielded similar success rates (Fig. S11), suggesting robust differences between these populations. Of the five islands, Little Cayman was the best discriminated with a mean SVM generalization success of 73.4% (Table S9). The results of the classification analyses on PCA data were very similar to results from SVMs and LDAs trained on reflectance values at 50nm-spaced wavelengths from 300 to 700nm (Fig. S12 and S13).

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

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

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

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

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

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

Sensitivity analyses on classifiers suggested an overall higher relative importance for PC2 and PC3 in determining between-group differences on Abaco, both in SVM and LDA classifiers (Fig. S14, S15), consistent with our ANOVA results (Fig. 3B). There was no strong signal of differences in relative importance among principal components on the other islands. Sensitivity analyses of SVMs trained on reflectance scores rather than principal components revealed, however, a consistently higher importance of ultraviolet reflectance in between-group differences on all islands (Fig. S16). This pattern was not recovered for LDAs trained on reflectance scores (Fig. S17).

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 (Table S11), 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. 3) 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. 3, Table 1). 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. We did detect a significant signal
397 of isolation-by-distance on Eleuthera, but we did not find differences in dewlap coloration between
398 habitats on this island.

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

408

409 The persistence of dewlap color divergence despite gene flow has been documented in multiple
410 species of anoles, sometimes in relation to environmental conditions. [Ng et al. \(2012\)](#) and [Ng](#)
411 [et al. \(2016\)](#) found divergent dewlap coloration in the face of gene flow between subspecies of *A.*
412 *distichus* across Hispaniola, and proposed this as a mechanism of reproductive isolation in the
413 early stages of speciation ([Ng and Glor, 2011](#); [Lambert et al., 2013](#); [Ng et al., 2017](#)). [Stapley et al.](#)
414 ([2011](#)) found that dewlap color polymorphism was maintained in the absence of genetic structure
415 between populations of *A. apletophallus* from Panama. [Thorpe and Stenson \(2002\)](#) found that
416 divergence in dewlap coloration matched habitat-type better than mitochondrial lineage in *A.*
417 *roquet* on Martinique, and a convergent pattern was found in *A. trinitatis* on the featureless island
418 of St Vincent ([Thorpe, 2002](#)). Finally, divergence in body coloration, but not dewlap coloration,
419 was also reported in *A. conspersus* on another small island, Grand Cayman ([Macedonia, 2001](#)).

Altogether, these results are consistent with a generally high adaptive potential of dewlap coloration in anoles over small distances, which could apply to *A.sagrei*. 420
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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. 422
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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; Baeckens et al., 2018). In particular, Baeckens et al. (2018) recently showed that dewlaps with spotted patterns occurred more often in *A. sagrei* on islands with more coexisting species of anoles. 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. 451
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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. 468
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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 have suggested 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 468
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475 predict contest-winning success between males. Driessens et al. (2015) further found that more
476 yellow and red dewlaps (relative to UV) predict better body condition, and that higher yellow and
477 UV reflectance at the margin of the dewlap predict higher hematocrit (the concentration of red
478 blood cells), indicating a better health.

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

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493 **Implications in the context of speciation** Local adaptation can be a precursor to ecological
494 speciation, a process that may have given rise to the adaptive radiation of *Anolis* lizards (Harmon
495 et al., 2003; Gavrilets and Losos, 2009). Ecologically-mediated divergence of a sexual signal may
496 be a potent path to the evolution of reproductive isolation through divergent sexual selection
497 (Reynolds and Fitzpatrick, 2007; Servedio et al., 2011). Evidence suggests that dewlap coloration
498 could take this role in anoles (Ng and Glor, 2011; Lambert et al., 2013; Geneva et al., 2015; Ng et al.,
499 2017), or at least that it is frequently involved in species recognition (Williams, 1969; Williams and
500 Rand, 1977; Losos, 1985; Macedonia and Stamps, 1994; Fleishman, 2000; Macedonia et al., 2013;
501 Ingram et al., 2016; Baeckens et al., 2018). Although this signal is not detected at the phylogenetic
502 scale of the whole genus (Nicholson et al., 2007; Harrison and Poe, 2012; Ingram et al., 2016),
503 sexual signals are often evolutionarily very labile (Kraaijeveld et al., 2011), and the anole dewlap
504 in particular is capable of rapid macroevolution; for example, *A. conspersus* on Grand Cayman
505 evolved a UV-blue dewlap from an ancestral orange dewlap in 2 to 3 million years (Macedonia,
506 2001). We present evidence of multiple cases of potentially adaptive maintenance of habitat-
507 associated dewlap divergence over small geographical scale in *A. sagrei* across the West Indies.
508 While these populations do not appear to be in the process of speciation, our results strongly
509 suggest that the anoline dewlap has enough micro-scale, local adaptive potential to participate in
510 the build-up of reproductive isolation, should it be recruited for assortative mating.

511

Acknowledgements

512 Collection permission was granted by the Bahamas Environment, Science and Technology Com-
513 mission, the Bahamas National Trust, and the Cayman Islands Department of the Environment.
514 The authors thank Sofia Prado-Irwin, Pavitra Muralidhar, Nicholas Herrmann, Richard E. Glor,
515 Alberto R. Puente-Rolón, Kevin Aviles-Rodriguez, Kristin Winchell, and Melissa Kemp for assis-
516 tance in the field. Funding for this work was provided by the Templeton Foundation (to JBL), NSF
517 DEB #1500761 (to AJG), NSF DBI #1609284 (to CMD), and a Harvard Museum of Comparative
Zoology Putnam Grant (to RGR).

Figures

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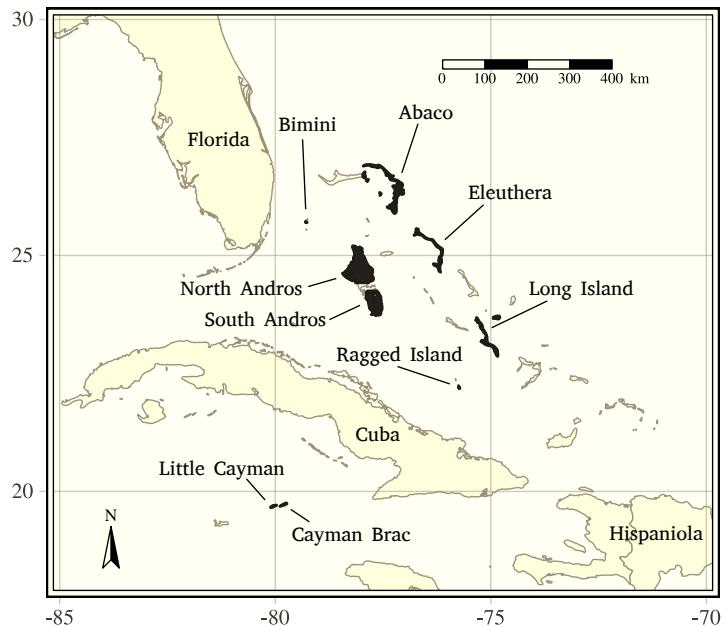


Figure 1: Map of the West Indies with sampled islands highlighted in black.

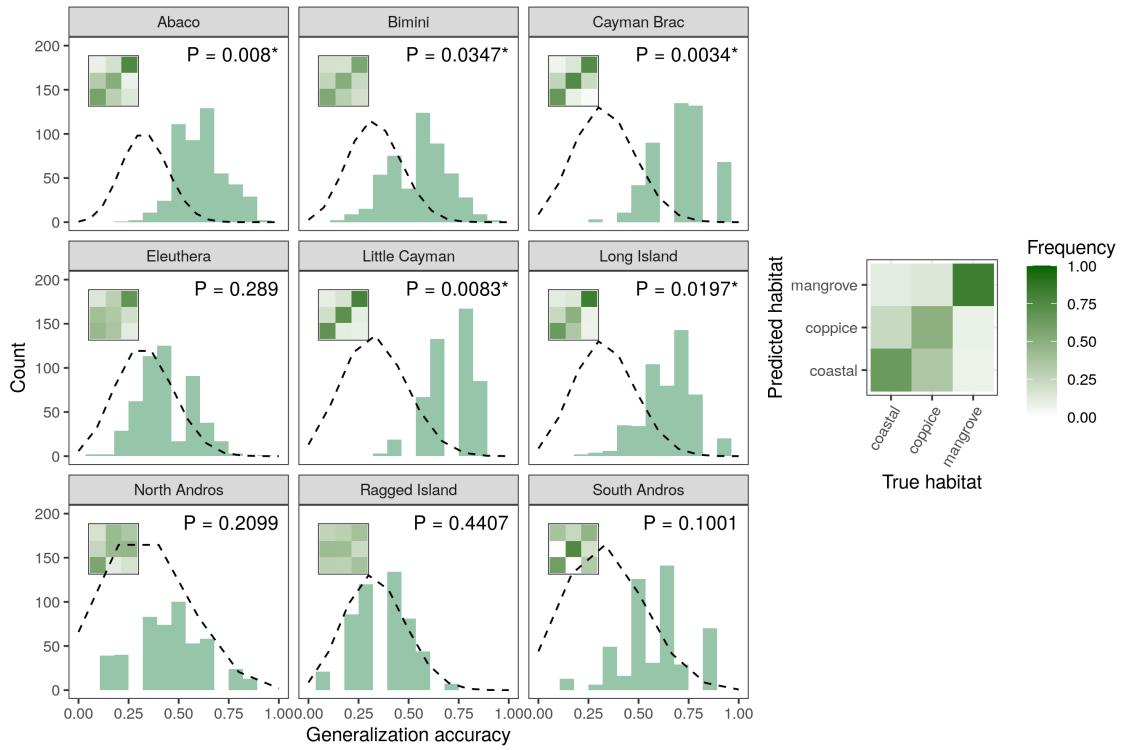


Figure 2: SVM classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The dashed line is the density of a corresponding null binomial distribution, which would be expected under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

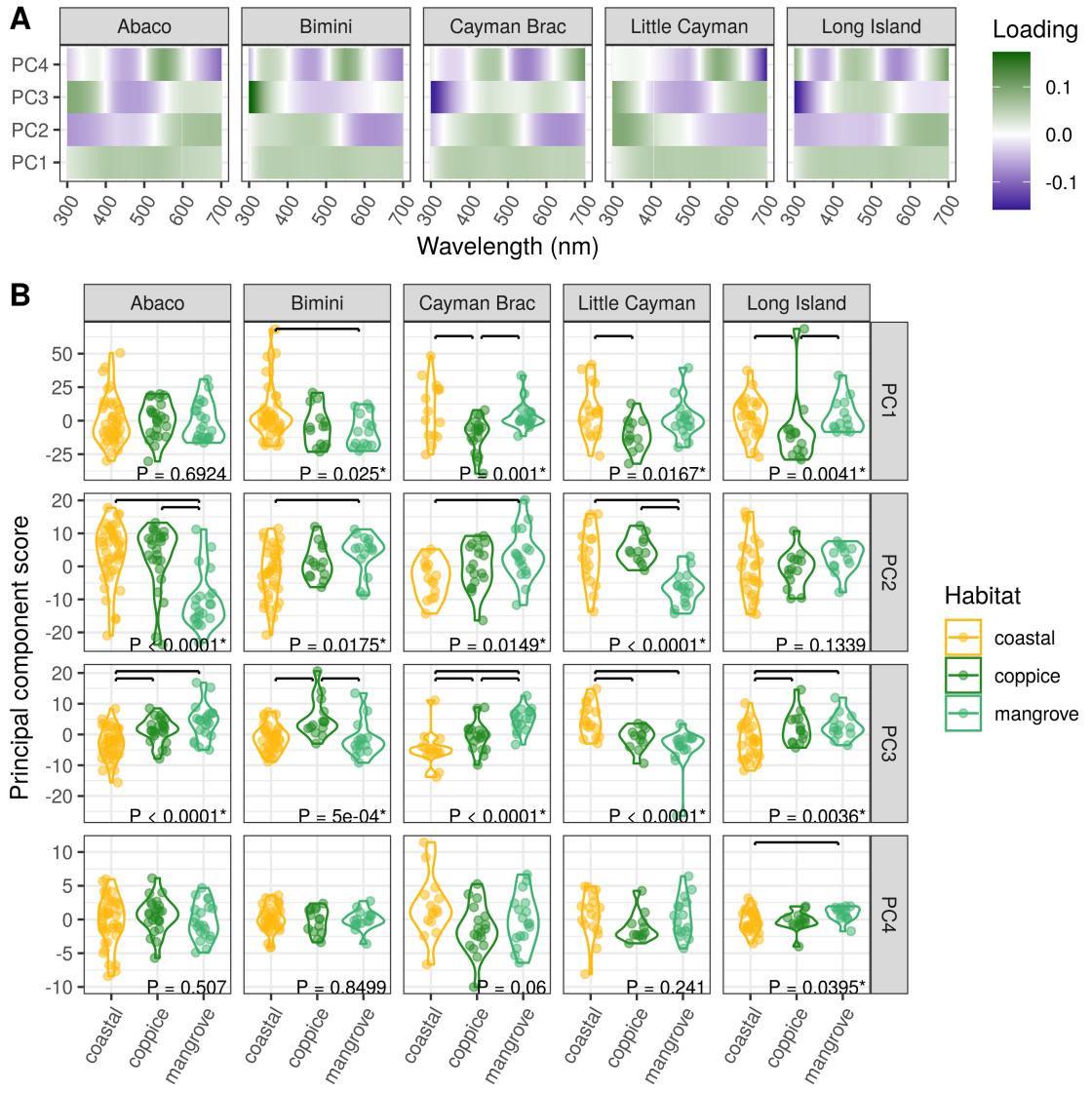


Figure 3: Dewlap color variation between habitat-types across the most significant islands. (A) Mapping of reflectance at various wavelengths onto the principal components (loadings from the PCA rotation matrix). (B) Distribution of PC scores between habitats along the first four PCs on each island where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

⁵¹⁹ **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	df _{LRT}	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

786

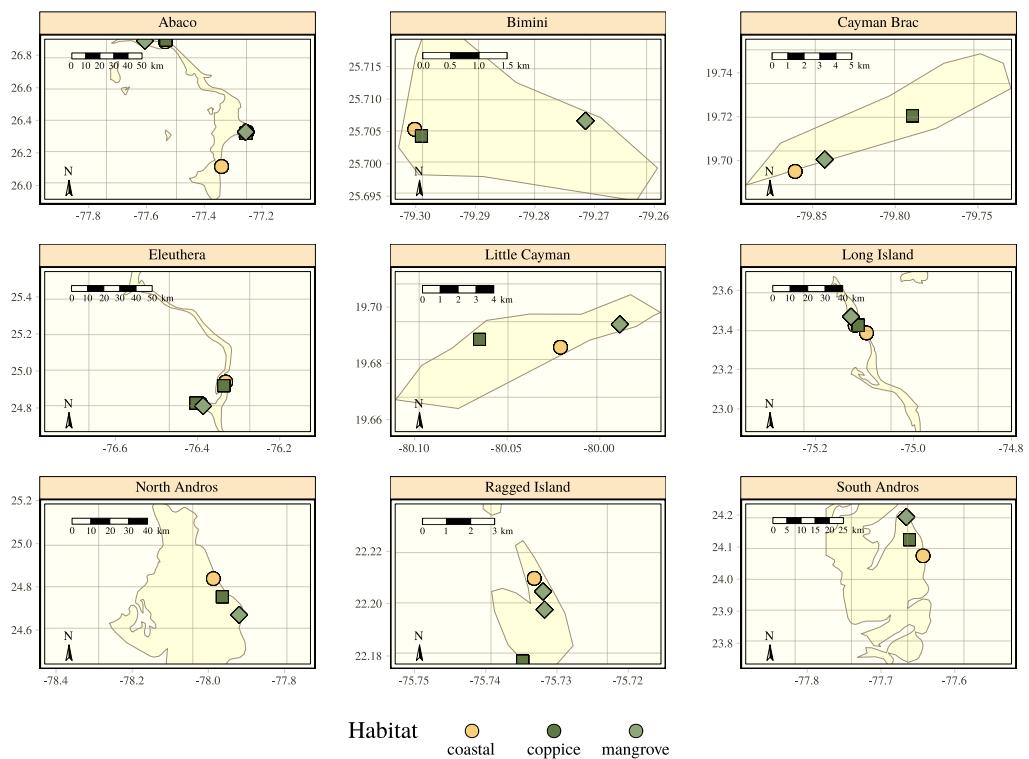


Figure S1: Map of the sampling sites and corresponding habitats across nine islands of the West Indies.

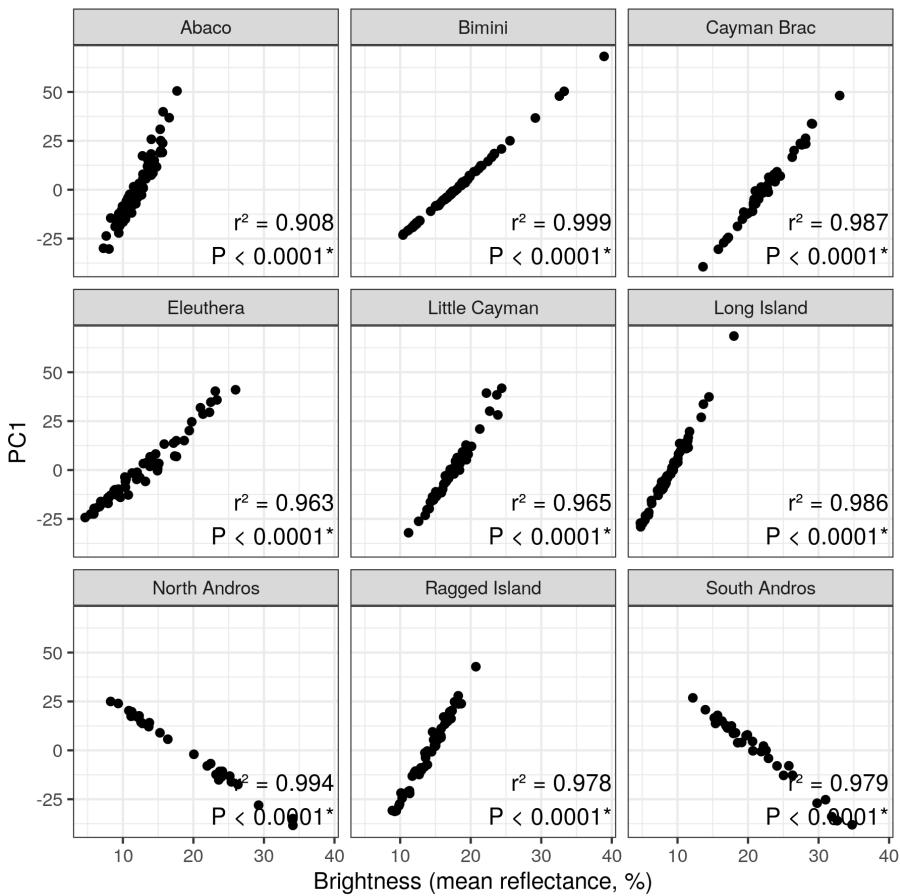


Figure S2: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score for each island. Pearson's squared correlation coefficients are reported. *, $P < 0.05$.

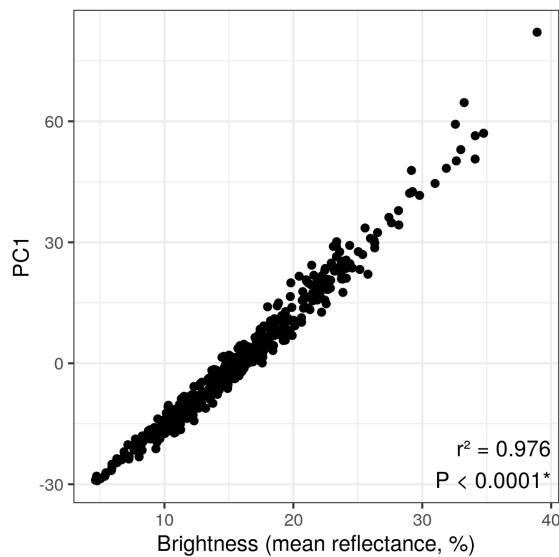


Figure S3: Correlation between dewlap brightness (as measured by the mean reflectance from 300 to 700nm in wavelength) and PC1 score across the whole archipelago. Pearson's squared correlation coefficient is reported. *, $P < 0.05$.

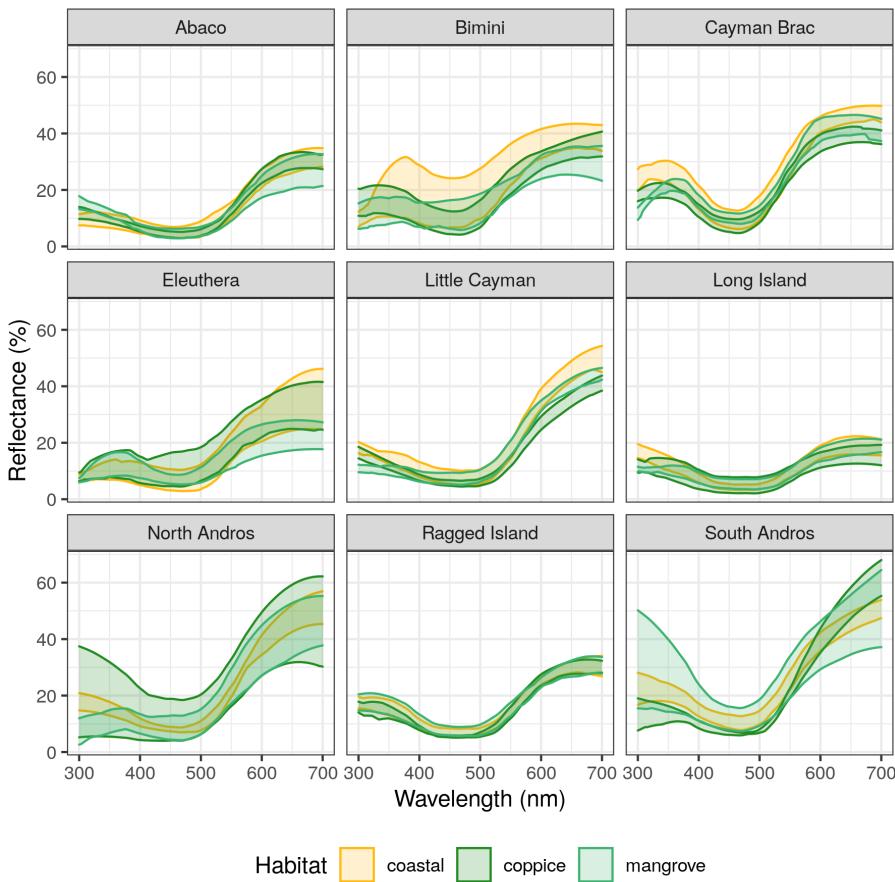


Figure S4: 5-95th percentile range of lizard dewlap reflectance values (in % of incoming light) across wavelengths for each island and each habitat.

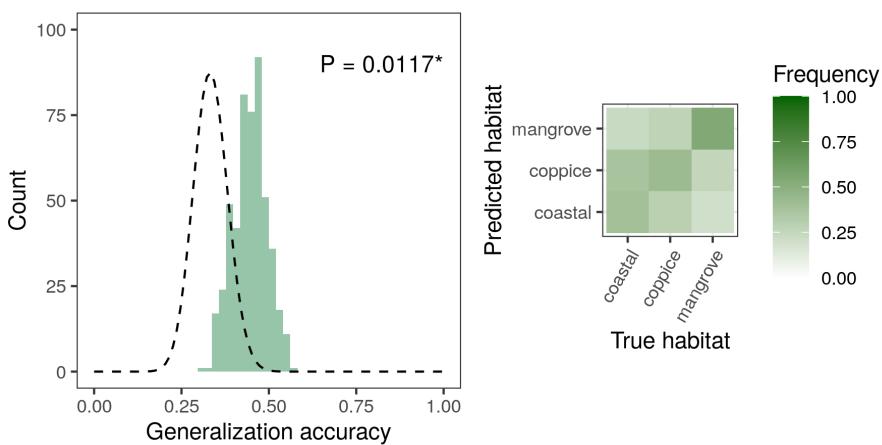


Figure S5: Archipelago-wide SVM classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 2.

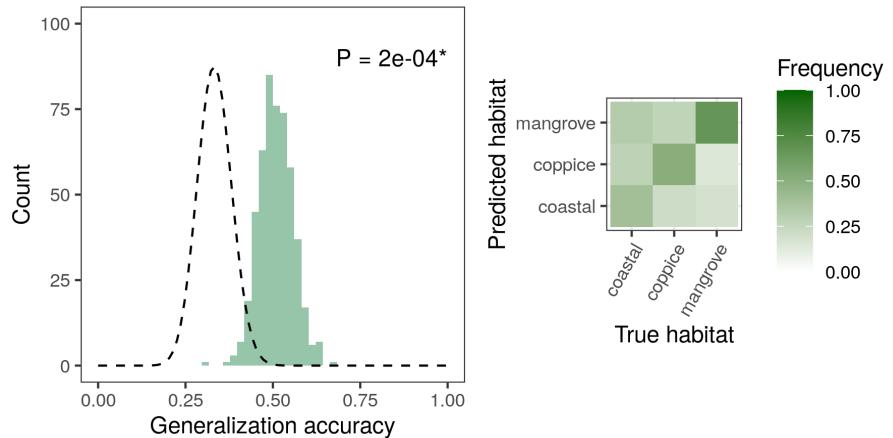


Figure S6: Archipelago-wide SVM classification accuracy based on reflectance data at 50nm-intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 2.

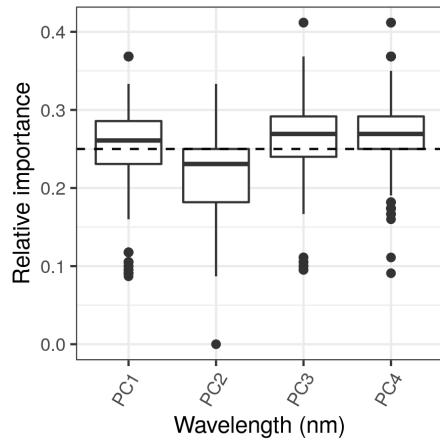


Figure S7: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on principal component data (Figure S5), with relative importance computed for every machine.

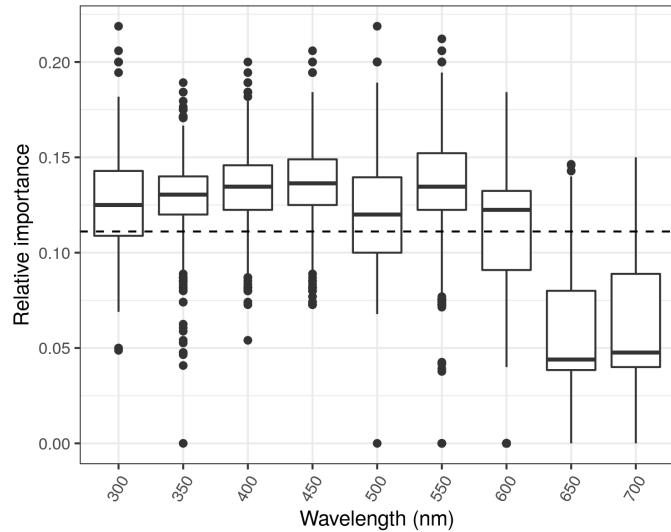


Figure S8: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance data at 50nm-intervals in wavelength (Figure S6), with relative importance computed for every machine.

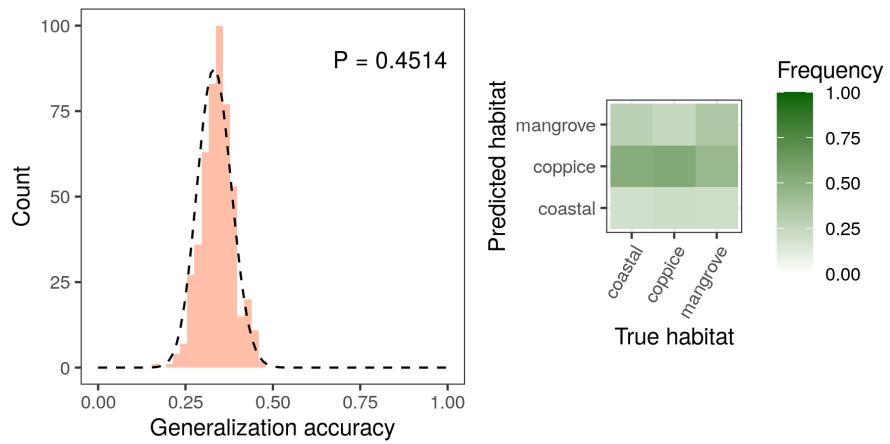


Figure S9: Archipelago-wide LDA classification accuracy based on principal component data. Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 2.

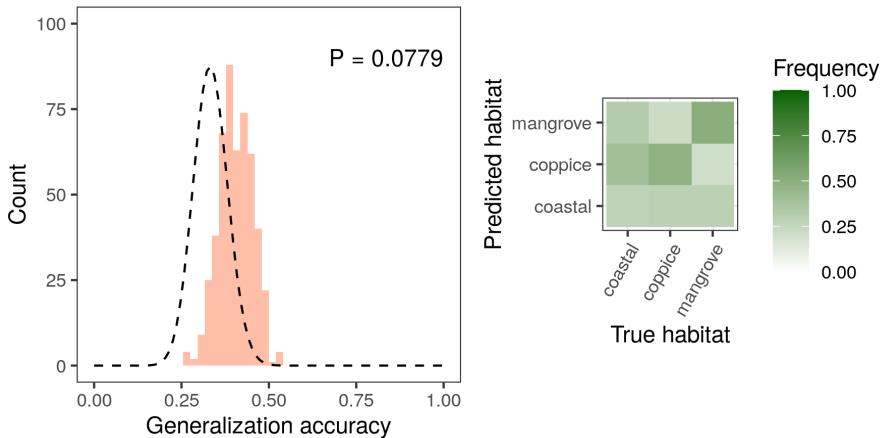


Figure S10: Archipelago-wide LDA classification accuracy based on reflectance data at 5nm intervals in wavelength (see Methods). Machines were trained on individual dewlaps regardless of island identity. The histogram shows the accuracy distribution over 100 replicates for each five cross-validation bins. The legend is the same as in Figure 2.

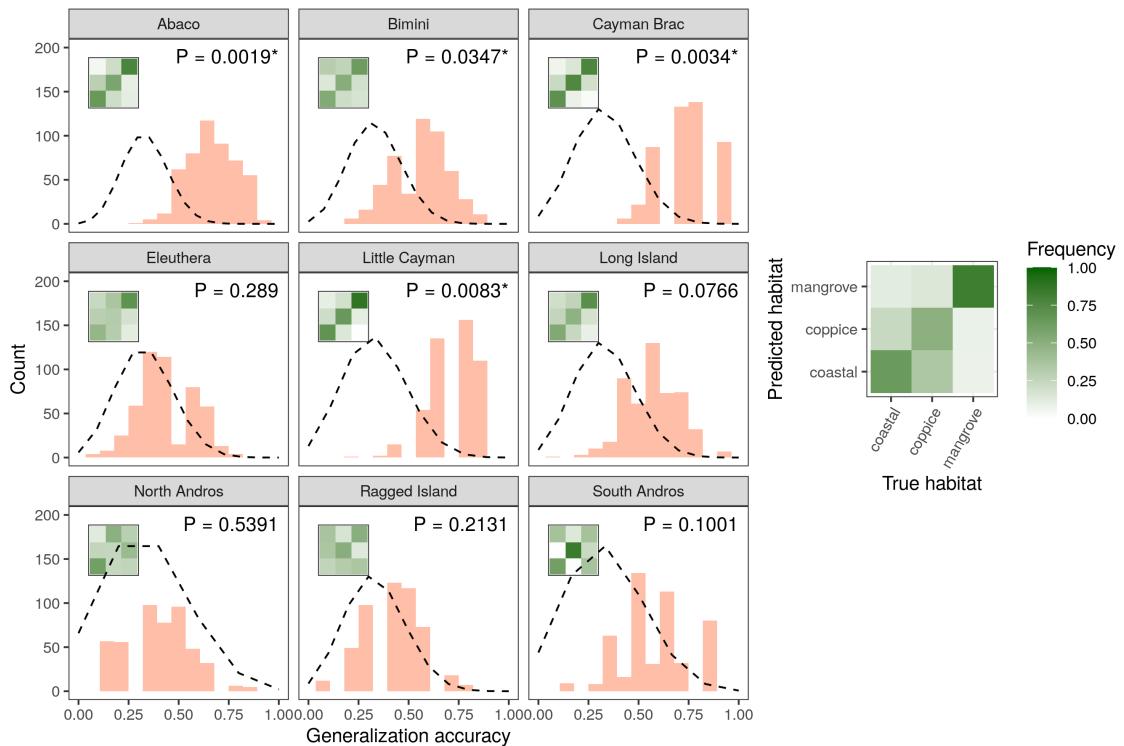


Figure S11: LDA classification accuracy across islands based on principal component data. Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

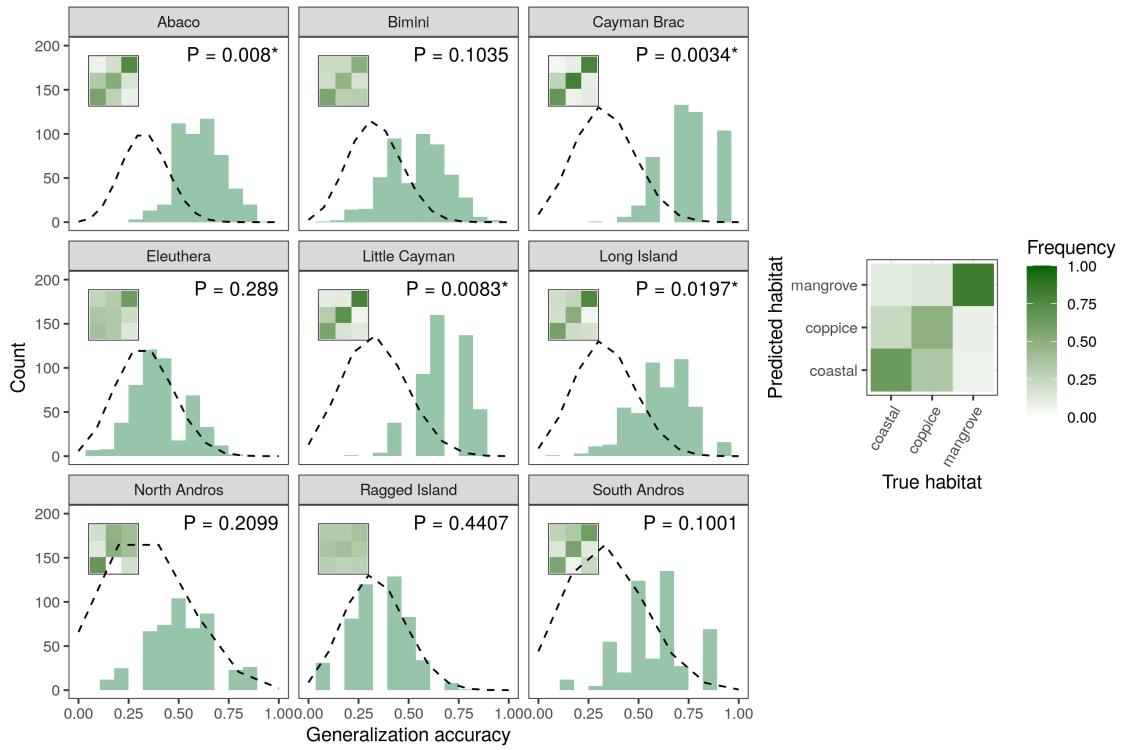


Figure S12: SVM classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

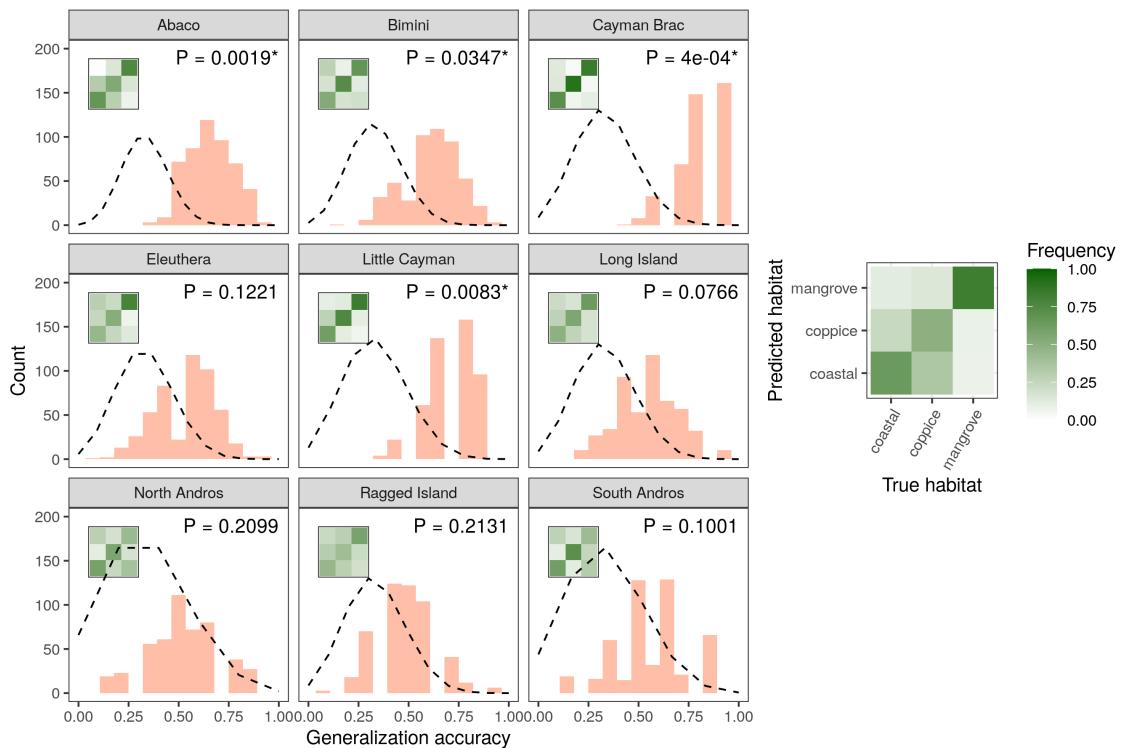


Figure S13: LDA classification accuracy across islands based on reflectance data at 50nm-intervals in wavelength (see Methods). Histograms show accuracy distributions over 100 replicates for each five cross-validation bins per island. The legend is the same as in Figure 2.

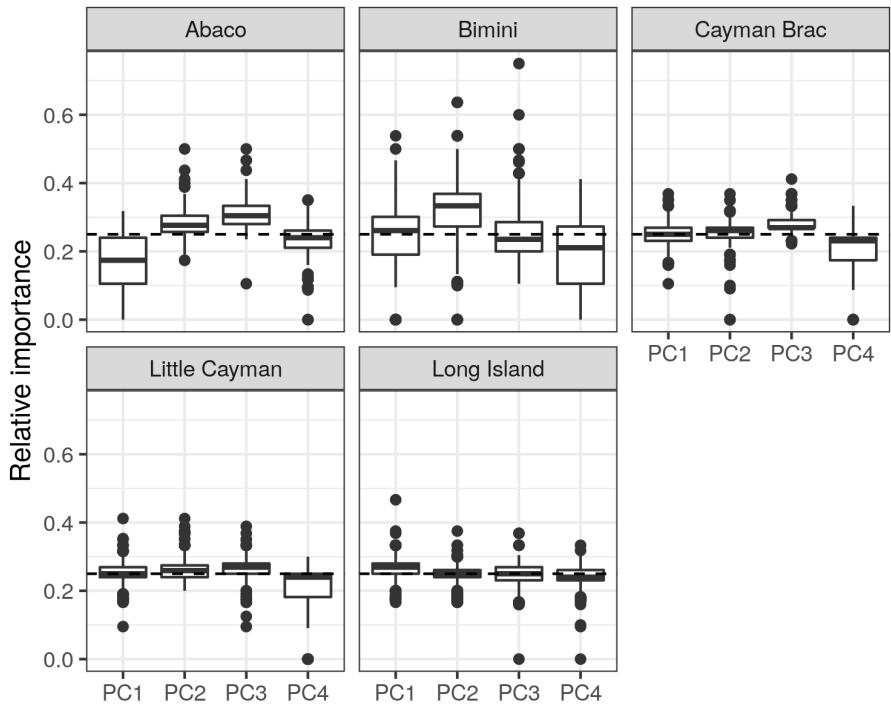


Figure S14: Sensitivity analyses of the different input variables in the within-island SVM classification on principal component data (Figure ??), with relative importance computed for every machine.

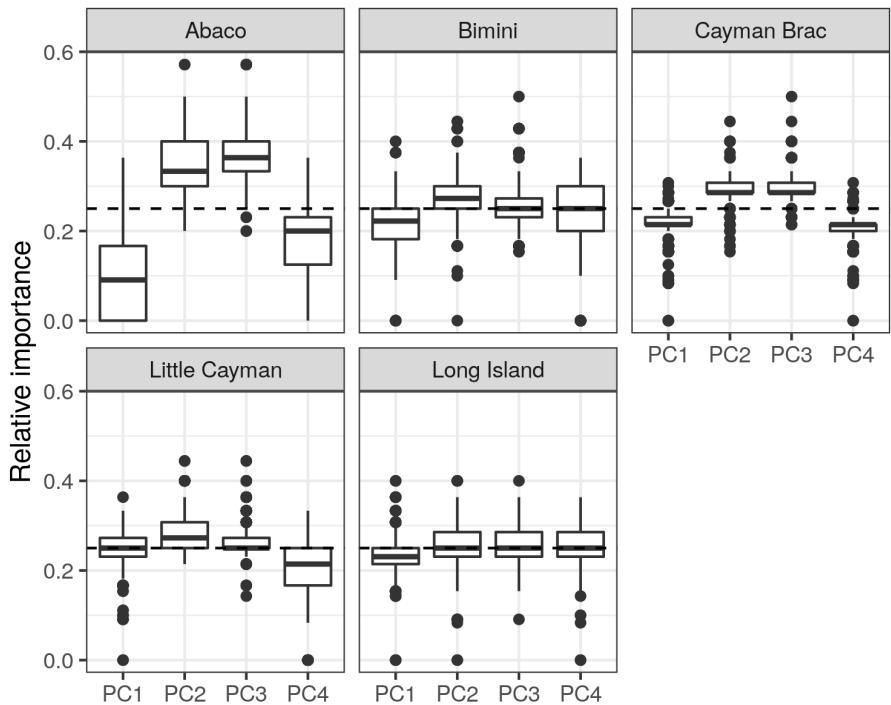


Figure S15: Sensitivity analyses of the different input variables in the within-island LDA classification on principal component data (Figure S11), with relative importance computed for every machine.

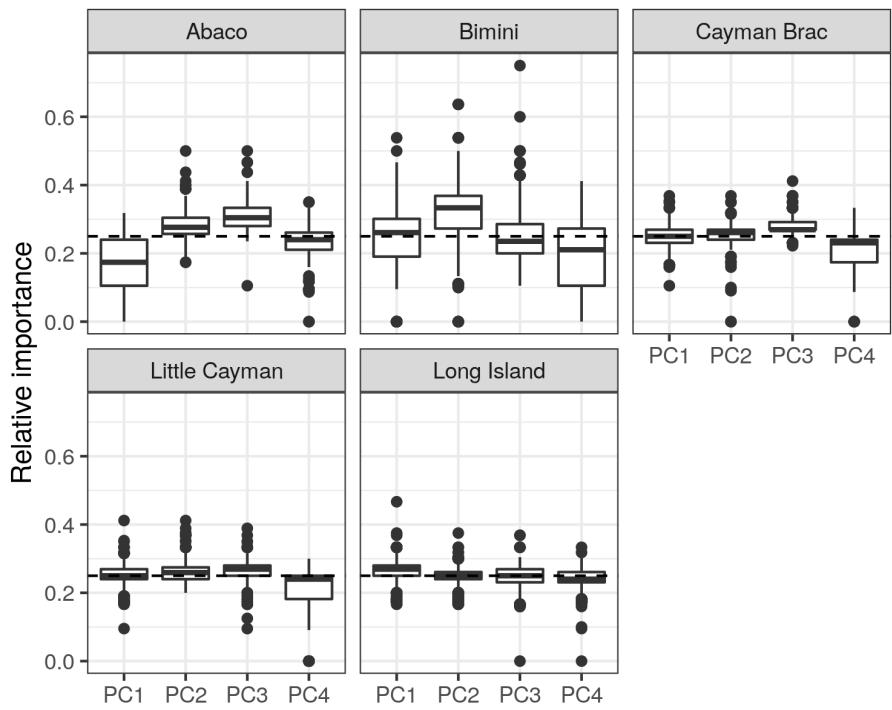


Figure S16: Sensitivity analyses of the different input variables in the archipelago-wide SVM classification on reflectance at 50nm-intervals in wavelength (Figure S12), with relative importance computed for every machine.

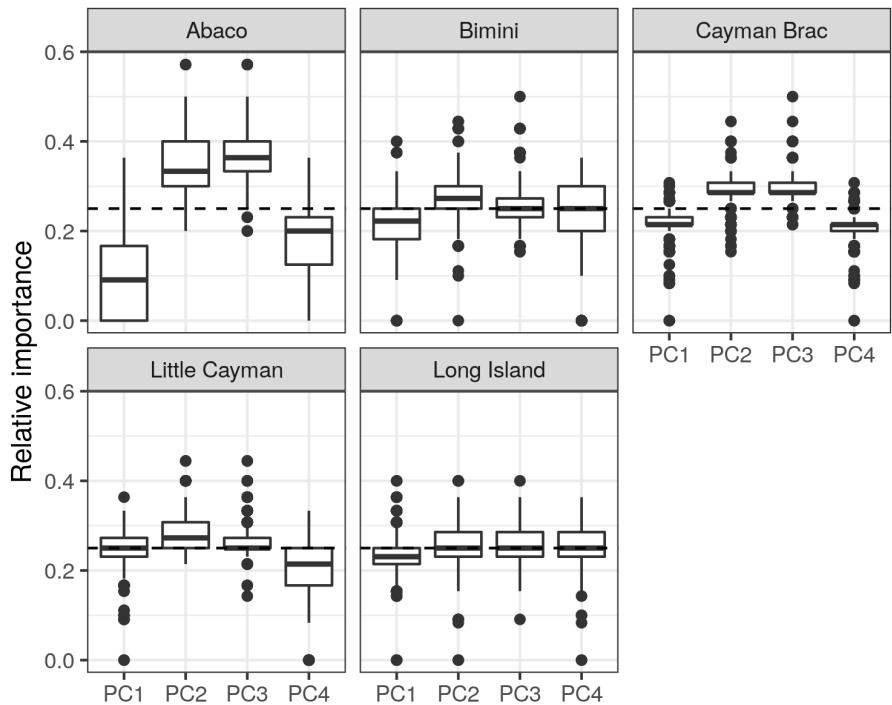


Figure S17: Sensitivity analyses of the different input variables in the archipelago-wide LDA classification on reflectance at 50nm-intervals in wavelength (Figure S13), with relative importance computed for every machine.

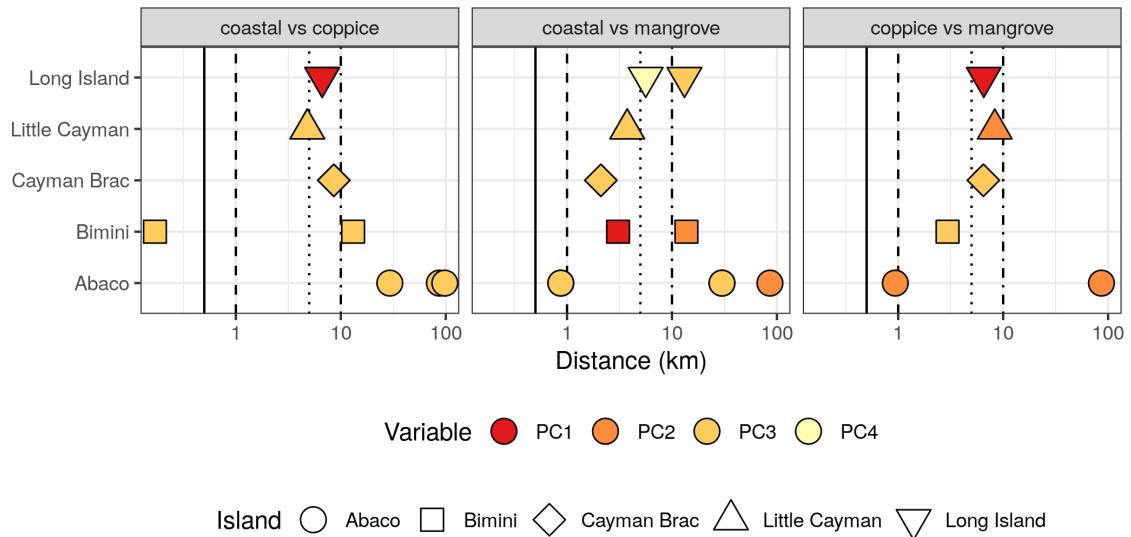


Figure S18: Spatial scale of between-habitat differences in dewlap coloration. For each variable and each pair of habitats where significant differences were detected (Figure 3), we performed multiple post hoc pairwise comparisons between the sites involved (Figure S1, Table S2), using nonparametric 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).

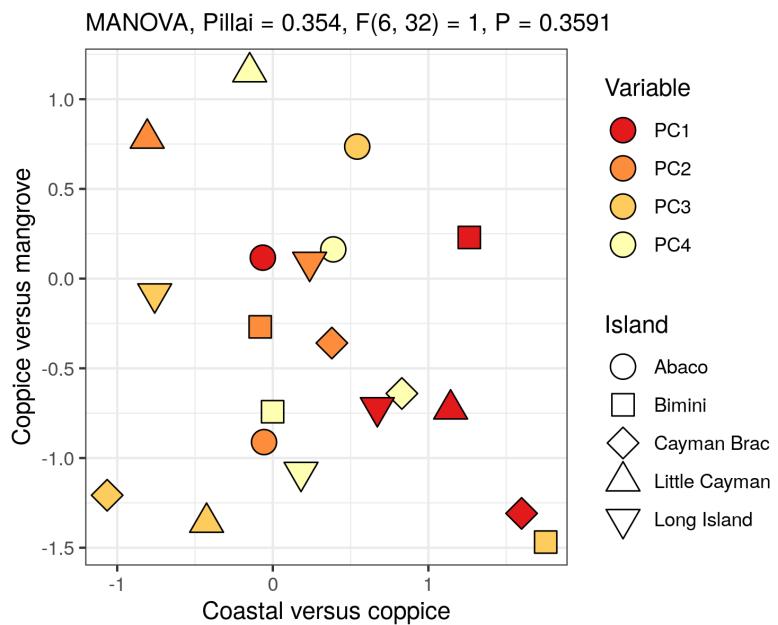


Figure S19: Test of parallel divergence between islands. Differences in habitat-means, or contrasts, are shown for two pairs of habitats for each principal component on each island, rescaled so the standard deviation of the means along each principal component is one. The contrasts represent the patterns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