Received Date : 07-Apr-2013

Revised Date : 22-Oct-2013

Accepted Date : 22-Nov-2013

Article type : Regular Paper

Genetic seascape of the threatened Caribbean elkhorn coral, *Acropora palmata,* on the Puerto Rico Shelf

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ABSTRACT: It has been proposed that the elkhorn coral, *Acropora palmata*, is genetically separated into two distinct provinces in the Caribbean, an Eastern and a Western population admixing in western Puerto Rico and around the Mona Passage. In this study, the genetic structure of *A. palmata* sampled at 11 Puerto Rican localities and localities from Curaçao, the Bahamas and Guadeloupe were examined. Analyses using five microsatellite markers showed that 75% of sampled colonies had unique genotypes, the rest being clone mates. Genetic diversity among genets was high (H_E = 0.761) and consistent across localities (0.685 to 0.844). F_{ST} ranged from -0.011 to 0.047 supporting low but significant genetic differentiation between localities within the previously reported Eastern and Western genetic provinces. Plots of genetic per geographic distances and significant Mantel tests supported isolation-by-distance (IBD) within Puerto Rico. Analysis with the software *Structure* favored a scenario with weak

differentiation between two populations, assigning eastern Puerto Rican locations (Fajardo and Culebra), Guadeloupe and Curaçao to the Caribbean Eastern population and western Puerto Rican locations (west of Vega Baja and Ponce), Mona and the Bahamas to the Caribbean Western population. Vieques and San Juan area harbored admixed profiles. Standardized F_{ST}s per 1,000 km unit further supported higher differentiation between localities belonging to different *Structure* populations, with IBD being stronger within Puerto Rico than on larger regional scales. This stronger genetic transition seems to separate localities between putative Eastern and Western provinces in the eastern Puerto Rican region, not around the Mona Passage.

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PROBLEM

Genetic diversity and structure in scleractinian corals vary significantly, reflecting the evolutionary differences between species, but also the type of genetic markers employed, microsatellite markers being more successful at detecting weak genetic structure than mitochondrial markers, ITS or allozymes (Palumbi 2003; Vollmer & Palumbi 2004; Van Oppen & Gates 2006). Interestingly, even related species with similar life-histories and dispersal potentials might exhibit different population structure (Severance & Karl 2006; Hemond & Vollmer 2010). With a few exceptions (Benzie *et al*. 1995; Ayre & Hughes 2000), panmixia is generally observed within small distances (10s of Km; Ng & Morton 2003; Magalon *et al.* 2005), where connectivity is assured over one-generation-spawning events (Palumbi 2003). In contrast, varying patterns of genetic structuring are generally the rule over larger geographic distances and are characterized by a combination of discrete populations with Isolation-By-Distance (IBD, MacKenzie *et al.* 2004; Maier *et al.* 2005). Studies in the Caribbean are less numerous than in the Indo-Pacific but have typically shown significant genetic structuring, perhaps as a result of limited gene flow (Vollmer & Palumbi 2007).

With over 100 species, *Acropora* is one of the most broadly distributed coral genera (Wallace 1999; Veron & Stafford-Smith 2000). *Acropora* species harbor diverse patterns of genetic structuring (e.g. Benzie *et al*. 1995; Ayre & Hughes 2000; MacKenzie *et al*. 2004; Baums *et al*. 2005b). Despite the extreme diversity of acroporids in the Indo-Pacific Ocean, there are only two species in the Caribbean, *A. palmata* and *A. cervicornis* (Van Oppen *et al*. 2000; Vollmer & Palumbi 2002). While molecular data of *A. cervicornis* across the Caribbean has supported significant genetic divergence between regions separated by several hundreds of kilometers or more (e.g. Florida vs. the Bahamas vs. Curaçao), genetic differentiation between reefs separated by a few kilometers is generally not significant, except when introgression of *A. palmata* alleles is observed (Vollmer & Palumbi 2007; Garcia Reyes & Schizas 2010; Hemond & Vollmer 2010). On the other hand, such short-scale structure was recently evidenced in *A. cervicornis* using spatial autocorrelation of nuclear and mtDNA data (Palumbi *et al.* 2012).

Microsatellites analysis of *A. palmata* sampled throughout the Caribbean in *Structure*, a software that has been widely used to find the number of biological populations in a given dataset (Evanno *et al*. 2005), suggested that the species comprised an Eastern and a Western population (Baums *et al*. 2005b, 2006a, 2006b). The population break in the southern Caribbean

seemed to occur at the Guajira Peninsula, Colombia, while in the northern Caribbean, the break was located around Puerto Rico. Depending on the model used, western Puerto Rican localities either clustered with the western population or presented admixed genotypes reminiscent of a hybrid zone between populations (Baums *et al*. 2005b, 2006a), suggesting that the Mona channel might act as a natural filter in *A. palmata*, as was reported for several other marine species (Colin 2003; Dennis *et al*. 2005; Galindo *et al*. 2006; Taylor & Hellberg 2006; Andras *et al.* 2013).

Further genetic characterization of elkhorn corals around Puerto Rico is necessary because it is unclear where the two proposed populations stop or merge. Indeed, it is arguable to assume two well differentiated biological populations based on *Structure* results, since the existence of discrete populations is an implicit assumption of *Structure,* which makes its use inadequate to describe continuously distributed genetic differentiation such as in the case of isolation-by-distance (Pritchard *et al*. 2000). Furthermore, only a few Puerto Rican reefs have been studied and they only represented the west and south coasts of the island (Baums *et al*. 2005b, 2006b). Additionally, a detailed description of the genetic diversity and structure of *A. palmata* in Puerto Rican reefs might improve local management of the species, following the example of the Tres Palmas Marine Reserve, implemented in 2004 to protect elkhorn coral stands (Valdés-Pizzini *et al*. 2009). In order to obtain this much needed and improved understanding of genetic structure on the Puerto Rico Shelf, we sampled *A. palmata* around Puerto Rico by alternating small geographic distances between reefs (few kilometers) and moderate distances (tens of km) between neighboring reefs as recommended by Guillot *et al*. (2009). Samples from the Bahamas, Curaçao and Guadeloupe were also included to represent distant reefs (hundreds to thousands of km), representing both inferred populations of the Eastern and Western regions (Baums *et al*. 2005b, 2006b). We assessed clonality and genetic diversity within these reefs, and explored patterns of IBD versus patterns of population structuring resulting from the existence of discrete populations in the dataset.

MATERIAL AND METHODS

Sampling

Twenty-four reefs were located in Puerto Rico (including Mona, Culebra and Vieques) (Figure 1, Table 1). Special effort was dedicated to (1) alternate small geographic distances (few kilometers) with moderate distances (tens of km) between reefs and (2) select reefs from areas all over the Puerto Rican archipelago in a comprehensive design. The other six reefs represented

samples from the Eastern (Curaçao, Guadeloupe) and Western populations (the Bahamas). All samples were taken between 2006 and 2009 and were collected opportunistically (non-randomized pattern). Particular efforts were made to sample both potential clone colonies (various ramets of a same genet) and potentially different genotypes. Hence, for each reef we sampled tissue from colonies within a 5 meter radius (likely to be clones) as well as colonies separated by tens to hundreds of meters (unlikely to be clones). Whenever possible, 20-50 colonies per reef were collected, preferentially by snapping the tip of branches. Samples from 412 colonies were obtained, including 86 from Garcia Reyes & Schizas (2010).

Molecular techniques

From each sample, 5-10 polyps were cut-off and total genomic DNA was extracted with the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's animal tissue protocol. Each sample was then screened for 5 polymorphic microsatellite markers, following a modified protocol from Baums *et al*. (2005a). The selected markers, namely #166, #181, #182, #192 and #207, were the same as those used in Baums *et al*. (2005a; 2006a; 2006b). PCR amplifications were done in 10 µl reactions, containing 1 µl genomic DNA (5-15 ng ul^{-1}), 0.8 mM dNTPs, 0.1 µM of forward primer with M13 tail, 0.1 µM of M13 fluorescently labeled with FAM (markers #166 and #182) or HEX (markers #181, #192 and #207), 0.2 µM of reverse primer, $MgCl_2$ (2 mM), 0.3 µl of 1U μl^{-1} Taq DNA polymerase (Fermentas), and 1X of the PCR buffer. Temperature cycling was performed by denaturing 1 min at 94°C, followed by 20 cycles of 20 s at 94°C, 35 s at 56°C and 30 s at 72°C, 15 cycles of 20 s at 94°C, 35 s at 50°C and 30 s at 72°C and a 10 min extension step at 72°C. Amplicons were diluted up to 50X to approach 10-20 ng μl^{-1}, pooled whenever possible (#166 with #207 and #182 with #192) and were run on an ABI3130*xl* Genetic Analyzer with ROX labeled size standards. Microsatellite alleles were scored using the software *GeneMapper® 4.0*.

Genetic diversity and structure

The Probability of Identity (PI) is the probability that two genetically different samples have identical multilocus genotypes given a set of genetic markers. Computation of PI was performed in *Genalex 6.1* (Peakall & Smouse 2006). Identical multilocus genotypes were then considered ramets of the same genet (clones of a same genotype) with a confidence probability PI. All subsequent analyses were performed by reducing the dataset to the number of unique genets. Because ramets were represented by a single genet, the final dataset had no further information

on genotype frequency. Genetic indices of diversity, tests of linkage disequilibrium (50,000 permutations), pair-wise F_{ST} between localities (50,000 permutations) and Hardy-Weinberg disequilibrium (HWE, 10,000 burn-in, 1,000,000 permutations) were estimated in *Arlequin 3.5* (Excoffier & Lischer 2010) and p-values were adjusted to control for False Discovery Rate (FDR; Benjamini & Hochberg 1995) with the *stats* package in R (R Development Core Team 2010). F_{ST} was preferred to R_{ST} because it is a more suitable measure of genetic distance between populations when the number of markers <20 (Gaggiotti *et al*. 1999). The number of K populations was estimated in *Structure* 2.3.3 (Pritchard *et al*. 2000; Hubisz *et al*. 2009) and repeated ten times for each value of K, ranging from K=1 to K=5. Using the FullSearch algorithm in *CLUMPP* 1.1.2 we permuted the independent replicate runs (Jakobsson & Rosenberg 2007). The mean of the permuted matrices across replicates was visualized in *Distruct 1.1* (Rosenberg 2004). To find the number of discrete populations in the dataset, we used *Structure* under three different models. The model ADM used only allelic information to find population structure. In contrast, the model POPINFO added *a priori* population assignments for some individuals to assign the remaining individuals to the *a priori* populations. Finally, the LOCPRIOR model enabled the incorporation of the sampling locations as *a priori* information. While these analyses were performed assuming 14 sampling localities (see Table 1), analyses were also repeated using the alternate "reef", "region" and "island" grouping for the LOCPRIOR model. *Structure* analyses, including the different methods to select the number of populations K that best describe the dataset, are further detailed in the supporting information (Text S1 and accompanying Figure S1 and S2). A matrix of genetic distances $F_{ST}/(1-F_{ST})$ was generated and tested against the geographic distance matrix to explore IBD patterns. Reflecting the short pelagic life of their larvae, *A. palmata* populations seem to be largely self-recruiting, while connectivity across large distances seems to approximate a rather one-dimensional path following shallow water habitats (Baums *et al*. 2006b), in particular along the Lesser Antilles. Hence we generated a geographic distance matrix based on the shortest shallow nautical (SSN) distances in Google Earth 6.2.1.6014 at the 1,000,000:1 scale. Alternatively, another matrix based on the shortest-nautical (SN) distances was constructed to explore IBD patterns involving direct connectivity, in particular for distant reefs such as the Bahamas, Guadeloupe and Curaçao. Matrices were appended in Table S1. Significance of IBD patterns was tested with Mantel tests (30,000 permutations) while Reduced Major Axis (RMA) regressions were used to

estimate the strength of genetic differentiation (RMA slope, jackknife 95% CI). Because the data is only known with error, RMA was favored over Ordinary Least Square regression (Hellberg 1994, Jensen *et al*. 2005). Mantel tests and RMA calculations were performed in IBDWS (Jensen *et al*. 2005) by comparing the matrix of pair-wise Slatkin's F_{ST} (F_{ST}/1-F_{ST}) with the geographic distance matrices after setting genetic distances <0 to 0. A log10 transformation was applied to the SN matrix to account for bi-dimensionality (Slatkin 1993, Rousset 1997). On the other hand, connectivity between distant reefs in the SSN matrix was rather one-dimensional since it followed shallow water habitats (from one island to the next), thus no logarithmic transformation was applied to the SSN matrix (Rousset 1997). Comparisons were first made with all studied localities, but because some populations had fewer individuals (n<15), part of the observed patterns was not highly supported (Cornuet *et al.* 1999). As a balance between improved statistical confidence and data loss, the results were compared anew by including only localities with a certain minimum of different genotypes (n=15, n=20 and n=30). When the data could not be shaped into a usable matrix for IBDWS, RMA for Java (Bohonak *et al*. 2004) was used instead (one-delete jackknife with 30,000 bootstraps for 95% CI), as was the case for RMA calculations excluding within-Puerto Rico pair-wise comparisons or to obtain the regression slopes between a specific location (Mona or Culebra) and mainland Puerto Rico.

Comment [PM1]: Added reference following the comments of reviewer 2 on populations with few individuals

RESULTS

Clonality and genetic diversity

Based on our dataset, the probability for two genetically different samples to have identical multilocus genotypes by chance (PI) using the five microsatellite markers was ~1.48 x 10^{-7}, which closely matched the ~1.5 x 10^{-7} estimate in Baums *et al*. (2005b, 2006a). Therefore, it was a reasonable assumption that identical genotypes represented biological clones. We identified 309 unique microsatellite multilocus genotypes (genets; 75%) and an additional 103 clones (25.0%) from a total of 412 colonies (Table 1). In order to avoid artificial, misleading signals resulting from the presence of those clones in our dataset, the remaining analyses, including estimates of genetic diversity and population structure, were based on the 309 unique genets identified in this study.

Comment [PM2]: This sentence was added as requested by reviewer 1 as a reminder that clones were taken out for analysis.

Mean genetic diversity across localities and loci was high (H_E = 0.761) and consistent across localities (0.685 to 0.844). Mean allelic diversity per locality across loci was 9.7 (Table

2). Allelic diversity ranged from 8 to 23, from the least (#181) to the most polymorphic locus (#166). Mean allelic range per location across loci was 11.3, ranging from 13 to 24 depending on the marker (#181 and #166, respectively). After correcting for multiple comparisons with the FDR, no test of pair-wise linkage disequilibrium was significant (140 pair-wise tests comparing all loci at each of the 14 localities). Therefore, the five loci were assumed to be unlinked. FDR corrected p-values for tests of HWE at each locus were not significant (70 tests, 5 loci for 14 localities), indicating that the sampled populations were at equilibrium.

Number of populations with *Structure*

Simulations performed under the ADM model (without *a priori* information on sampling locations) had a higher probability L(K) for K = 1 (Figure 2A, B). Furthermore, the summary statistics α and F did not stabilize, indicating that *Structure* did not detect population structure with this algorithm (Pritchard *et al*. 2000) as visually confirmed in Figure 3A. This contrasted with the findings of Baums *et al*. (2005b) who found an optimal number of population K = 2 using the same algorithm. In the POPINFO model, which assigns some genotypes to user-specified *a priori* populations, the results were similar (Figure 3B and S2D, E). Again, the summary statistics did not stabilize, indicating that *Structure* could not find a likely assignment for the given genotypes. Under the LOCPRIOR model which uses sampling locations as *a priori* information, the optimum burn-in period was found to be relatively long, with r (the contribution of the predefined sampling locations to the end probabilities assigned to the individuals), α and F generally stabilizing after 5 to 10 million iterations. These control steps were necessary to have confidence in the resulting probability for K (Hubisz *et al*. 2009). Also, the variance of L(K) within and between runs was relatively high when K > 2, requiring a high number of iterations and the use of replicate runs. Based on the arithmetic mean of L(K) across replicate runs, the optimal number of K found by *Structure* in all models was K = 2 (Figure 2H). This was also true for other recommended methods, such as using only the best L(K) among replicate runs (Figure 2G) or the ΔK parameter described in Evanno *et al*. (2005) (Figure 2I). When performing alternate LOCPRIOR analyses, i.e. by assigning individuals to *a priori* locations corresponding to "reefs", "regions" or "islands" (Figure 2G, H) the original "localities" model was generally found to reach superior probabilities across the range of tested K (1 to 5), although at K = 2, the "reef" model had a slightly higher L(K) than the "localities" model (mean $L(K)_{reefs}$ = -6,412 and mean $L(K)_{localities}$ = -6,421, respectively). Following the population model at K = 2, the Bahamas,

Mona, Rincón, Lajas, Guánica, Ponce, Isabela and Vega Baja were clustered into one population. Curaçao, Guadeloupe, Culebra, Fajardo clustered into another population. Finally, Vieques and the San Juan area had admixed origin of populations (Figure 3C).

Genetic structure and isolation-by-distance

The genetic distances between localities were small (F_{ST} ranging from -0.011 to 0.047) but often significant, even after correcting for multiple comparisons (Table 3). Conversely, they were not significant for those locations with the lowest numbers of genets, such as Guánica or Isabela (n=3 and n=5, respectively). In general, genetic distances were higher between the most distant localities, in conformity with a model of IBD. In the West, pair-wise F_{ST}s indicated that despite a relatively low number of genotypes (n=11) the Bahamas showed significant divergence with all localities, except Mona, Rincón and Vega Baja. Interestingly, Mona showed no significant difference with the closest localities in eastern Puerto Rico (e.g. Rincón, Lajas). In the East, Culebra (n=19), Guadeloupe (n=47) and Curaçao (n=10) had non-significant F_{ST}s among them. On the other hand, these locations exhibited significant pair-wise differences against most other localities.

IBD plots showed consistent patterns of genetic differentiation via IBD, regardless of the type of geographic distance matrix or of the minimum sample size for each population (Figure 4). Mantel tests were high and significant ($p<0.05$) except when minimum sample size per population was set to 15 different genotypes. In such cases, removing the pair-wise comparisons involving localities outside Puerto Rico effectively resulted in significant Mantel test results within Puerto Rico (Figure 5, dash line r=0.53, p=0.0064), suggesting different patterns and strengths of differentiation within and outside Puerto Rico. This was supported by marginally non-overlapping confidence intervals ranges (95% CI) between the slopes of their respective RMA regression, respectively $6.6\text{x}10^{-5} – 1.7\text{x}10^{-4}$ for within Puerto Rico pair-wise comparisons and $1.9\text{x}10^{-5} – 6.5\text{x}10^{-5}$ for the remaining pair-wise comparisons (Figure 5). However, there was no clear, separate clustering pattern characteristic of discrete populations (Figure 4 and 5). Ideally, population structure in discrete populations would show separate clustering of within-population genetic distances from clustering of between-population genetic distances. However, an intermediate clustering pattern can be observed: for near-identical geographic distances, pair-wise comparisons between *a priori* Western and Eastern populations in Figures 4 and 5 (orange symbols) were characterized by higher genetic distances than pair-wise comparisons within each

of these groups (light blue and blue symbols, respectively). Although stronger statistical support could be achieved by increasing the low number of genets in the Bahamas and Curaçao (respectively n=11 and n=10), pair-wise F_{ST}s per 1,000 km unit were higher within Puerto Rico than when locations thousands of kilometers apart were compared, supporting the presence of a zone of further differentiation in Puerto Rico (Table 3). In this regard, Culebra was characterized by the highest F_{ST} per 1,000 km. In fact, the RMA regression slope of pair-wise comparisons between Culebra and mainland Puerto Rico ($s=1.2x10^{-4}$, $SE=2.5x10^{-5}$) was superior to the slope for Mona-mainland Puerto Rico ($s=7.0x10^{-5}$, $SE=2.7x10^{-5}$), although their respective 95% CI overlapped ($8.3x10^{-5} – 1.5x10^{-4}$ and $-4.6x10^{-5} – 1.9x10^{-4}$, respectively).

DISCUSSION

Sampling strategy and clone proportions

Clone distribution in the three-dimensional reef space depends on a variety of factors (Coffroth & Lasker 1998). For example, the genetic disposition of individual genets is likely to be important for the successful settlement of new ramets. Environmental factors such as hurricane disturbance, reef orientation and inclination, current dynamics and competition for space with other reef organisms will be responsible for part of the observed clone distribution, frequency and density (Highsmith *et al*. 1980). Because we wished to avoid overrepresentation of clones for the benefit of genetic structure analyses, we favored a non-random, opportunistic sampling strategy. Hence, population dynamics implications based on the frequency and density of ramets in this study should be interpreted with caution. We found that unique genets represented 75% of the samples (Ng/N = 0.75, mean Ng/N per locality = 0.75). In contrast, Baums *et al*. (2006a) used randomized circle plots and opportunistic sampling and found that both sampling strategies yielded similar results (mean Ng/N per reef = 0.52 and 0.51 respectively). Since genotypes were scored with the same markers in both studies, differences at the molecular level are unlikely to explain the difference in the proportion of clones. Because clonality varies grandly among reefs, the choice of sampling localities might explain some amount of discordance. The rest of the differences can probably be explained by a sampler effect (personal preferences for certain coral colonies during sampling) and/or other uncontrolled factors, e.g. the depth of sampling. A lower value of unique haplotypes (42%) was estimated from reefs of west and southwest Puerto Rico, where 46 unique mitochondrial haplotypes were detected from 110 distinct colonies (Garcia Reyes & Schizas 2010). The difference with the

current results is not surprising, because microsatellites are usually more variable than mitochondrial markers, detecting more unique genets (Baums *et al*. 2005a).

The frequency of clones in each locality was unrelated to a purely geographic division between Western and Eastern provinces. Rather, difference in clonal structure is more likely explained by differences in environmental conditions such the size and depth of the shelf area (Baums *et al*. 2006a). The areas with the most clones were found in shallow, extensive shelves, such as Vega Baja and Escambrón. The back reefs of these locations varied in depth between <1 to two meters, providing asexual recruits with space to settle and shelter from waves. Tres Palmas was also well represented by clonal genets, although its shallow shelf is short and rapidly reaches unsuitable depths for dense *A. palmata* stands. On the contrary, reefs with no or few clones were often representative of areas with less suitable habitat. For example, the Anse-Bertrand reef is positioned against a small cliff, and elkhorn coral colonies were found at depth where they were growing in an encrusting fashion, limiting their ability to produce asexual recruits via fragmentation. Reefs at Piñones and Sun beach had simply no space for settlement of asexual recruits. Reefs at Shack, Pointe-des-Châteaux and Grand-Cul-de-Sac-Marin were positioned in shallow, extensive shelves, but numerous dead stands of *A. palmata* separated the sampled colonies, most likely the ghosts of disease or bleaching past. In the event of disease outbreaks, sensitive genets will disappear first (Reusch *et al*. 2005). Hence, because of their low genetic diversity, highly clonal reefs are more likely to lose extensive coral cover than heterogeneous reefs.

High genetic diversity

An important step in the genetic diversity and structure analyses is that the frequency of each genotype was removed from the dataset prior to analyses. This precaution avoided unreasonable assumptions for the estimation of gene flow and genetic structure (Baums *et al*. 2005b). In *A. palmata,* asexual reproduction happens by fragmentation, when broken branches rise into new, identical clones near the original colony (Bruckner 2002; Reusch *et al*. 2005). Thus, the genetic fingerprint of clones within a reef will mostly be the result of environmental hazards, not of sexual reproduction and gene flow. On the other hand, the probability of inbreeding and gene flow depends on the effective population size, and genets having many ramets will contribute more to sexual reproduction (Coffroth & Lasker 1998). Hence, some amount of information is inevitably lost in the analysis. The genetic diversity found in this study

(H_E = 0.761) was higher than the genetic diversity in *Acropora nasuta* (MacKenzie *et al*. 2004) or *A. cytherea* (Concepcion *et al*. 2009) but surprisingly lower than for *A. muricata* and *A. digitifera* using the same microsatellite markers used in this study (Tang *et al.* 2010). On the other hand, our results were consistent with those of Baums *et al*. (2005b) for *A. palmata.* Interestingly, genetic diversity was also consistent across all localities in our study and was higher than the genetic diversity found in a study of 14 rare and common species of Indo-Pacific *Acropora* using nine neutral microsatellite markers (including the five markers used in the present study; Richards *et al.* 2012) With respect to conservation efforts, these results suggest that there is high genetic diversity in *A. palmata* despite the dramatic losses during the last decades, in particular to the White Band disease (Bruckner 2002). The high genetic diversity estimated with microsatellites, however, is not depicted in the mitochondrial nucleotide diversity (π = 0.00075) of *A. palmata* in Puerto Rico, a value amongst the lowest reported values for scleractinian corals (Garcia Reyes & Schizas 2010). Part of the discrepancy can be explained by the low levels of genetic variability observed in mitochondrial DNA of corals compared to nuclear genes (Hellberg 2006).

Clustering of Western and Eastern populations in Puerto Rico

In the study of Baums *et al*. (2005b), two populations, roughly divided east and west of Puerto Rico, were detected using *Structure*. Sampling around the Mona Passage, the proposed region of population admixture, was limited to 36 unique genets from Mona and 90 unique genets along the west and southwest coast of Puerto Rico (Rincón, Lajas, Bajo Gallardo). In order to improve our understanding of population differentiation in the region, further sampling efforts in the south of Puerto Rico and in the Dominican Republic were conducted in Baums *et al*. (2006b). In their study, western and southwestern Puerto Rican reefs were pre-assigned to the Eastern population via the POPINFO option in *Structure*, in disagreement with the initial *Structure* results of Baums *et al*. (2005b), where the same Puerto Rican reefs clustered with the Western population when no *a priori* assignments were made. The added samples from the Dominican Republic clustered with the Western population (Baums *et al*. 2006b), but the *a priori* assignment of western and southwestern Puerto Rican samples to the Eastern cluster did little to improve our understanding of population differentiation around Puerto Rico. By including new samples and locations around the proposed region of population admixture (Baums *et al* 2005b), in particular where sampling was missing (northern and eastern Puerto

Rico), the present study further details the population structure of elkhorn coral in this region of particular interest.

In the present study, in accord with high genetic diversity and low estimates of population structure (F_{ST}s, AMOVA analyses presented in supporting information in Text S2 and accompanying Table S2), genetic differentiation with *Structure* was detected but weak: the basic admixture model (ADM) in *Structure* was not able to detect population structure in the data, while a high number of iterations was needed to reach statistical stability and find population structure when using prior information on locations via the LOCPRIOR model, recommended in case of weak population structure (Hubisz *et al*. 2009). In contrast, Baums *et al*. (2005b) were able to find population structure separating a Western from an Eastern population using the same markers and admixture model without *a priori* information, thus proposing that two populations of *A. palmata* meet at Puerto Rico. Differences between the two studies might be due to the more extensive geographic coverage and larger number of unique genotypes in Baums *et al*. (2005b) than in this study (n = 709 and n = 309, respectively) or user-related differences in the assessment of marker states.

Baums *et al*. (2005b) performed additional runs in *Structure*, using the built-in POPINFO option to define *a priori* assignments of non-Puerto Rican localities to their respective Eastern or Western cluster, thus allowing the software to estimate population admixture of the unassigned Puerto Rican genotypes. The resulting assignments of evenly admixed Puerto Rican colonies were then interpreted as hybrid genotypes between the Eastern and the Western populations. Similarly, we also used the POPINFO option to define *a priori* assignments of selected locations. Various configurations combining Western and Eastern *a priori* assignments invariably resulted in (1) the lack of stabilization of control parameters during the runs and (2) similar patterns of totally admixed genotypes, in equal proportions between K populations, for all individuals that were not assigned to *a priori* cluster. This pattern suggests that those admixed assignments resulted from a lack of discriminative power of the algorithm, and represented undecided assignments rather than perfectly admixed genotypes (with 1:1 proportions of each population of origin in the case of K=2) (Pritchard *et al*. 2000).

Using the LOCPRIOR option in *Structure*, which was not available when Baums *et al.* (2005b, 2006b) were published, we further detailed the population structure of *A. palmata* in Puerto Rico. Our results agree with Baums *et al*. (2005b), with K = 2 having the highest

Comment [PM3]: Clarified to answer the comment of reviewer 2 on admixed individuals in the POPINFO model.

probability to explain the data, even when reefs outside Puerto Rico were not included. In Puerto Rico, the Western population would include Mona Island and the west coast of Puerto Rico, as was described in Baums *et al*. (2005b). The Western population most likely extends (1) northwest and north of Puerto Rico reaching past Vega Baja; (2) southwest and south past Lajas and Guánica, reaching past Ponce. In contrast, the Eastern population probably includes Culebra and reaches past Fajardo. A transitional area occurs somewhere between Vega Baja and Fajardo in the North, explaining the admixed profiles of the San Juan area. In the south/southeast of the island, the transitional area is located between Ponce, Fajardo and Vieques. Keeping in mind that the low number of genets for some of these localities might affect our results (Cornuet *et al.* 1999), we also find significant F_{ST} and AMOVA results supporting the genetic structure evidenced by our *Structure* results.

Comment [PM4]: Added reference following the comments of reviewer 2 on populations with few individuals

Patterns of IBD vs. discrete populations

Structure explicitly allocates individuals into an *a priori* number of groups that are discrete and whose members minimally violate the assumptions of HWE (Pritchard *et al*. 2000). In instances where genetic differentiation is correlated with geographic distance, individuals separated by sufficiently large geographic distances may violate the assumptions of a population at HWE when placed into one group, and therefore two or more groups may better explain the clustering of these individuals. This, however, does not mean that these groups are discrete, and that individuals with intermediate genotypes represent admixed individuals between two discrete populations, but rather indicates that the program is forcing continuous variation into discrete and discontinuous clusters. Because Bayesian clustering programs such as *Structure* can overestimate the number of clusters in datasets characterized by IBD, IBD should also be tested and interpretations based on the results of all analyses (Frantz *et al.* 2009).

Comment [PM5]: Added to clarify our view following general comments of reviewer 2 on Structure results.

Structure analyses indicate two clusters with genetically intermediate individuals and localities occurring in western Puerto Rico (Figure 3). This result is broadly comparable to that of Baums *et al*. (2005b, 2006a). However, our interpretation, based mainly on the addition of localities and samples in the proposed area of admixture, differs slightly. Analyses of pair-wise F_{ST}s and of correlations between genetic and geographic distances suggested a pattern of IBD, which normally characterizes populations with limited connectivity, such as in a stepping stone model (Hellberg 2007). This is consistent with what is now known of limited larvae dispersal in corals and other marine species (Palumbi 2003; Cowen *et al*. 2006; Galindo *et al*. 2006; Hellberg

2007; Andras *et al.* 2013). Furthermore, the choice of a different geographic distance matrix or limiting analyses to localities with a certain minimum sample size (no minimum, 15, 20 or 30 genotypes) did not affect the interpretation of genetic differentiation by IBD. Our data suggests that the strength of IBD measured as the slope of the correlation between genetic and geographic distances differs along the studied seascape inhabited by *A. palmata*. The slope was steeper in comparisons involving the small geographic distances around Puerto Rico than Caribbean wide comparisons, suggesting that IBD is much stronger within the region of Puerto Rico than outside this region. This could reflect the stronger genetic changes associating with the hybridization of the Western and Eastern populations in the Puerto Rican region (Baums *et al*. 2005b). While *Structure* consistently placed the Bahamas within the putative Western population along several Puerto Rican localities, the Bahamas were found to be significantly differentiated from almost all other locations in this study, a result reminiscent of the genetic separation of the Bahamas from other Caribbean locations in *A. cervicornis* (Galindo *et al*. 2006; Vollmer & Palumbi 2007; Garcia Reyes & Schizas 2010; Hemond & Vollmer 2010) or *Orbicella* (previously *Montastraea*) *annularis* (Foster *et al.* 2012). East of Puerto Rico (Culebra, Guadeloupe and Curaçao), there was little genetic divergence between localities, suggesting near-panmixia within the eastern region (Table 3), a result largely consistent with the Eastern cluster described by Baums *et al*. (2005b) but also with the strong separation of Eastern locations from the West in both experimental data and dispersal models in *O. annularis* (Foster *et al.* 2012).

Although our findings within Puerto Rico were well supported, further studies should be conducted to confirm our observations regarding non-Puerto Rican localities because of the low number of sites (n=3) and genets per site (n=11 in the Bahamas and n=10 in Curaçao) analyzed outside of Puerto Rico in this study.

Re-assessing population structure in the elkhorn coral

Genetic structure in this study was characterized by a non-uniform IBD across the seascape inhabited by *A. palmata*, suggesting that connectivity, at least among some *A. palmata* reefs, is limited. IBD seemed to be stronger among localities from Puerto Rico, in particular in eastern Puerto Rico. IBD was weaker to insignificant outside of the Puerto Rico Shelf, as suggested by mostly non-significant genetic structuring within the Eastern population. When the distribution of the genetic diversity is interpreted in the framework of a discrete population structure as implemented in *Structure*, one then observes a zone of transition in eastern Puerto

Rico between apparently two discrete genetic groups. This zone of transition did not seem to associate directly with the Mona Passage, west of Puerto Rico, which was suggested to be a significant barrier to gene flow in *A. palmata* (Baums *et al*. 2006b). Foster *et al.* (2012) also found an East-West break in the genetic structure of *O. annularis*, but noted that its location was ambiguous since their results pointed to a separation between the British Virgin Islands and Dominica. Understanding why the genetic transition could be more pronounced in the east of Puerto Rico, as suggested by the slopes of RMA regressions between mainland Puerto Rico and Mona vs. mainland Puerto Rico and Culebra, is an intriguing challenge. The filtering effect of the Mona Passage, coupled with asymmetric net gene flow in the easterly direction, as is generally the case for surface currents during the spawning season on the north coast of Puerto Rico, could provide an explanation. Admittedly, the genetic structure in the south of the island would then be expected to respond to gene flow following opposite currents in the westerly direction, which does not seem to be the case, unless gene flow in the southeast is hindered by another, unidentified barrier. Residual genetic structure inherited from historical shifts in the geographical ranges of Caribbean species during the late Quaternary (Lighty *et al*. 1982; Toscano & Macintyre 2003) could explain some of the present genetic patterns, as did the last glaciation event on Indo-Pacific population dynamics of marine species (Benzie *et al*. 1999). Less than 15 genotypes were recovered from two of the three non-Puerto Rican localities in this study (Curaçao and the Bahamas). Because *Structure* and other analyses of population structuring are context-dependent, adding molecular markers, new locations in the larger Caribbean and increasing sample size per population seems essential for further, improved analyses (e.g. when assigning individuals to populations of origin, see Cornuet *et al.* 1999). These additions will likely provide us with a better understanding of possible IBD and other genetic structuring of *A. palmata* populations outside of Puerto Rico. New sampling efforts concentrated in the northeast, southeast and east of Puerto Rico are also needed to identify with more accuracy the location(s) of the strongest genetic shift(s) between Eastern and Western populations. Meanwhile, building on the knowledge that most of the extant genetic diversity of *A. palmata* is present on the Puerto Rico Shelf, we exert local decision-makers to implement new conservation measures for the elkhorn coral, as effective protection at a local scale could be beneficial for preserving the global genetic diversity of *A. palmata*.

Comment [PM6]: The section was edited to take into account the comments of reviewer 2 on the difficulty of "in locating the break between the Eastern and Western Caribbean". The referenceto Cornuet et al 2009 was also added.

ACKNOWLEDGMENTS

We thank Jenny Acevedo, Silvia Planas, Ximena Velez Zuazo, Juan Marrero and Elsie Rivera for their help, Claude Bouchon and Olivier Gros for their logistic support in Guadeloupe, and Edwin Hernandez for providing samples from Mona. The manuscript was also greatly improved from the comments of various reviewers. Funding was provided by a Caribbean Coral Reef Institute Grant, a CMRC Program Development Grant (05-PRKS-01-05A) awarded to NVS and via NIH-SCORE grant S06GM08102 and NSF-HRD grants 0734826 and 0931659 to TH. Collection permits were issued by PR–D.N.E.R. and D.R.A.M. of Guadeloupe.

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631 **SUPPORTING INFORMATION**

632 **Text S1.** This supplement details the methods used to estimate and choose the number of discrete
633 populations K in Structure, *Structure* configurations for the manuscript, as well as results from
634 additional runs**. Figure S1.** Additional *Structure* runs under the LOCPRIOR model on a reduced
635 dataset as further described in Text S1. **Figure S2.** *Structure* results for K=2 under the
636 LOCPRIOR model comparing global results to the results for Puerto Rican locations only. **Table**
637 **S1**. Geographic distance matrices between the 14 locations reported in the manuscript. **Text S2.**
638 Tests of Hierarchical genetic structuring using FST-based Analysis of Molecular Variance
639 (AMOVA). **Table S2.** Text S2-accompanying configurations and results for AMOVA.

640 **FIGURE LEGENDS**

641 **Figure 1**. **Sampling locations of *Acropora palmata.*** 30 reefs were sampled (24 reefs in Puerto
642 Rico, including Mona, Vieques and Culebra, 4 reefs in Guadeloupe, 1 reef in Curaçao, and 1 reef
643 in the Bahamas). Reefs are represented by codes of three-upper case letters, referring to the reef
644 names in Table 1. The corresponding 14 localities grouping those reefs in the same table were
645 also marked as full lower cape names on the map. Maps modified from maps generated on
646 reefbase.org and Garcia Reyes & Schizas (2010).

647 **Figure 2. Results of *Structure* for different parameter sets**. From top to bottom, the first row
648 contains the results of the ADM model (A, B, C), the second row contains the results of the three
649 POPINFO models (D, E, F), the third row (G, H, I) contains the results of the LOCPRIOR model
650 for the four grouping variants proposed to explore the dataset at different scales. A, D and G plot
651 the highest L(K) out of ten runs for each K assumed *a priori.* B, E and H plot the mean L(K) and
652 standard deviation (vertical whiskers) over the ten runs for each K. C, F, and I plot ΔK,
653 following Evanno *et al.*(2005).

654 **Figure 3. *Structure* results for K = 2 with the three parameter sets defined in this study**. All
655 parameter sets used the admixture algorithm and performed 5,000,000 burn-in followed by
656 15,000,000 iterations per run. Each figure plots the mean assignments of each individual
657 genotype to K *a priori* populations (here K=2) among 10 replicate runs. A. ADM parameter set:
658 admixture without *a priori* information. B. POPINFO 2 parameter set: admixture model with the
659 Bahamas and Mona *a priori* assigned in a first population (Western population, blue), and
660 Guadeloupe and Curaçao *a priori* assigned in a second population (Eastern population, red). C.
661 LOCPRIOR parameter set run with *a priori* information for the 14 localities.

662 **Figure 4. Pair-wise genetic distances in function of pair-wise geographical distances**. The
663 first, second, third and fourth columns (left- right) show the plots of genetic versus geographic
664 distances when the analysis include all localities (n=14), only localities with >=15 genotypes
665 (n=8), >=20 genotypes (n=6) and >=30 genotypes (n=5). The top (A to D) and bottom rows (E to
666 H) respectively show the plots for shortest-nautical (SN) and shortest-shallow-nautical (SSN)
667 geographic distance. SN values were log10 transformed due to the bi-dimensionality of the
668 model assumed. In all cases, $F_{ST}(1\text{-}F_{ST})$ between pair-wise served as a genetic distances. For
669 each plot, RMA regressions were drawn with black lines. Results of the Mantel tests realized in
670 each case were directly appended on the graphs. W-W: comparisons between two localities from

the Western region. E-E: comparisons between two localities from the Eastern region. W-E: comparisons between one Western locality and one Eastern locality. H-*: comparisons between one hybrid locality and any other locality. The region of origin of each locality was determined using *Structure* results (Figure 3).

Figure 5. Pair-wise genetic distances in function of pair-wise shortest-shallow-nautical distances. The following population assignments were based on *Structure* results using the LOCPRIOR option and K = 2. E-E: Pair-wise comparisons within the Eastern population. W-W: Pair-wise comparisons within the Western population. W-E: Pair-wise comparisons between Western and Eastern populations. H-*: Pair-wise comparisons involving admixed localities (San Juan area and/or Vieques). RMA regression for all points is represented by the full line. Two additional regressions were also performed to test the difference in IBD strength within Puerto Rico (circles, dash line) or with alternate locations (involving Curaçao, Bahamas and/or Guadeloupe; triangles, dot line). For each group, Mantel test and RMA regression results between $F_{ST}/(1-F_{ST})$ and geographic distances are appended next to the corresponding line whenever possible.

687 **Table 1.** Samples origin, number of sampled colonies and number of unique genotypes (genets)
688 in this study.

Island	Region	Locality	Reef	Abb.	Latitude (N)	Long. (W)	Genets	Colonies
Bahamas	Bahamas	Bahamas	Lee Stocking Island	LEE	23°45'41"	76°05'15"	11	16
Curaçao	Curaçao	Curaçao	Curaçao	CUR	12°11'10"	69°00'05"	10	10
Puerto Rico	North PR	San Juan area	Piñones	PIN	18°27'44"	65°59'47"	6	6
			Escambrón	ESC	18°28'05"	66°05'29"	11	25
		Vega Baja	Chalets	CHA	18°29'27"	66°24'52"	14	37
		Culebra	Luis Peña	LUI	18°19'05"	65°19'31"	1	1
			Soni beach	SON	18°19'09"	65°15'07"	1	1
			Punta Soldado	PUN	18°16'58"	65°17'24"	17	19
		Fajardo	Cayo Lobos	CAY	18°22'32"	65°34'03"	7	11
			Ratón	RAT	18°22'53"	65°35'03"	3	4
	NorthEast PR	Vieques	Pirates cove	PIR	18°06'32"	65°23'55"	6	7
			Secret beach	SEC	18°06'31"	65°23'59"	9	9
			Sun beach	SUN	18°05'20"	65°27'55"	15	15
	NorthWest PR	Isabela	Shack	SHA	18°30'58"	67°05'58"	5	5
		Rincón	Tres Palmas	TRE	18°20'47"	67°15'48"	38	52
	SouthWest PR	Lajas	Atravesao	ATR	17°56'38"	67°05'12"	3	3
			Enrique	ENR	17°57'11"	67°02'48"	1	2
			Laurel	LAU	17°56'24"	67°03'44"	1	1
			Margarita	MAR	17°55'04"	67°06'24"	8	13
			Media Luna	MED	17°56'20"	67°02'36"	15	24
			Tumurote	TUR	17°56'10"	67°01'09"	1	1
			El Palo	ELP	17°55'53"	67°05'38"	1	1
	South PR	Guánica	Guilligan	GUI	17°56'26"	66°52'07"	3	9
		Ponce	Caja de Muerto	CAJ	17°54'05"	66°30'35"	25	30
Guadeloupe	Guadeloupe	Guadeloupe	Anse-Bertrand	ANS	16°29'16"	61°29'42"	15	15
			Grand-Cul-de-Sac-Marin	GRA	16°21'36"	61°35'37"	20	20
			Pointe-des-Châteaux	POI	16°15'03"	61°10'53"	9	11
			Port-Louis	POR	16°25'35"	61°32'04"	3	3
Mona	Mona	Mona	Sardinera	SAR	18°05'29"	67°56'23"	41	50
			Fortuna Reefer	FOR	18°03'24"	67°52'05"	9	11
TOTAL							309	412

689 Abb.: three letters reef abbreviation; Long.: longitude.

690 **Table 2**. Summary of genetic diversity indices among the
691 14 localities.

Locality	Ng	Ng/N	A	H_O	H_E	R
Bahamas	11	0.69	9.2	0.873	0.844	11.6
Curaçao	10	1.00	7.4	0.695	0.741	8.8
San Juan area	17	0.55	9.2	0.694	0.746	11.2
Vega Baja	14	0.38	9.6	0.771	0.795	12.0
Culebra	19	0.90	9.4	0.768	0.740	10.6
Fajardo	10	0.67	7.2	0.700	0.685	10.8
Vieques	30	0.97	12.2	0.740	0.754	13.2
Isabela	5	1.00	5.8	0.790	0.777	7.6
Rincón	38	0.73	12.2	0.754	0.766	13.2
Lajas	30	0.67	11.8	0.780	0.764	13.0
Guánica	3	0.33	4.2	0.667	0.760	5.4
Ponce	25	0.83	12.0	0.704	0.764	13.0
Guadeloupe	47	0.96	12.2	0.706	0.744	12.2
Mona	50	0.82	13.8	0.756	0.780	16.0
TOTAL	309					
MEAN		0.75	9.7	0.743	0.761	11.3

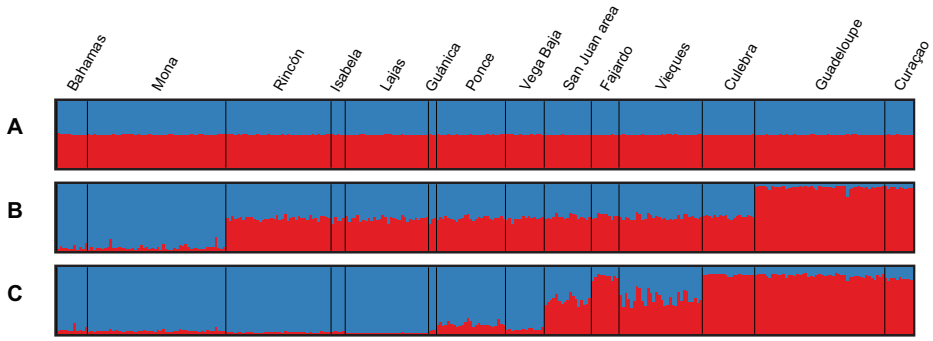
692 Ng: number of genets. Ng/N: ratio of the number of
693 genets by the number of colonies. Allelic diversity (A),
694 observed heterozygosity (H**O**), expected heterozygosity
695 (H**E**) and allelic range (R) were averaged across loci.

696

Table 3. Pair-wise genetic distances and pair-wise genetic distances per 1,000 km unit between localities.

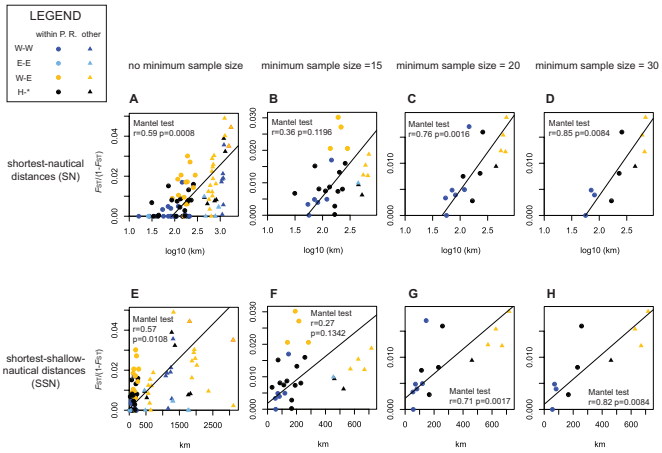
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Bahamas	-	0.016	0.016*	0.001	0.017*	0.005	0.026*	0.015	0.029*	0.023*	0.036*	0.029*	0.024*	0.011
2	Mona	0.017	-	**0.070**	-0.208	0.049	-0.084	**0.117***	0.006	**0.064**	**0.062***	**0.054**	**0.071***	0.025*	0.011*
3	Rincón	0.018*	0.005	-	-0.899	-0.071	-0.368	0.042	-0.050	**0.067**	0.035	0.032	**0.120***	0.018*	0.013*
4	Isabela	0.001	-0.020	-0.025	-	-0.201	-0.238	-0.061	-0.401	-0.179	0.040	**0.056**	**0.090**	0.000	0.015
5	Lajas	0.021*	0.004	-0.004	-0.017	-	-1.914	**0.055**	-0.013	0.037	0.018	0.034	**0.151***	0.024*	0.015*
6	Guánica	0.006	-0.009	-0.030	-0.026	-0.031	-	-1.018	-0.187	-0.107	-0.066	-0.056	**0.097**	0.007	0.005
7	Ponce	0.034*	0.017*	0.005	-0.009	0.003	-0.039	-	-0.038	0.000	**0.062**	**0.082**	**0.145***	0.021*	0.013
8	V. Baja	0.019	0.001	-0.005	-0.029	-0.002	-0.034	-0.008	-	-0.205	0.038	**0.102**	**0.154**	0.014	0.009
9	S. J. area	0.037*	0.013	0.009	-0.019	0.007	-0.022	0	-0.007	-	**0.093**	0.023	**0.206**	0.011	0.004
10	Vieques	0.031*	0.016*	0.008	0.008	0.003	-0.010	0.007	0.005	0.008	-	-0.255	**0.224**	0.020	0.005
11	Fajardo	0.047*	0.014	0.006	0.009	0.006	-0.009	0.01	0.009	0.001	-0.011	-	-0.303	-0.012	-0.004
12	Culebra	0.039*	0.020*	0.026*	0.017	0.029*	0.017	0.020*	0.018	0.015	0.007	-0.008	-	0.022	-0.003
13	Guadeloupe	0.043*	0.018*	0.012*	0.000	0.015*	0.004	0.012*	0.008	0.006	0.009	-0.006	0.01	-	0.004
14	Curaçao	0.034	0.022*	0.025*	0.029	0.028*	0.010	0.024	0.016	0.008	0.008	-0.007	-0.006	0.005	-

Below diagonal: F_{ST}. Above diagonal: standardized F_{ST} per 1,000 km (SSN distances). F_{ST} per 1,000 km > 0.05 and are printed in bold to indicate areas of higher genetic differentiation. *False Discovery Rate corrected p-value < 0.05 for multiple comparisons.


Bahamas
Mona
Rincón
Isabela
Lajas
Guánica
Ponce
Vega Baja
San Juan area
Fajardo
Vieques
Culebra
Guadeloupe
Curaçao
A
B
C

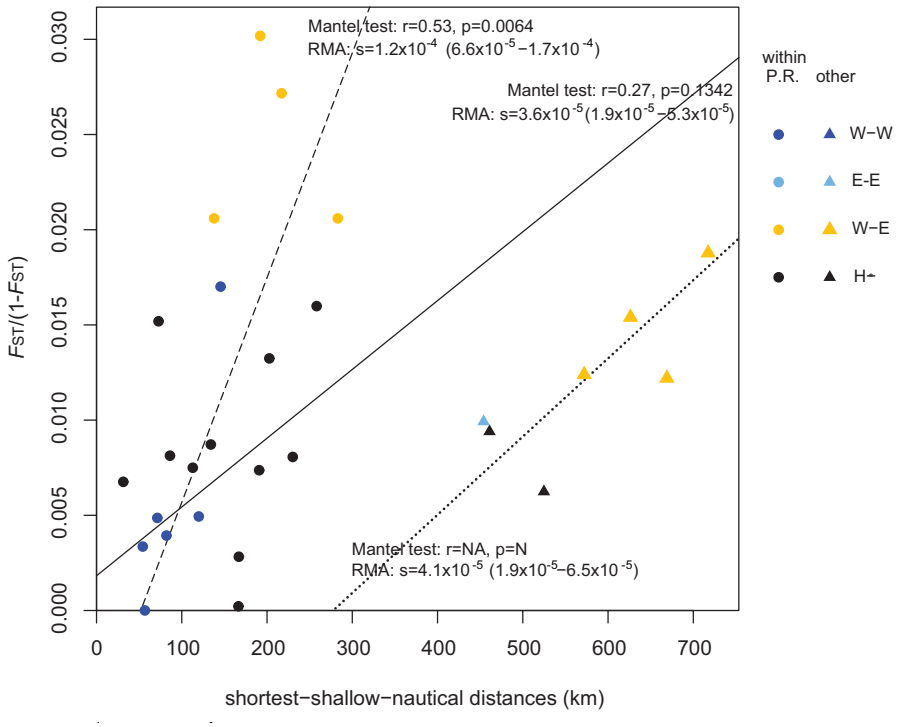
703

704


LEGEND
within P. R.
other
W-W
E-E
W-E
H-*
no minimum sample size
minimum sample size =15
minimum sample size = 20
minimum sample size = 30
A
B
C
D
E
F
G
H
shortest-nautical distances (SN)
shortest-shallow-nautical distances (SSN)
Mantel test r=0.59 p=0.0008
Mantel test r=0.36 p=0.1196
Mantel test r=0.76 p=0.0016
Mantel test r=0.85 p=0.0084
Mantel test r=0.57 p=0.0108
Mantel test r=0.27 p=0.1342
Mantel test r=0.71 p=0.0017
Mantel test r=0.82 p=0.0084
log10 (km)
km

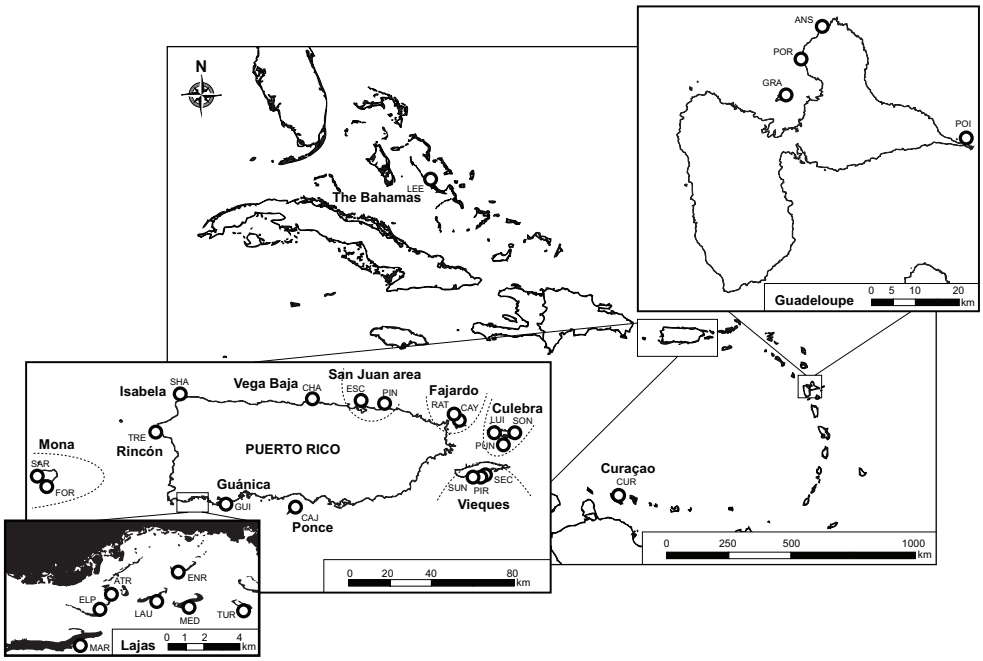
705

706


Mantel test: r=0.53, p=0.0064
RMA: s=1.2x10-4 (6.6x10-5–1.7x10-4)
Mantel test: r=0.27, p=0.1342
RMA: s=3.6x10-5(1.9x10-5–5.3x10-5)
Mantel test: r=NA, p=N
RMA: s=4.1x10-5 (1.9x10-5–6.5x10-5)
within P.R.
other
W–W
E-E
W–E
H–
FST/(1-FST)
0.000
0.005
0.010
0.015
0.020
0.025
0.030
0
100
200
300
400
500
600
700
shortest−shallow−nautical distances (km)

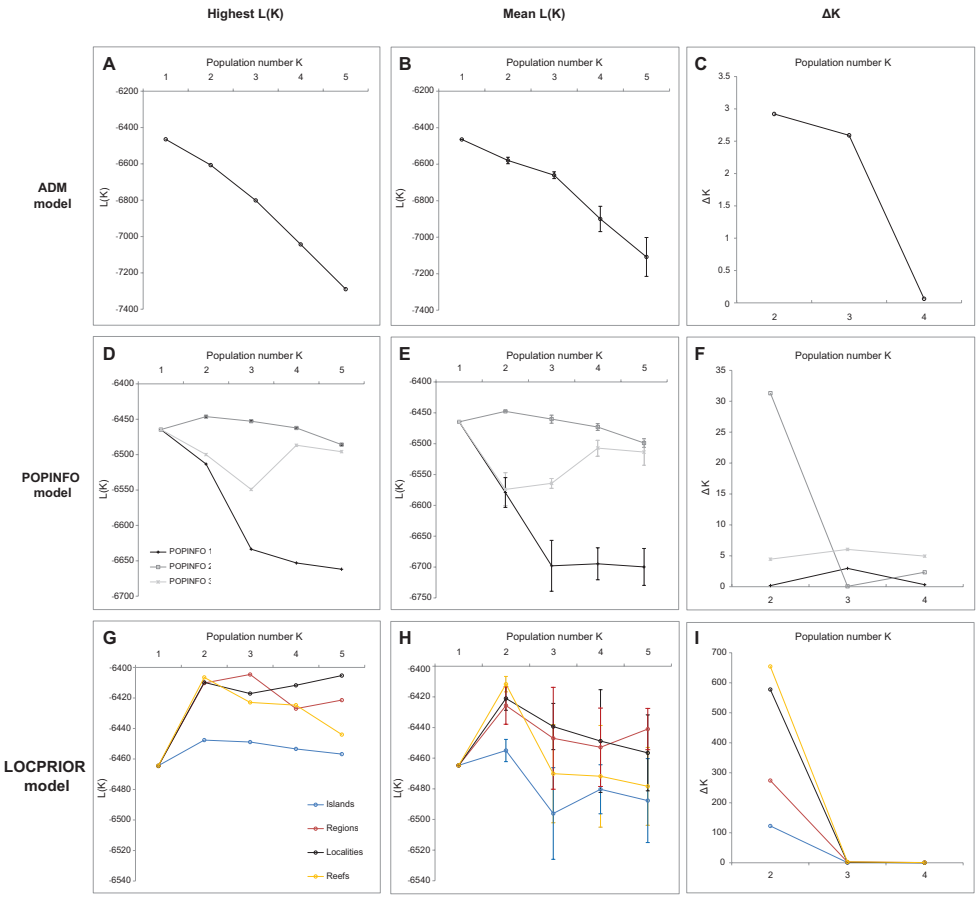
707

708


N
The Bahamas
LEE
ANS
POR
GRA
POI
Guadeloupe
0 5 10 20
km
San Juan area
Isabela
SHA
Vega Baja
CHA
ESC
PIN
Fajardo
RAT
CAY
Culebra
LUI
SON
PUN
Mona
SAR
FOR
TRE
Rincón
PUERTO RICO
Guánica
GUI
CAJ
Ponce
SUN
PIR
SEC
Vieques
Curaçao
CUR
0 20 40 80
km
0 250 500 1000
km
ATR
ENR
ELP
LAU
MED
TUR
MAR
Lajas
0 1 2 4
km

709

710


Highest L(K)
Mean L(K)
ΔK
ADM model
POPINFO model
LOCPRIOR model
A
B
C
D
E
F
G
H
I
Population number K
L(K)
ΔK
POPINFO 1
POPINFO 2
POPINFO 3
Islands
Regions
Localities
Reefs

711