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

Pascal Mège[1], Nikolaos V. Schizas[2], Joselyd Garcia Reyes[2] & Tomas Hrbek[1,3]

1 Department of Biology, University of Puerto Rico-Río Piedras, San Juan, Puerto Rico
2 Department of Marine Sciences, University of Puerto Rico-Mayagüez, Mayagüez, Puerto Rico
3 Laboratório de Evolução e Genética Animal, Universidade Federal do Amazonas, Manaus, Brazil

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Correspondence
Pascal Mège, Department of Biology, University of Puerto Rico-Río Piedras, San Juan, Puerto Rico.
E-mail: pascal.mege@gmail.com

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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 (0.685–0.844). 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} per 1000 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, but not around the Mona Passage.

Introduction

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 may 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 (tens of kilometers; 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, *Acropora palmata* and *Acropora 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 *versus* the Bahamas *versus* Curaçao), genetic differentiation between reefs separated by a few kilometers is generally not significant, except when introgression of *Acropora 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).

Microsatellite 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,b). The population break in the Southern Caribbean seemed to occur at the Guajira Peninsula, Colombia, whereas 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 debatable whether one can 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). 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 kilometers) 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 kilometers), 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) (Fig. 1, Table 1). Special effort was dedicated to (i) alternate small geographic distances (few kilometers) with moderate distances (tens of kilometers) between reefs and (ii) 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-m 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, Valencia, CA, USA) following the manufacturer's animal tissue protocol. Each sample was then screened for five polymorphic microsatellite markers, following a modified protocol from Baums *et al.* (2005a). The selected markers (#166, #181, #182, #192 and #207) were the same as those used in Baums *et al.* (2005a, 2006a,b). PCR amplifications were done in 10-μl reactions, containing 1 μl genomic DNA (5–15 ng·μl^{-1}),

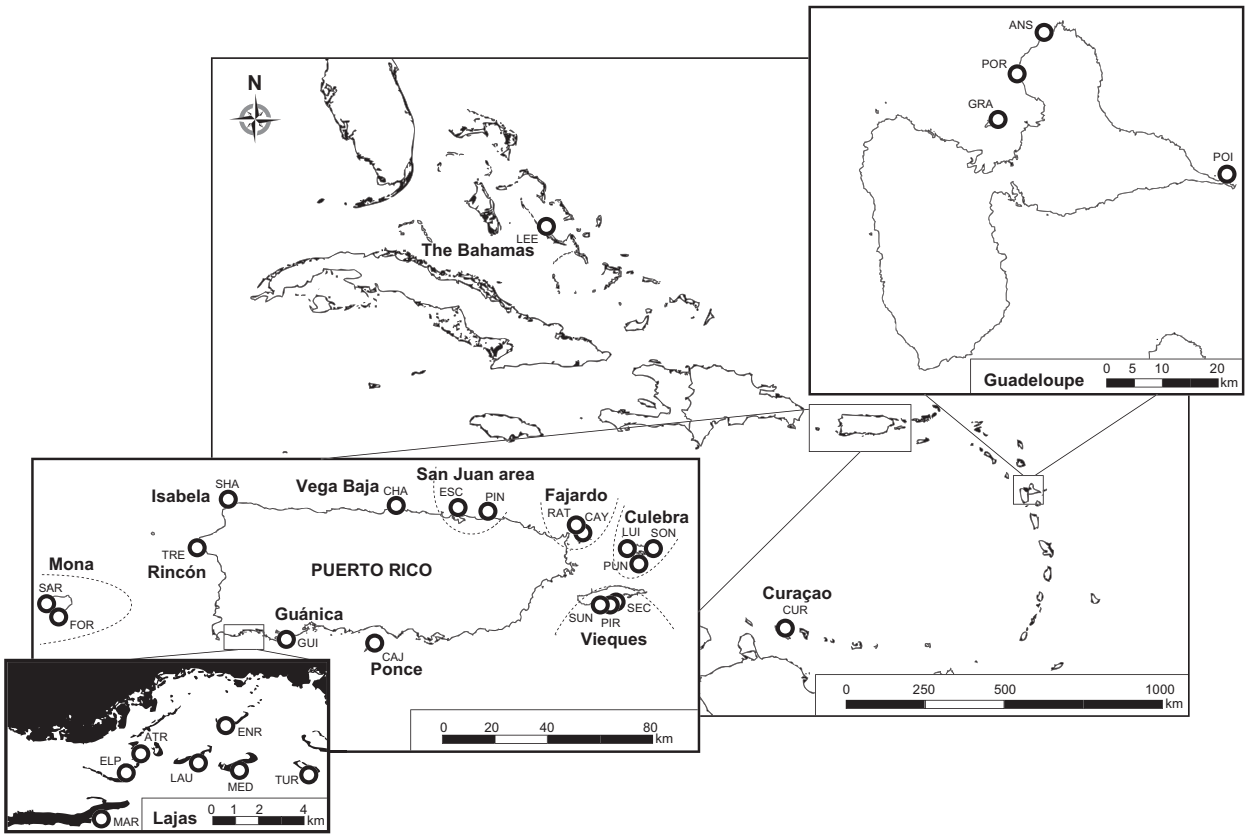

Fig. 1. Sampling locations of *Acropora palmata:* 30 reefs were sampled (24 reefs in Puerto Rico, including Mona, Vieques and Culebra, four reefs in Guadeloupe, one reef in Curaçao, and one reef in the Bahamas). Reefs are represented by codes of three uppercase letters, referring to the reef names in Table 1. The corresponding 14 localities grouping those reefs in the same table were also marked as full lowercase names on the map. Maps modified from maps generated on reefbase.org and Garcia Reyes & Schizas (2010).

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 1 $U \cdot \mu l^{-1}$ Taq DNA polymerase (Fermentas, Vilnius, Lithuania), and 1× 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 50× to approach 10–20 $ng \cdot \mu l^{-1}$, 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 (Applied Biosystems, Carlsbad, CA, USA).

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), pairwise 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 is <20 (Gaggiotti *et al.* 1999). The number of K populations was estimated in STRUCTURE 2.3.3

Table 1. Origin of samples, number of sampled colonies and number of unique genotypes (genets) in this study.

island	region	locality	reef	abb.	latitude (N)	longitude (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

abb. = three-letter reef abbreviation.

(Pritchard *et al.* 2000; Hubisz *et al.* 2009) and repeated 10 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 (Data S1 and accompanying Figs 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, *Acropora palmata* populations seem to be largely self-recruiting, and 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 are appended in Table S1. Significance of IBD patterns was tested with Mantel tests (30,000 permutations) and reduced major axis (RMA) regressions were used to estimate the strength of genetic

differentiation (RMA slope, jackknife 95% CI). Because the data are 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 pairwise Slatkin's F_{ST} ($F_{ST}/1-F_{ST}$) with the geographic distance matrices after setting genetic distances of <0–0. A $\log_{10}$ 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) and 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 were not highly supported (Cornuet *et al.* 1999). As a balance between improved statistical confidence and data loss, the results were compared again 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 & Van Der Linde 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.

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 approximately 1.48×10^{-7}, which closely matched the approximately 1.5×10^{-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). 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.

Mean genetic diversity across localities and loci was high ($H_E = 0.761$) and consistent across localities (0.685–0.844). 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 (#166) polymorphic locus.

Table 2. Summary of genetic diversity indices among the 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

Ng = number of genets; Ng/N = ratio of the number of genets to the number of colonies.
Allelic diversity (A), observed heterozygosity (H_O), expected heterozygosity (H_E) and allelic range (R) were averaged across loci.

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, five 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 (Fig. 2A and 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 Fig. 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 (Figs 3B and S2D and 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

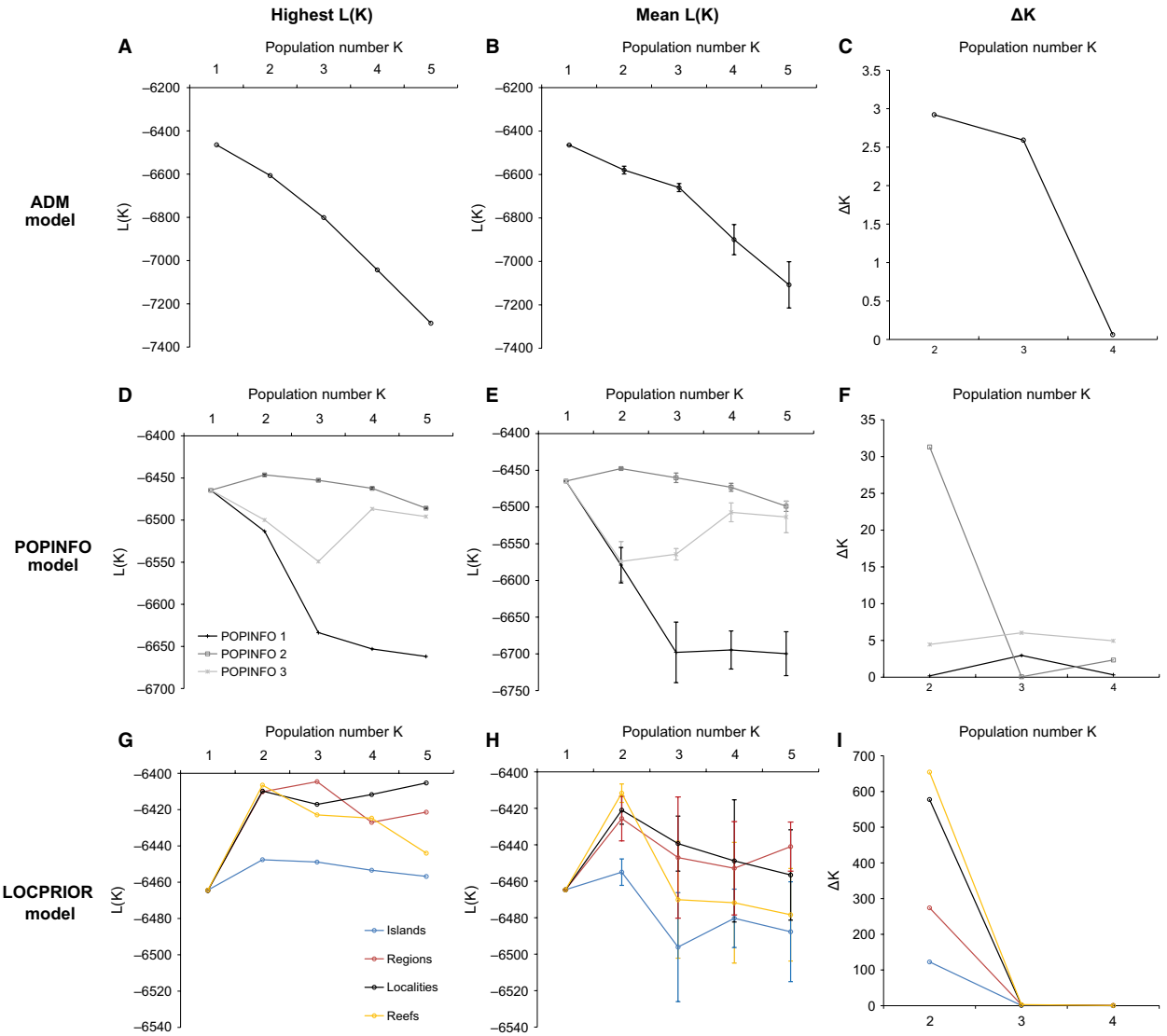

Fig. 2. Results of STRUCTURE for different parameter sets. From top to bottom, the first row contains the results of the ADM model (A–C), the second row the results of the three POPINFO models (D–F), and the third row (G–I) the results of the LOCPRIOR model for the four grouping variants proposed to explore the dataset at different scales. A, D and G plot the highest L(K) of 10 runs for each K assumed *a priori*. B, E and H plot the mean L(K) and standard deviation (vertical whiskers) over the 10 runs for each K. C, F, and I plot ΔK, following Evanno *et al.*(2005).

the predefined sampling locations to the end probabilities assigned to the individuals), α and F generally stabilizing after 5–10 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 (Fig. 2H). This was also true for other recommended methods, such as using only the best L(K) among replicate runs (Fig. 2G) or the ΔK parameter described in Evanno *et al.* (2005) (Fig. 2I). When performing alternate LOCPRIOR analyses, *i.e.* by assigning individuals to *a priori* locations corresponding to 'reefs', 'regions' or 'islands' (Fig. 2G and H), the original 'localities' model was generally found to reach superior probabilities across the range of tested K (1–5), although at K = 2, the 'reef' model had a slightly higher L(K) than the 'localities' model (mean $L(K)_{reefs} = -6412$ and mean $L(K)_{localities} = -6421$, respectively). Following the population model at K = 2, the Bahamas, Mona, Rincón, Lajas, Guánica, Ponce, Isabela and Vega Baja were clustered into one population and

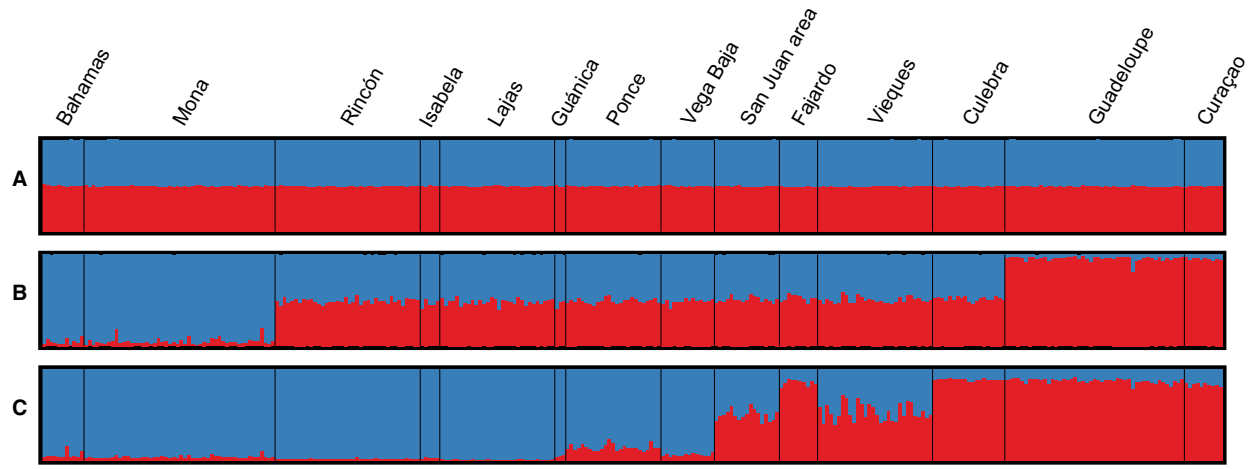

Fig. 3. STRUCTURE results for K = 2 with the three parameter sets defined in this study. All parameter sets used the admixture algorithm and performed 5,000,000 burn-ins followed by 15,000,000 iterations per run. Each figure plots the mean assignments of each individual genotype to K *a priori* populations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

Curaçao, Guadeloupe, Culebra, Fajardo clustered into another. Finally, Vieques and the San Juan area had admixed origins of populations (Fig. 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 from 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 with 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 (Fig. 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 (Fig. 5, dashed 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 6.6×10^{-5} to 1.7×10^{-4} for within Puerto Rico pair-wise comparisons and 1.9×10^{-5} to 6.5×10^{-5} for the remaining pair-wise comparisons (Fig. 5). However, there was no clear, separate clustering pattern characteristic of discrete populations (Figs 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 Figs 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 1000-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 1000 km. In fact, the RMA regression slope of pair-wise comparisons between Culebra and mainland Puerto Rico ($s = 1.2 \times 10^{-4}$,

Table 3. Pair-wise genetic distances and pair-wise genetic distances per 1000-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 1000 km (SSN distances).
F_{ST} per 1000 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.

SE = 2.5×10^{-5}) was superior to the slope for Mona-mainland Puerto Rico (s = 7.0×10^{-5}, SE = 2.7×10^{-5}), although their respective 95% CI overlapped (8.3×10^{-5} to 1.5×10^{-4}, and -4.6×10^{-5} to 1.9×10^{-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 greatly 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

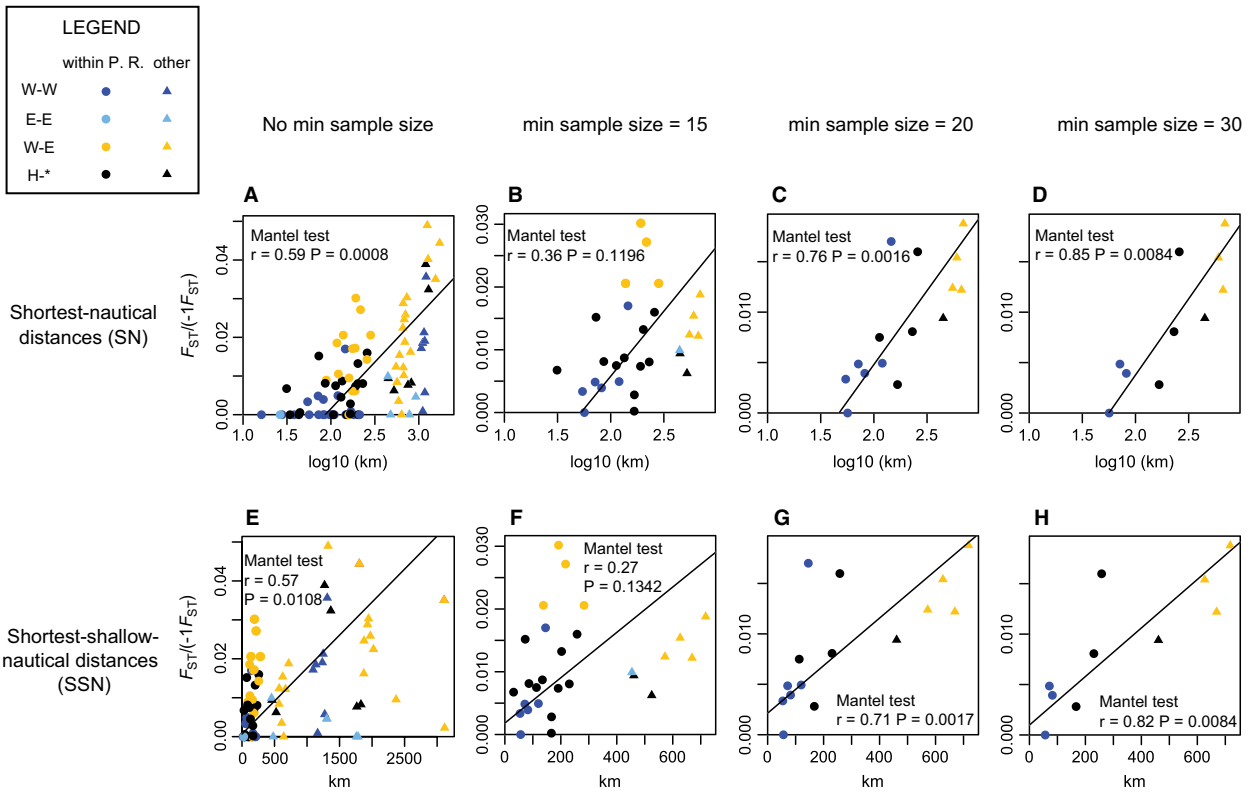

Fig. 4. Pair-wise genetic distances as a function of pair-wise geographical distances. The first, second, third and fourth columns (left–right) show the plots of genetic *versus* geographic distances when the analysis included all localities (n = 14), only localities with ≥15 genotypes (n = 8), ≥20 genotypes (n = 6) and ≥30 genotypes (n = 5). The top (A–D) and bottom rows (E–H) respectively show the plots for shortest-nautical (SN) and shortest-shallow-nautical (SSN) geographic distance. SN values were $\log_{10}$ transformed due to the bi-dimensionality of the model assumed. In all cases, F_{ST} $(1-F_{ST})$ between pair-wise localities served as genetic distances. For each plot, RMA regressions were drawn with black lines. Results of the Mantel tests realized in each case were directly appended to 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 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 (Fig. 3).

Baja and Escambrón. The back reefs of these locations varied in depth between <1 and 2 m, 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 *Acropora palmata* stands. In contrast, 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 depths where they were growing in an encrusting fashion, limiting their ability to produce asexual recruits via fragmentation. Reefs at Piñones and Sun beach simply had 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 in the 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 compared with 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; Fong & Lirman 2005; Reusch *et al.* 2005). Thus, the genetic fingerprint of clones within a reef will mostly be the result

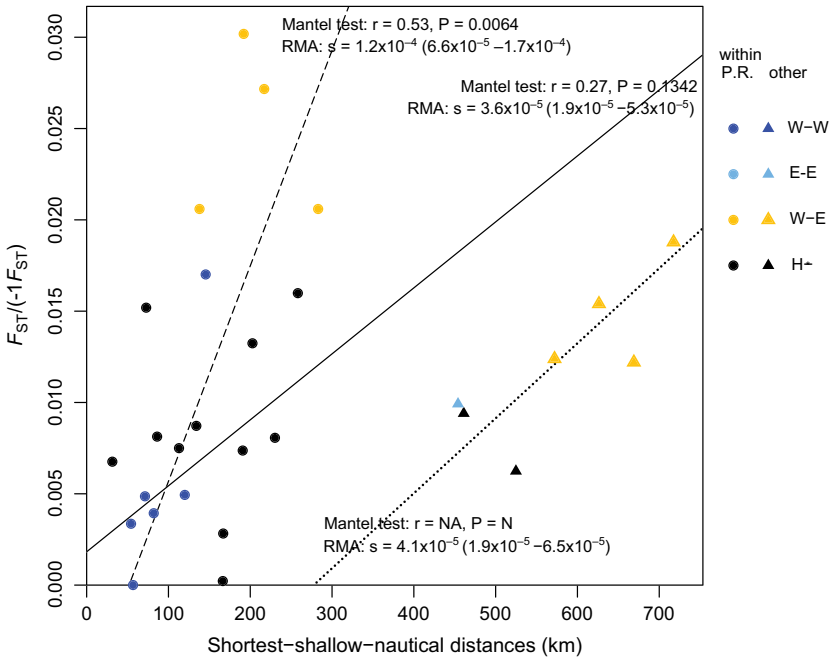

Fig. 5. Pair-wise genetic distances as a function of pair-wise shortest-shallow-nautical distances. The following population assignments were based on STRUCTURE results using the LOCPRIOR option and K = 2. E–E: Pair-wise comparisons within the eastern population. W–W: Pair-wise comparisons within the 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, dashed line) or with alternate locations (involving Curaçao, Bahamas and/or Guadeloupe; triangles, dotted 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.

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 *Acropora cytherea* (Concepcion *et al.* 2010) but surprisingly lower than for *Acropora muricata* and *Acropora 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 & van Oppen 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 due to White Band disease (Bruckner 2002). However, the high genetic diversity estimated with microsatellites is not reflected in the mitochondrial nucleotide diversity ($\pi = 0.00075$) of *A. palmata* in Puerto Rico, with among 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 with 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). 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 accordance with high genetic diversity and low estimates of population structure (F_{ST}, AMOVA analyses presented in Supporting Information in Data S2 and accompanying Table S2), genetic differentiation was detected with STRUCTURE but was weak: the basic admixture model (ADM) in STRUCTURE was not able to detect population structure in the data, and a high number of iterations were needed to reach statistical stability and determine 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, and thus suggested that two populations of *Acropora 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, 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 used the POPINFO option to define *a priori* assignments of selected locations. Various configurations combining western and eastern *a priori* assignments invariably resulted in (i) lack of stabilization of control parameters during the runs and (ii) 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) 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 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 (i) northwest and north of Puerto Rico reaching past Vega Baja; (ii) 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.

Patterns of IBD *versus* 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. However, this 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).

STRUCTURE analyses indicate two clusters with genetically intermediate individuals and localities occurring in Western Puerto Rico (Fig. 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} 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 two apparently 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 should be more pronounced in the east of Puerto Rico, as suggested by the slopes of RMA regressions between mainland Puerto Rico and Mona *versus* 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 1999). Fewer 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 seem 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 more accurately 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 exhort 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*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods used to estimate and choose the number of discrete populations K in STRUCTURE, STRUCTURE configurations for the manuscript, as well as results from additional runs.

Data S2. Tests of hierarchical genetic structuring using F_{ST}-based Analysis of Molecular Variance (AMOVA).

Fig. S1. Additional STRUCTURE runs under the LOCPRIOR model on a reduced dataset as further described in Data S1.

Fig. S2. STRUCTURE results for K = 2 under the LOCPRIOR model comparing global results with the results for Puerto Rican locations only.

Table S1. Geographic distance matrices between the 14 locations reported in the manuscript.

Table S2. Configurations and results for AMOVA for Data S2.