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Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*

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Abstract

The movements of larvae between marine populations are difficult to follow directly and have been the subject of much controversy, especially in the Caribbean. The debate centres on the degree to which populations are demographically open, such that depleted populations can be replenished by recruitment from distant healthy populations, or demographically closed and thus in need of local management. Given the depressed state of many tropical reef populations, the understanding of these movements now bears critically on the number, placement, and size of marine reserves. Most genetic analyses assume that dispersal patterns have been stable for thousands of generations, thus they commonly reflect past colonization histories more than ongoing dispersal. Recently developed multilocus genotyping approaches, however, have the demonstrated ability to detect both migration and population isolation over far shorter timescales. Previously, we developed five microsatellite markers and demonstrated them to be both Mendelian and coral-specific. Using these markers and Bayesian analyses, we show here that populations of the imperiled reef-building coral, *Acropora palmata*, have experienced little or no recent genetic exchange between the western and the eastern Caribbean. Puerto Rico is identified as an area of mixing between the two subregions. As a consequence of this regional isolation, populations in the western and eastern Caribbean should have the potential to adapt to local conditions and will require population-specific management strategies.

Keywords: gene flow, genotypic clustering, larval dispersal, *Acropora palmata*, marine reserves, microsatellite

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Introduction

Historically, marine populations have been thought to be well connected via long-distance dispersal of planktonic larval stages (Heck & McCoy 1978; Veron 1995; Scheltema *et al*. 1996). High connectivity between populations would have important implications for the management of marine resources. For example, a smaller number of marine reserves would theoretically be required to achieve adequate protection of larval supplies, in contrast to highly structured populations which would require a larger number of reserves. The extent of interconnectedness of coral-reef-associated organisms is the subject of ongoing controversy (Roberts 1997; Cowen *et al*. 2000). Roberts (1997) proposed a high correlation between ocean surface currents and larval dispersal routes, such that species with planktonic larvae inhabiting regions connected by strong currents should show high genetic similarity. In contrast, Cowen *et al*. (2000) suggested that high diffusion and mortality rates, aided by behavioural adaptations, should result in local larval retention and closed populations over ecologically relevant timescales. Indeed, an increasing number of studies report far less dispersal than previously predicted (reviewed in Hellberg *et al*. 2002; Swearer *et al*. 2002; Thorrold *et al*. 2002).

Marine fishes reveal a range of genetic similarity in Caribbean populations that is weakly correlated with the larval lifespan (Shulman & Bermingham 1995). Recently,

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Taylor & Hellberg (2003) presented mitochondrial DNA (mtDNA) evidence for strong differentiation among Caribbean populations of a cleaner goby (*Elacatinus evelynae*). The proposed phylogeographical break is the Mona Passage between Puerto Rico and Mona Island (Colin 1975; Starck & Colin 1978; Taylor & Hellberg 2003; Taylor & Hellberg submitted). This break is also observed using other genetic markers and in other members of this genus (Taylor & Hellberg submitted). Some populations of *E. evelynae* were even reciprocally monophyletic, suggesting thousands of generations of isolation despite modest geographical separation and larvae with a 3-week pelagic duration. However, these gobies may be regarded as poor dispersers compared to most benthic reef inhabitants for several reasons. First, they belong to an especially species-rich lineage, so their strong subdivision could be considered a propensity to differentiate. Second, their larvae are overrepresented in inshore ichthyoplanktonic samples, which suggests that they may be adapted for near shore retention (Smith & Potts 1987; Leis *et al*. 2003). Third, their eggs are attached to the substrate prior to fertilization, so that embryos do not spend the first six days of their ontogeny as plankton (Valenti 1972). These conditions do not apply to many species, including broadcast-spawning corals that comprise the very foundation of Caribbean coral reefs.

Acropora palmata Lamarck is one of the major reef-building corals in the Caribbean, providing essential habitat for a multitude of reef organisms (i.e. foundation species, Gladfelter *et al*. 1978; Lirman 1999). Historically, it dominated the reef crest and the shallow fore reef (0–5 m) (Goreau 1959; Bruckner 2002), but populations have drastically declined during the 1980s and remain in a depressed state (Bruckner 2002). Recovery of populations depends in part on successful reproduction. Asexual reproduction by breakage (fragmentation) and reattachment of branches can be the predominant mode of recruitment in *A. palmata* (Highsmith 1982). In contrast, sexual reproduction, which provides the potential for larval influx to depressed populations, appears relatively limited (Highsmith 1982). *A. palmata* releases gametes into the water column once a year, generally after the August full moon, in a synchronized spawning effort (Szmant 1986). Each colony simultaneously releases eggs and sperm in bundles that float to the surface where they break apart and mix with gametes from other colonies (*A. palmata* is a poor self-fertilizer, Baums *et al*. in press). After fertilization, *A. palmata* larvae undergo a 78-h period of development before showing first signs of motility (e.g. Baums *et al*. in press). Pelagic larvae become competent to settle within 5 d, but can remain planktonic for up to 20 d (M. Vermeij, unpublished; I. B. Baums, M. W. Miller, personal observation). First settlement of Pacific *Acropora* larvae occurs between 3 and 27 d (Harrison & Wallace 1990; Hayashibara *et al*. 1997; Nishikawa *et al*. 2003) in aquarium studies.

Estimation of genetic population structure in corals poses a number of difficulties. Soft-bodied coral larvae do not permit chemical (Swearer *et al*. 2002) or visual (Taylor & Hellberg 2003) analyses of their larval life as is possible in fish larvae via inspection of ear bones (otoliths). Genetic techniques are the only current alternative. Most reef corals, however, harbour intracellular algal symbionts, complicating coral-specific marker development (Shearer *et al*. in press). mtDNA is generally invariant within coral species, making it useless for population genetic studies (Shearer *et al*. 2002). Furthermore, because of frequent asexual reproduction in *A. palmata*, the genetic markers employed must be sufficiently polymorphic to identify individual genets. This allows for the exclusion of multilocus genotypes that are identical by descent and thus prevents inflation of inferred interpopulation differences caused by local asexual reproduction. Here, we examine population structure in the broadcast-spawning *A. palmata*. We used five microsatellite loci, previously demonstrated to be coral-specific, single copy, Mendelian, and unlinked by controlled crosses (Baums *et al*. in press), to genotype *A. palmata*.

Knowledge of the population structure of *A. palmata* is both potentially informative as to the generality of proposed phylogeographical breaks in the Caribbean and essential in estimating the likelihood of natural population recovery or the success of potential conservation measures. In this study, we test the null hypothesis of Caribbean panmixia in *A. palmata*. The expectation of no population structure is based on the presumed dispersal potential of this coral's larvae (5 d to 2 weeks) and the strong and consistent surface currents running through its range during the spawning season (Fig. 1). Contrary to this expectation, we present evidence for the existence of two distinct population clusters of *A. palmata* that show little recent migration between them, and a high degree of self-recruitment within populations. The break between the two populations is not abrupt, with mixing evident in samples from Mona Island and Puerto Rico.

Materials and methods

Sampling

Acropora palmata colonies were sampled and genotyped from 44 reefs in 11 localities spanning much of the Caribbean and the Bahamas through scuba diving (Table 1, Fig. 2). Sample sizes in Table 1 indicate the number of unique genets identified ($n = 709$, see following discussion) and the total number of colonies (ramets) sampled ($n = 1300$). A description of clonal structure in *A. palmata* will be provided in a separate report. A 1-cm-long tip was snipped off each sampled colony using a bolt cutter and placed in a labelled bag. Coral samples were immersed in 70% ethanol upon returning to shore and stored at −80 °C until genotyping.

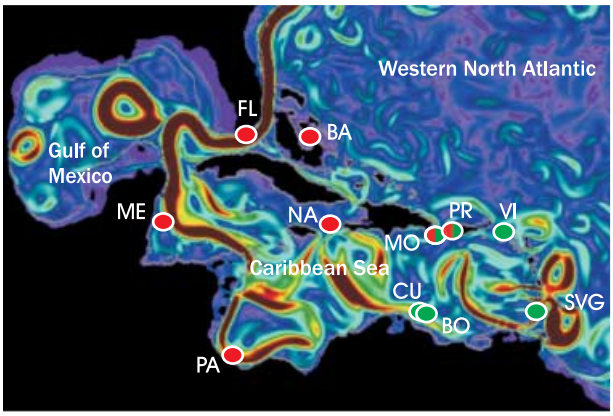

Fig. 1 Surface currents in the Caribbean on an average August 1. Dark red indicates highest energy (fastest currents), and blue indicates lowest. *Acropora palmata* spawns most often in August. Sampling localities are indicated by ovals (compare to Fig. 2). Instantaneous surface velocity derived from a high-resolution simulation with the Miami isopycnic coordinate ocean model (MICOM) (Garraffo *et al*. 2001, 2003). Red ovals, localities in the western Caribbean and the Bahamas: Panama (PA), Mexico (ME), Florida (FL), the Bahamas (BA), Navassa (NA); green ovals, localities in the eastern Caribbean: US Virigin Islands (VI), St Vincent and the Grenadines (SVG), Bonaire (BO) and Curacao (CU). Red/green ovals, localities in an area of mixing (see text): Mona Island (MO) and Puerto Rico (PR). Plot courtesy of Z. Garraffo and E. Chassignet, University of Miami.

Genotyping

Tissue samples were extracted and genotyped as described in Baums *et al*. (in press). Briefly, two multiplex polymerase chain reactions (PCRs) were performed per sample using fluorescently labelled primers to assay five loci containing AAT repeats. These five microsatellite loci have previously been demonstrated to be Mendelian and coral-specific using controlled crosses (Baums *et al*. in press). PCR products were visualized with an automated sequencer (ABI 3730). An internal size standard (Gene Scan 500-Liz, Applied Biosystems) ensured accurate sizing. Electropherograms were analysed with GENEMAPPER software 3.0 (Applied Biosystems). Alleles were scored as PCR product size and converted to repeat number by subtracting the size of the flanking region when necessary (e.g. for R_{ST} estimates, see following discussion).

Samples that shared the same diploid genotype across all five loci were considered to be clone mates (ramets) belonging to the same genet. Replicated genotypes were never shared between reefs, only within reefs. We calculated the probability of identity (P_{ID}) to give a conservative estimate of the probability that two individuals sampled in the same population share a multilocus genotype by chance, not by descent (i.e. are clone mates, Waits *et al*. 2001). The combined P_{ID} is obtained after sequentially multiplying P_{ID} values over all loci. Biased (Paetkau & Strobeck 1994) and unbiased (Kendall & Stewart 1977) estimates were 1.5×10^{-7} and 1.4×10^{-7}, respectively (Table 2). P_{ID} was calculated by GIMLET (Valiere 2002). Because of the low probability of misidentifying colonies as clone mates when in fact they are not, each distinct five-locus genotype was included only once in the data set for statistical analysis.

Samples were tested for deviation from the expectations of Hardy–Weinberg equilibrium (HWE) using FSTAT (Goudet 1995) (alleles were randomized 1000 times within populations). Controlled crosses had shown that the five loci used here are inherited in Mendelian fashion in genets from Florida (Baums *et al*. in press). However, null alleles might be present in other localities. We thus tested for heterozygote deficiencies for each locus ($n = 5$) in each locality ($n = 11$) using GENEPOP (http://wbiomed.curtin.edu.au/genepop/). Out of 55 tests, five were significant at the $P < 0.05$ level. After sequential Bonferroni correction (Holm 1979), no test remained significant. Therefore, we found no evidence for null alleles.

Clustering Analysis

No a priori information was available as to the likely number of populations of *A. palmata*. Thus, the number of genetically differentiated *A. palmata* populations, *K*, was estimated by employing a Bayesian approach, implemented in the program STRUCTURE (Pritchard *et al*. 2000; Falush *et al*. 2003). STRUCTURE uses a clustering method to assign individuals (in our case genets) with similar multilocus genotypes to probable common populations. Mean and variance of log likelihoods and posterior probabilities of the number of populations for $K = (1, 2, \ldots 11)$ were inferred from multilocus genotypes by running STRUCTURE five times with 10^6 repetitions each (burn in = 100 000 iterations, or generations). The mean membership (q) for each genet describes the likelihood of that genet belonging to the respective cluster. Genets can be assigned partial membership in multiple clusters, with membership coefficients summing to one across clusters. The 'admixture ancestry model' (because the planktonic larval stage of *A. palmata* might lead to highly interconnected populations) was run under the assumption of 'correlated allele frequencies' rather than 'independent allele frequencies' to improve clustering of closely related populations (Falush *et al*. 2003). A second approach was taken to evaluate the ancestry of genets sampled in Puerto Rico because the Mona Channel separating Puerto Rico and Mona Island had been identified previously as a phylogeographical break in reef fish (see Introduction). Genets from Puerto Rico were designated as 'unknowns' and STRUCTURE was used to assign these genotypes to their place of origin. In this kind of analysis, all other genets were used as baseline data, that is STRUCTURE was told where they originated. Migrants

Table 1 Localities, sample sizes (the number of colonies genotyped) and number of genets (repetitive genotypes excluded) for *Acropora palmata* analysed in this study. NA, not available. VI, US Virgin Islands; SVG, St Vincent and the Grenadines

Subregion	Locality	Reef name	Latitude (°)	Longitude (°)	No. of samples	No. of genets	Per locality
West	Panama	Tobobe	9.122683	−81.818333	10	6	39
		Bastimente	9.265	−82.12005	41	17	
		Cayo Wild Cayne	9.3459	−82.17183	17	7	
		Bocas Del Drago	9.41615	−82.3309	22	9	
	Mexico	Chinchorro	18.383333	−87.45	35	7	7
	Florida	Dry Tortugas	24.6209	−82.8675	4	2	34
		Rock Key	24.456017	−81.859633	25	4	
		Western Sambo	24.479867	−81.718667	34	9	
		Sand Island	25.0179	−80.368617	56	12	
		Little Grecian	25.118433	−80.31715	24	1	
		LG Snail Patch	25.118517	−80.301367	1	1	
		Horseshoe	25.139467	−80.29435	25	1	
		Boomerang Reef	25.352467	−80.17845	11	2	
		Marker 3	25.373333	−80.160217	42	2	
	Bahamas	Great Iguana	26.70747	−77.15358	44	27	96
		Halls Pond	24.35387	−76.56992	19	12	
		Rocky Dundas	24.2788	−76.5387	17	4	
		Little Darby	23.84738	−76.2088	25	10	
		Bock Cay	23.8075	−76.16014	23	8	
		Black Bouy	23.80219	−76.146	21	11	
		Charlie's Beach	23.78082	−76.10391	32	14	
		Perry Shallow	23.77326	−76.09543	11	6	
		Children's Bay	23.84733	−76.20807	4	4	
	Navassa	NW Point	18.413567	−75.029433	35	35	68
		N Shelf	18.413483	−75.02285	18	18	
		Lulu Bay	18.395833	−75.019883	15	15	
	Total				611		244
Mixed	Mona	Carmelita	18.103222	−67.936472	24	6	36
		Fortuna Reefer	18.03330	−67.869017	24	23	
		Pajores	NA	NA	23	7	
	Puerto Rico	Bajo Gullardo	18.00325	−67.3317	23	18	90
		San Cristobal	17.56493	−67.04515	48	26	
		Rincon	18.21007	−67.15849	47	46	
	Total				189		126
East	VI	Johnsons Reef	18.361733	−64.7743	23	16	41
		Grounding VI	NA	NA	19	11	
		Hawksnest BayI	18.347233	−64.780717	46	14	
	SVG	Blue Lagoon	13.12848	−61.19932	16	10	166
		Petit Byahaut W	NA	NA	28	24	
		Bequia	13.01503	−61.24906	21	17	
		Mustique	12.89151	−61.18625	32	24	
		Canouan	12.69425	−61.33644	29	21	
		Mayreaux Gardens	12.63216	−61.38161	19	14	
		Tobago Cays	12.62533	−61.34991	36	32	
		Union Island	12.5916	−61.41596	29	24	
	Bonaire	Taylors Made	12.22382	−68.40507	43	32	32
	Curacao	Boka Patrick	12.287333	−69.042667	21	15	100
		Awa Blanca	12.04056	−68.78336	43	28	
		Sea Aquarium	12.08376	−68.89575	54	25	
		Blue Bay	12.13516	−68.9898	41	32	
	Total				500		339
Total					1300		709

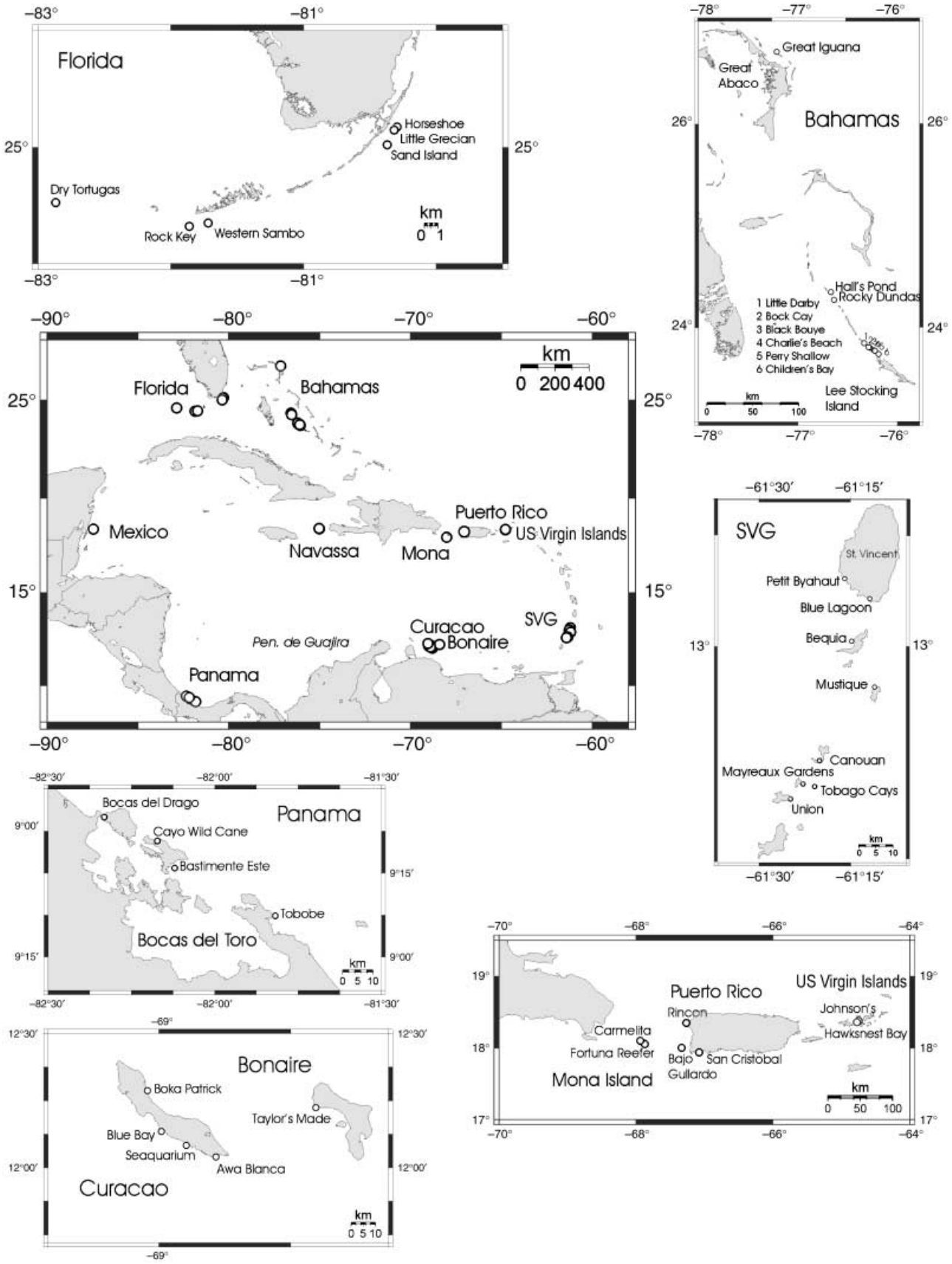

Fig. 2 Maps of sampling localities of *Acropora palmata* in the Caribbean. Maps created with OMC (http://www.aquarius.geomar.de/omc).

Table 2 Characteristics of *Acropora palmata* microsatellite markers in the 11 sampling localities. Given are the number of samples (*N*), the number of observed (H_O) and the number of expected (H_E) heterozygotes and the number of alleles (*A*) found per marker and locality. The presence of heterozygote deficits (Het deficit) over all loci at each locality was evaluated with a Hardy–Weinberg exact test implemented by GENEPOP (http://wbiomed.curtin.edu.au/genepop/). No test remained significant after sequential Bonferroni correction (Holm 1979). The probability of identity (P_{ID}, see text for explanation) was calculated by GIMLET (Valiere 2002)

			166			181			182			192			207			Het deficit		
Population	Locality	*N*	H_E	H_O	*A*	H_E	H_O	*A*	H_E	H_O	*A*	H_E	H_O	*A*	H_E	H_O	*A*	*P* value	SE	P_{ID}
West	Panama	39	34	36	12	9	10	3	36	31	15	35	36	15	34	35	11	0.06	0.01	
	Mexico	7	7	7	8	4	2	3	5	5	5	6	7	7	6	7	7	0.73	0.01	
	Florida	34	31	30	15	18	20	7	27	26	13	31	30	14	30	33	14	0.83	0.02	
	Bahamas	96	88	89	17	50	52	12	80	78	19	87	88	16	85	81	17	0.17	0.03	
	Navassa	68	60	59	17	36	39	7	59	59	19	62	63	17	59	55	14	0.08	0.01	
	Mona	36	33	29	15	11	9	4	31	32	17	32	35	12	32	33	14	0.67	0.03	
PR	PR	90	83	82	22	34	35	6	70	72	16	81	80	15	79	75	15	0.17	0.02	
East	VI	41	32	32	15	14	12	4	31	31	16	36	39	10	34	31	12	0.27	0.03	
	SVG	166	122	128	13	59	59	8	112	111	17	146	143	14	136	142	14	0.82	0.02	
	Bonaire	32	20	23	6	11	11	4	21	24	13	29	26	12	28	27	11	0.28	0.02	
	Curacao	100	78	75	19	41	38	6	73	77	20	90	86	15	88	93	15	0.01	0.00	
Sum	All	709			25			18			23			21			18			
Biased																				1.5×10^{-7}
Unbiased																				1.4×10^{-7}

between populations were identified in a third round of analyses using the PopInfo option and the program was run with default values.

We used another Bayesian model, implemented by BAYESASS (Wilson & Rannala 2003), to estimate recent migration rates, *m*, among clusters and sampling localities. This model assumes loci are not physically linked (as demonstrated previously using crosses, Baums *et al*. in press) and that generations are nonoverlapping. The latter is likely violated because *A. palmata* genets are potentially long-lived resulting from asexual reproduction. The model was run under the default parameters. We ran two separate BAYESASS analyses: one examining migration between the population clusters and one among all 11 sampling localities. BAYESASS performs a likelihood ratio test using a chi-squared (χ^2) statistic to determine if the posterior probabilities of migration rates are significantly different from their priors. χ^2 values were significant ($P = 0.001$), indicating that enough genotypic variation is present to detect migration rates between sampled localities and populations.

Estimates of migration matrices can be sensitive to the particular populations included in the analysis (Cornuet *et al*. 1999). The relative influence of each locality on inferred immigration rates was assessed by a jackknifing procedure. A migration matrix consists of estimates of immigration rates among pairwise population combinations. Migration matrices were obtained by sequentially omitting each of the sampling localities ('jackknifed results', $n = 11$ matrices) and for all 11 localities ('overall results', $n = 1$ matrix). The resulting 11 jackknifed matrices were summarized by considering the values along the diagonals only (the diagonals summarize the amount of self-recruitment into a locality since immigration values per locality always add to one), resulting in a single 'jackknifed self-recruitment' matrix. Similarly, the overall self-recruitment rates were extracted from the overall migration matrix by considering only the diagonal. The difference between the overall self-recruitment rate and each of the 11 jackknifed self-recruitment estimates was calculated for each locality. Immigration rates of 30% represent the maximum value allowed into any locality/population as defined in the model (see the documentation for BAYESASS).

F- *and* R-*statistics*

Estimates of F_{ST}, F_{IT}, F_{IS} and R_{ST} were performed using MSA (Dieringer & Schlötterer 2003) and FSTAT (Goudet 1995), respectively. F_{ST} describes the amount of population differentiation based on the variance in allele frequencies among populations and was estimated using Weir & Cockerham's (1984) θ. The estimator of R_{ST}, ρ (Slatkin 1995), takes allelic relationships into account by assuming a stepwise-mutation model (SMM) characteristic of some microsatellite loci. F_{IS} and F_{IT} measure deviations from Hardy–Weinberg proportions within subpopulations and in the total population, respectively. An analysis of molecular variance (AMOVA, Excoffier *et al*. 1992) was performed based on the number of different alleles (F_{ST}) and on the sum of squared size differences (R_{ST}) as implemented by ARLEQUIN version 2.0 (Schneider *et al*. 2000).

Results

Identification of populations using STRUCTURE

Patterns of microsatellite differentiation reject the hypothesis that the Caribbean elkhorn coral, *Acropora palmata*, constitutes a single, interbreeding population throughout its geographical range. Results from the STRUCTURE analysis suggested that the genotyped individuals fell into two differentiated clusters (Fig. 3a). Further division of the data set into more than two clusters was not supported (data not shown). For $K = 2$, genets had high membership coefficients (0.83 and 0.84, respectively, Fig. 3a) in the cluster from which they were sampled. The sampling localities were identified as belonging to either a western cluster (Panama, Florida, the Bahamas, Navassa, Mona Island and Puerto Rico) or an eastern cluster (Virgin Islands, St Vincent and the Grenadines, Curacao and Bonaire) (Fig. 3a). The geographical break between these two clusters occurs between Panama and Curacao in the south and between Mona Island and Puerto Rico in the north (Fig. 3a). Previous studies in the Caribbean had indicated that Mona Channel separating Mona Island and Puerto Rico might represent a phylogeographical break (see Introduction). When genets from Puerto Rico and Mona Island were treated as 'unknowns' with all other genets assigned to their respective subregions a priori, STRUCTURE returned mixed ancestry for Puerto Rico and Mona Island genets with equal likelihood of originating from east and west (Fig. 3b). No other localities showed such extensive mixing. To further explore the a priori hypotheses of the Mona Channel as a phylogeographical barrier between the eastern and western Caribbean, we focused on the status of Puerto Rico in the following *F*-statistics analyses by assigning it in turn to the western, the eastern or its own cluster.

Population structure as estimated by F*-statistics*

There was significant differentiation between clusters, as estimated by F_{ST}, R_{ST} and AMOVA, regardless of the assignment of Puerto Rico (Tables 3 and 4). Measures of population differentiation were highest when Puerto Rico was assigned to the western cluster (Table 3). Intermediate F_{ST} and R_{ST} values were obtained when sampling localities were divided into three clusters, with Puerto Rico being the sole member of the third cluster (Table 3). For comparison, F_{ST} and R_{ST} estimates are given when grouping is based on the 11 sampling localities. This resulted in mostly reduced F_{ST} and R_{ST} values, as expected since populations are now artificially divided into subpopulations.

AMOVA attributed between 95% (based on the number of different alleles or F_{ST}) and 89% (based on the sum of squared size differences, R_{ST}) of the variation to within localities (Table 3). Variation is maximally distributed among clusters when Puerto Rico is assigned to the western cluster (F_{ST}, 4.10%; R_{ST}, 9.17%; Table 4A, B). Congruently, the amount of variation among localities within clusters increases when Puerto Rico is assigned to the eastern cluster (F_{ST}, 2.15%; R_{ST}, 3.15%; Table 4C, D) instead of the western cluster (F_{ST}, 1.33%; R_{ST}, 1.85%; Table 4A, B). Because of the high levels of expected within-population heterozygosity ($H_{E\ within}$, Table 2), these values approach the theoretical maximum of F_{ST} $(1 - H_{E\ within})$ (Balloux & Lugon-Moulin 2002). In addition to using *F*-statistics, we also made a cruder test for the presence of Wahlund effects [net deficiencies in heterozygotes that result from lumping subdivided samples which may themselves show HWE (Wahlund 1928)]. All 11 sampling localities were found to be in HWE ($P > 0.1$ over all loci). The same was true for both clusters when Puerto Rico was assigned to the western cluster ($P > 0.05$ over all loci). However, significant deviations

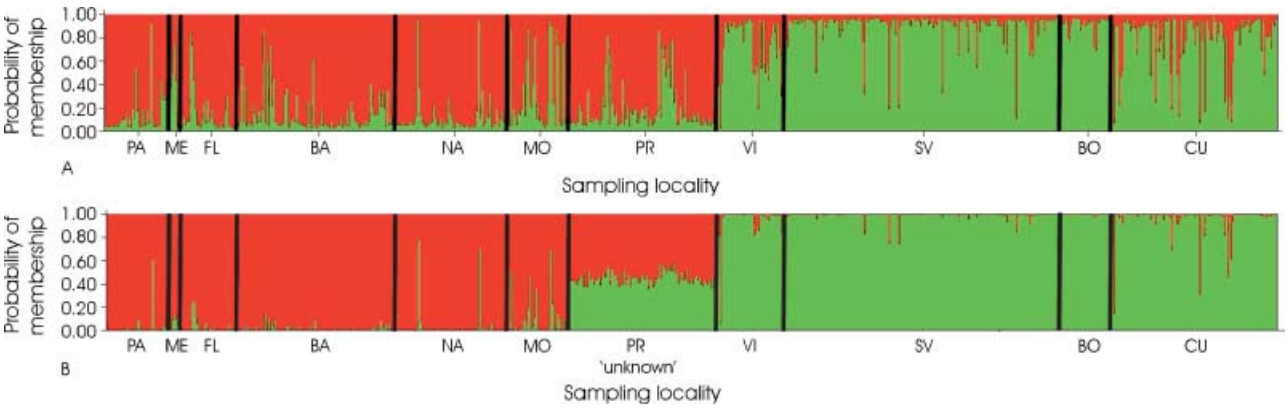

Fig. 3 Geographical subdivision of western and eastern Caribbean populations of *Acropora palmata* as inferred using STRUCTURE. (A) Results when STRUCTURE was run without providing information about the geographical origin of samples. Two clusters are distinguishable, a western cluster with genets from Panama (PA), Mexico (ME), Florida (FL), the Bahamas (BA), Navassa (NA), Mona Island (MO) and Puerto Rico (PR) and an eastern cluster with genets from the US Virigin Islands (VI), St Vincent and the Grenadines (SVD), Bonaire (BO) and Curacao (CU). (B) Results when STRUCTURE was asked to classify the genets sampled in Puerto Rico (designated as 'unknown') while having the geographical origin of all other genets designated *a priori*.

Grouping	Statistic	F_{ST}	F_{IT}	F_{IS}	R_{ST}
2 clusters (PR in west)	Mean	0.040	0.049	0.010	0.221
	Lower CI	0.012	0.020	0.006	
	Upper CI	0.080	0.088	0.014	
	P value	< 0.001			
2 clusters (PR in east)	Mean	0.032	0.046	0.015	0.150
	Lower CI	0.015	0.021	0.007	
	Upper CI	0.056	0.078	0.024	
	P value	< 0.001			
3 clusters (PR separate)	Mean	0.036	0.044	0.008	0.195
	Lower CI	0.012	0.018	0.004	
	Upper CI	0.091	0.097	0.011	
	P value	< 0.001			
11 localities	Mean	0.036	0.035	−0.001	0.153
	Lower CI	0.015	0.015	−0.004	
	Upper CI	0.074	0.069	0.002	
	P value	< 0.001			

Table 3 Population subdivision of *Acropora palmata*. Estimates of F_{ST}, F_{IT}, F_{IS} and R_{ST} were performed using MSA (Dieringer & Schlotterer 2003) and FSTAT (Goudet 1995), respectively. CI, confidence interval; PR, Puerto Rico. For sample sizes see Table 1. Samples had significant within-population heterozygote deficits (measured by F_{IS}) when PR was assigned to eastern cluster (F_{IS}, $P < 0.01$) but not when PR was assigned to the western cluster ($P > 0.05$), suggesting a Wahlund effect when PR is assigned to the eastern cluster.

from HWE were detected when Puerto Rico was assigned to the eastern cluster ($P < 0.01$), indicating a Wahlund effect.

Assessment of immigrants and asymmetrical migration

STRUCTURE identified a small number of individuals (18 of 709; 2.5%) with likely immigrant ancestry in the western ($n = 6$) and the eastern ($n = 12$) clusters. All other genets were assigned to the cluster from which they were sampled.

Immigration between the two *A. palmata* clusters was essentially absent (< 0.02%) as estimated with the Bayesian approach implemented in BAYESASS (Wilson & Rannala 2003) when Puerto Rico is assigned to the western cluster. This implied that absence of migration is not attributable to lack of information as indicated by significant χ^2 values when comparing the posterior probabilities of migration rates to their priors (Wilson & Rannala 2003). The same result of no exchange between west and east localities is obtained when immigration is assessed for all 11 sampling localities (Table 5). Again, log-likelihood ratio tests were significant. However, immigration rates between individual localities were the *ca.* 30% in four of seven localities in the west and three of four localities in the east (Table 5), indicating that localities may not be differentiated enough to allow for robust immigration analysis at this spatial scale (documentation for BAYESASS).

Despite the overall lack of differentiation between localities, the inclusion or exclusion of some localities had a larger effect on immigration rates among localities than others. Immigration rates per locality among all 11 localities were compared to immigration rates obtained when omitting one locality in turn (jackknifing over localities). Estimates of immigration rates changed little for Panama, Mexico, Florida, Mona Island, the US Virgin Islands and Curacao (Fig. 4a) compared to estimates derived from all 11 localities. In general, self-recruitment rate estimates were higher when all 11 localities were included. The magnitude of change in self-recruitment in the other localities was not dependent on the sample size of the omitted localities (linear regression, $r^2 = 0.08$, $P > 0.1$). The average influence of each of the localities on the overall immigration rate is shown in Fig. 4b. Immigration rates decreased on average when St Vincent and the Grenadines (SVG) samples were omitted as compared to values obtained when all 11 localities were considered (Fig. 4b). By this measure, SVG plays the most important role in connecting localities in east. In contrast, Panama did not contribute significantly to immigration into the localities sampled in this study (Table 5) and had consistently high self-recruitment rates (Fig. 4a). Taken together, these observations are congruent with asymmetrical larval exchange patterns between the sampled localities in this study.

Discussion

Two populations of Acropora palmata

Patterns of microsatellite differentiation reported here reject the hypothesis that the Caribbean elkhorn coral, *Acropora palmata*, constitutes a single, interbreeding population throughout its broad geographical range. Instead, our results reveal a significant genetic discontinuity that indicates populations from the eastern Caribbean (roughly the US Virgin Islands and the Lesser Antilles) and from the western Caribbean (Panama, Mexico, Florida, the Bahamas, and Navassa) have experienced little if any gene flow between them in the recent past. Genets from Puerto Rico and Mona Island have mixed ancestry, but show closer affinity to the western cluster. Subdivision within the two regional clusters is subtle. It is evident, however, that

Table 4 Analysis of molecular variance (AMOVA) among regions and localities for *Acropora palmata*. Significance tests are based on 10 100 permuations. The *P* values are the random value ≤ observed value. (A and B) Puerto Rico is assigned to the western cluster; (C and D) Puerto Rico is assigned to the eastern cluster; (A and C) based on the number of different alleles (F_{ST}); (B and D) based on the sum of squared size differences (R_{ST})

(A)

Source of variation (F_{ST})	Degrees of freedom	Sum of squares	Variance components	Significance tests (*P* value)	Variation (%)
Among clusters	1	64.66	0.08	0.003 ± 0.001	4.10
Among localities within clusters	9	44.32	0.03	0.000 ± 0.000	1.33
Within localities	1407	2644.94	1.88	0.000 ± 0.000	94.57
Total	1417	2753.92	1.99		

(B)

Source of variation (R_{ST})	Degrees of freedom	Sum of squares	Variance components	Significance tests (*P* value)	Variation (%)
Among clusters	1	19642.64	25.97	0.002 ± 0.001	9.17
Among localities within clusters	9	7699.28	5.25	0.000 ± 0.000	1.85
Within localities	1407	354446.55	251.92	0.000 ± 0.000	88.98
Total	1417	381788.46	283.13		

(C)

Source of variation (F_{ST})	Degrees of freedom	Sum of squares	Variance components	Significance tests (*P* value)	Variation (%)
Among clusters	1	46.85	0.06	0.025 ± 0.002	2.85
Among localities within clusters	9	62.12	0.04	0.000 ± 0.000	2.15
Within localities	1407	2644.94	1.88	0.000 ± 0.000	95.00
Total	1417	2753.92	1.98		

(D)

Source of variation (R_{ST})	Degrees of freedom	Sum of squares	Variance components	Significance tests (*P* value)	Variation (%)
Among clusters	1	15640.06	20.72	0.011 ± 0.001	7.36
Among localities within clusters	9	11701.85	8.88	0.000 ± 0.000	3.15
Within localities	1407	354446.5	251.92	0.000 ± 0.000	89.49
Total	1417	381788.46	281.51		

immigration patterns between localities within clusters are asymmetrical.

Two methods of analysis provide support for population differentiation of *A. palmata* in the Caribbean. Two distinct population clusters were identified as the most plausible scenario by the Bayesian modelling approach of STRUCTURE (Pritchard *et al*. 2000) without providing prior geographical information (Fig. 3a). Based on the high mean membership *q* in each cluster, nine of the 11 sampling localities were unambiguously identified as belonging to one of the two clusters. STRUCTURE identified only 18 individuals (or 2.5%) as not originating from the cluster where they were sampled. *F*- and *R*-statistics (Table 3) support this grouping as well. Based both on F_{ST} (0.040) and R_{ST} (0.221), differentiation between the two clusters was highly significant and larger than between the 11 original sampling localities. In agreement with the STRUCTURE results, higher values of differentiation are obtained when Puerto Rico is assigned to the western cluster rather than when Puerto Rico is assigned to the eastern cluster or when treated as a third population (Table 3). Differentiation estimates based on R_{ST} (Slatkin 1995) are expected to, and often do (Balloux & Goudet 2002), result in higher values than when the test is based on F_{ST} (Table 3).

Results of the Bayesian assignment model (Wilson & Rannala 2003) strongly support the split of *A. palmata* into

Table 5 Immigration rates of *Acropora palmata* (means ± 1 SD) among 11 sampling localities as estimated by BAYESASS

	PA (39)		ME (7)		FL (34)		BA (96)		NA (68)		MO (36)		PR (90)		VI (41)		SVG (166)		BO (32)		CU (100)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PA	**0.95**	**0.04**	0.05	0.04	0.02	0.03	0.02	0.02	0.01	0.01	0.02	0.02	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
ME	0.00	0.00	**0.71**	**0.04**	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
FL	0.00	0.00	0.02	0.03	**0.68**	**0.01**	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
BA	0.02	0.03	0.02	0.03	0.08	0.06	**0.80**	**0.08**	0.26	0.04	0.03	0.03	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
NA	0.00	0.01	0.02	0.03	0.01	0.01	0.06	0.03	**0.68**	**0.01**	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
MO	0.00	0.00	0.02	0.02	0.01	0.01	0.00	0.00	0.00	0.00	**0.68**	**0.01**	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
PR	0.01	0.01	0.05	0.05	0.18	0.06	0.09	0.08	0.03	0.03	0.18	0.05	**0.99**	**0.01**	0.01	0.01	0.00	0.00	0.00	0.01	0.02	0.02
VI	0.00	0.00	0.01	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	**0.68**	**0.01**	0.00	0.00	0.00	0.00	0.00	0.00
SVG	0.01	0.01	0.04	0.04	0.01	0.01	0.00	0.01	0.01	0.01	0.06	0.03	0.00	0.00	0.28	0.03	**0.99**	**0.00**	0.18	0.14	0.22	0.08
BO	0.00	0.01	0.02	0.03	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.00	**0.79**	**0.15**	0.06	0.07
CU	0.00	0.01	0.03	0.04	0.01	0.01	0.02	0.02	0.00	0.01	0.01	0.02	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.01	**0.68**	**0.01**

Source localities are given in rows, recipient localities in columns. Values along the diagonal are self-recruitment rates for each locality (bold). Likely immigrant sources are underlined. PA, Panama; ME, Mexico; FL, Florida; BA, Bahamas; NA, Navassa; MO, Mona Island; PR, Puerto Rico; VI, US Virgin Islands; SVG, St Vincent and the Grenadines; BO, Bonaire; CU, Curacao. Sample sizes in parentheses. Note that sample size for ME is small ($n = 7$).

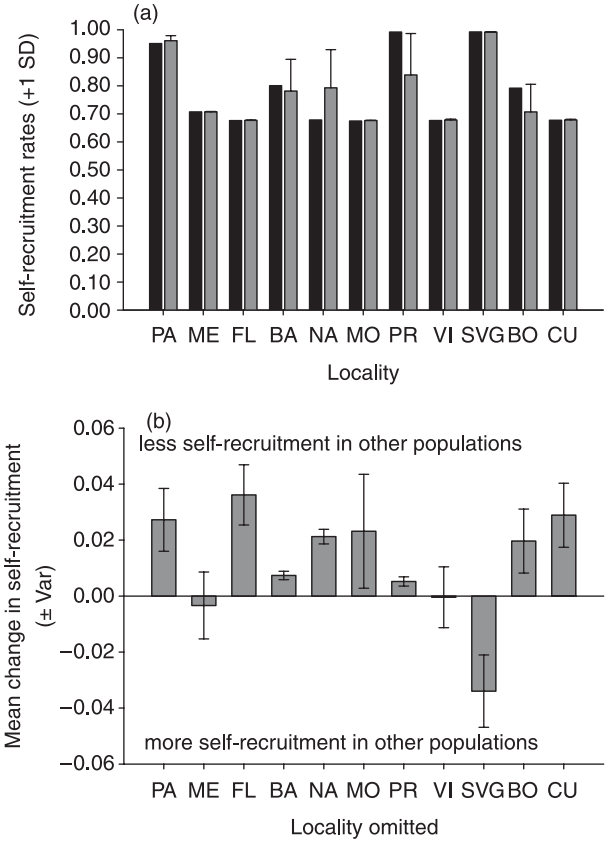

Fig. 4 Jackknife analysis of self-recruitment rates. (a) Comparison of *Acropora palmata* self-recruitment rates from all 11 sampling localities (black bars) and the mean rates (± 1 SD, grey bars) of self-recruitment as estimated by jackknifing (omitting one locality at a time). (b) The influence of each locality on self-recruitment rates of others. Shown are the difference between the self-recruitment rates of the remaining localities when omitting one locality (jackknifing, mean ± variance) and the rates obtained when considering all 11 localities. Positive values indicate less self-recruitment into that locality as estimated by jackknifing compared to values obtained when considering all 11 localities.

a western and an eastern cluster, with Puerto Rico assigned to the western cluster. Over 98% of genets originated from within their respective clusters.

Recent work on terrestrial organisms demonstrated that BAYESASS can accurately estimate migration rates between populations when source populations are well known and are exhaustively sampled (Berry *et al*. 2004). In contrast, studies of broadcast-spawning marine invertebrate species like *Acropora palmata* cannot hope to sample populations exhaustively. The results of our assignment tests were sensitive to missing localities (and/or reduction in sample size), as indicated by lower rates of self-recruitment estimates when one sampling locality at a time was omitted from the analysis (Fig. 4a, b). Such jackknifing procedures are reported to result in conservative estimates

of assignment accuracies in situations when individuals (not populations) are sequentially omitted from the analysis (Guinand *et al*. 2004). The largest change occurs when SVG are excluded from the analysis. Self-recruitment estimates at other localities increased by an average of *ca*. 3% when SVG is omitted, suggesting an important role for this island chain as a larval source and so connecting *A. palmata* stands in the eastern Caribbean.

Gene flow patterns in highly clonal acroporids

Geographic variation has been described in a number of hard corals in temperate (Hellberg 1996; LeGoff *et al*. 2004), and tropical studies (Ayre & Dufty 1994; Benzie *et al*. 1995; Ayre *et al*. 1997; Adjeroud & Tsuchiya 1999; Ayre & Hughes 2000; Takabayashi *et al*. 2003; Mackenzie *et al*. 2004) using nuclear markers. Comparison of our results to other coral studies is complicated by two factors. First, geographical structure estimates from allozymes using F_{ST} statistics are likely to yield higher values than if the same pattern would have been ascertained with microsatellites. This is attributable to the higher mutation rates of microsatellites that result in a high degree of polymorphism, thereby deflating F_{ST} values (Neigel 1997; Balloux & Lugon-Moulin 2002). Second, while studies of clonal corals routinely measure the contribution of asexual reproduction to population structure (e.g. using the statistic N*, Johnson & Threlfall 1987; Uthicke *et al*. 1998), clone mates are rarely removed from the data set before F_{ST} values are calculated. This would likely lead to upward bias in population differentiation measures. Here, the great polymorphism of the markers used (Baums *et al*. in press) led to high confidence in the identification of clone mates and thus each unique genotype was included only once. Hence, only qualitative comparisons to other studies of tropical reef corals with a broadcast spawning mode are warranted.

Ayre & Hughes (2000) used allozymes and found significant subdivision in two broadcasting acroporids sampled from populations spanning 500–1200 km along the Great Barrier Reef. Mackenzie *et al*. (2004) found similar differentiation in *Acropora nasuta* over a similar spatial scale using a combination of two intron sequences and one microsatellite locus. These three acroporids showing subdivision had little clonal structure. However, two other broadcast spawners (*Acropora millepora* and *Acropora valida*) surveyed by Ayre & Hughes (2000) were moderately to highly clonal, as is *A. palmata* (Baums *et al*. in preparation). These two clonal species showed F_{ST} values that did not differ from zero over the 500–1200-km range, even with the inclusion of clone mates in the data set. In the present study, sampling localities were separated by a maximum distance of about 1800 km within the western cluster (Bahamas–Panama) and about 840 km within the eastern cluster (Bonaire/Curacao–SVG, estimated midpoints of each locality, Fig. 2). Comparison of spatial scale is not straightforward, however, as the Great Barrier Reef is essentially linear in contrast to the oval-shaped Caribbean basin. The linear aspect of the Great Barrier Reef may also peripherally isolate coral populations, leading to their genetic differentiation (Ayre & Hughes 2004). Our findings for *A. palmata* are unique among works on corals in finding a genetic discontinuity in the centre of distribution of a broad-ranging species.

Gene flow in the Caribbean

Marine biogeographers have viewed the Caribbean as one province, with too few geographical barriers to allow for further subdivision (Veron 1995). Studies of Caribbean metazoans have until recently found little genetic differentiation, even over evolutionary timescales. Of eight species of reef fish surveyed by Shulman & Bermingham (1995), using mtDNA RFLPs, only three showed slight evidence of population structure, but this structure was not related to geography or larval characteristics. Invertebrates like the queen conch (*Strombus gigas*) (Mitton *et al*. 1989; Campton *et al*. 1992) and the spiny lobster (*Panulirus argus*) are similarly highly connected throughout their range (Silberman *et al*. 1994).

However, reports of population differentiation within the Caribbean have been accumulating. Gutierrez-Rodriguez & Lasker (2004) provided evidence for three subdivided populations of a brooding gorgonian across the Bahamas. Additionally, range endpoints of species distributions suggest a possible break at the Mona Channel (Starck & Colin 1978), where swift currents pass between Puerto Rico and Hispaniola. Here, a division between two colour forms of the goby *Elacatinus evelynae* (Colin 1975; Taylor & Hellberg 2003) was found. Phylogeographical breaks are also evident in other members of this genus (Taylor & Hellberg, submitted). These observations on other organisms are in general agreement with where we see a genetic break in *A. palmata*, but the picture in this coral is more complicated than in the *Elacatinus* gobies. *A. palmata* genets from Puerto Rico showed closer affinity to western populations than to eastern ones, indicating that the Mona Channel does not pose an impassable barrier to *A. palmata* larvae.

The break extends southward to somewhere between the sampling localities in Panama and the Netherlands Antilles. Unsuitable habitat of the Venezuelan coast seems to cause disruptions in species' distribution for other organisms. For example, Punta Guajira in the southern Caribbean (Fig. 2) divides coral-reef-dwelling species from those tolerant of productive upwelling waters (Colin 1975).

We feel that the most likely explanation for the genetic differentiation described here is limited dispersal of larvae. Evidence for localized recruitment of marine larvae, obtained with diverse methods, has been accumulating (Swearer *et al*. 2002). Both theoretical models (Cowen *et al*. 2000, 2003) and empirical studies (Swearer *et al*. 1999),

demonstrate larval retention caused by physical and behavioural mechanisms. Preliminary attempts to verify the plausibility of an oceanographic barrier in the vicinity of Mona Passage using a larval migration model based on both life history characteristics and physical forcing indicate that larvae released from localities in the eastern Caribbean also recruit within the eastern Caribbean (C. Paris *et al*., unpublished). Clearly, detailed current patterns in this region deserve further study. Other coral reef species with a variety of life history characteristics should also be investigated to ascertain the generality of these suggested phylogeographical breaks in the Caribbean.

Geographically restricted introgression from a species with which *A. palmata* hybridizes is unlikely to be responsible for the observed pattern of eastern vs. western differentiation. The genus *Acropora* contains two extant Caribbean species, *A. palmata* and *Acropora cervicornis*, and one hybrid form, *Acropora prolifera* (Van Oppen *et al*. 2000; Vollmer & Palumbi 2002). Hybridization within the genus presumably occurs during mass spawning events when hermaphroditic colonies release eggs and sperm into the water column. While introgressed genes from *A. palmata* appear in *A. cervicornis* (Vollmer & Palumbi 2002), the reverse flow of genes from *A. cervicornis* into the *A. palmata* genome is not observed. Thus, introgression does not adequately explain the geographical patterns found in *A. palmata*.

Conservation and reserve design

Marine reserves are used for conservation and management of coral reef organisms. Optimal design strategies, that is their placement, number, and size, are the subject of much debate (Palumbi 2003). The existence of two populations of *A. palmata* in the Caribbean, possibly separated by a semipermeable phylogeographical barrier, suggested that marine reserves designed to provide a source of larvae for other imperiled reefs may be effective only within the western or eastern subregion. For example, preserving *A. palmata* stands in the US Virgin Islands is not likely to help reseed devastated Jamaican reefs. The subdivision we have revealed thus implies a need for a greater number of marine reserves to protect this critical Caribbean reef builder (Cowen *et al*. 2000).

Summary

Bayesian analysis of population structure, along with *F*-statistics and genotype assignment tests, suggested that *A. palmata* in the Caribbean formed two distinct populations with limited gene flow between them. These two populations met at Puerto Rico, where mixing was observed. Assignment tests suggested self-recruitment may be high in some localities, although St Vincent was identified as an important source for *A. palmata* larvae within the eastern cluster. Taken together, our results indicated that (i) phylogeographical barriers may exist for reef corals in the Caribbean, (ii) the region surrounding Puerto Rico may be an especially productive location for studying the mechanisms and processes that maintain genetic differentiation between marine populations, and (iii) larval exchange between *A. palmata* stands may be asymmetrical between localities.

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