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Local and regional genetic connectivity in a Caribbean coral reef fish

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Abstract Coupled bio-physical models of larval dispersal predict that the Costa Rica–Panama (CR–PAN) reefs should constitute a demographically isolated region in the western Caribbean. We tested the hypothesis that CR–PAN coral reef fish populations would be isolated from Mesoamerican Barrier Reef System (MBRS) populations. To test that, we assessed population genetic structure in bicolor damselfish (*Stegastes partitus*) from both regions. Adult fish were genotyped from five reefs in CR–PAN and from four reefs along the MBRS at 12 microsatellite loci. Between-region F_{ST} ($F_{ST} = 0.0030$, $P < 0.005$) and exact test ($x^2 = 74.34$, d$f = 18$, $P < 0.0001$) results indicated that there is weak but significant genetic differentiation between regions, suggesting some restriction in connectivity along the Central American coastline, as predicted by bio-oceanographic models. Additionally, there is among-site genetic structure in the CR–PAN region, relative to the MBRS and between regions, suggesting higher self-recruitment within CR–PAN. This finding may be explained by differences in habitat characteristics.

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Introduction

Most coral reef fishes have a pelagic larval stage with potential to disperse long distances (Leis 1991). Because adult reef fishes are relatively sedentary (Sale 1980), the pelagic stage is an important link for population connectivity between patchily distributed habitat. Connectivity plays key roles in local adaptation and speciation, population replenishment and the likelihood of local extinction. Connectivity is also important for coral reef management; for example, it provides a mechanism for no-take reserves to enhance fish production outside their borders (Botsford et al. 2001; Hilborn et al. 2004; Kritzer and Sale 2004). Caribbean reef ecosystems are experiencing serious decline (Gardner et al. 2003) and need urgent management action. The Mesoamerican Barrier Reef System (MBRS) and the reefs fringing the eastern coast of Central America constitute critically important regions of biodiversity concentration. A quantitative understanding of patterns of connectivity in that region is of particular relevance for management and conservation.

Based on coupled bio-physical modeling of oceanographic data, habitat availability and larval behavior of coral reef fish in the Caribbean, four distinct regions of population isolation have been identified: the eastern Caribbean, the western Caribbean, the Bahamas–Turks and Caicos Islands, and the region at the periphery of the Panama–Colombia gyre (Cowen et al. 2006). Based on this, the Panama–Colombia reefs were predicted to be isolated from the remainder of the Caribbean, due to limited larval exchange (Cowen et al. 2006). Additionally, this region is predicted to have higher self-recruitment than the others, due to low importation from upstream locations (Cowen et al. 2006). Genetic work on corals has validated some of these regional connectivity predictions, for example the

observed breaks in gene flow for *Montastrea annularis* throughout the Caribbean, match the model predictions (Foster et al. 2008). Furthermore, an oceanographic–genetic model designed for low-dispersal species also predicted a cluster of genetically similar populations within the Panama coastal region and was validated with *Acropora palmata* data (Galindo et al. 2006). Therefore, the Panama–Colombia region is a good candidate for elevated self-recruitment and partial isolation (Cowen et al. 2006; Galindo et al. 2006) and thus genetic differentiation. However, limited genetic data exist to test this possibility for species with high dispersal potential.

Genetic markers can be used to indirectly estimate levels of self-recruitment since genetic differentiation is highly sensitive to migration (Hellberg et al. 2002). For example, many reef-associated species in the Indo-Pacific show significant levels of genetic differentiation at small and large scales (e.g., Planes et al. 1998; Bernardi et al. 2001; Planes and Fauvelot 2002; Magalon et al. 2005; Gerlach et al. 2007), as do some species from the Caribbean, including gobids, wrasses and damselfishes (Shulman and Bermingham 1995; Taylor and Hellberg 2003). Although there is a tendency for species with short pelagic larval duration (PLD) to show higher levels of population differentiation (e.g., Doherty et al. 1995; Riginos and Victor 2001), there is not always a clear relationship between PLD and dispersal (Shulman and Bermingham 1995). Indeed, in some cases, the relationship only gains significance when comparing species with and without a larval dispersal stage (Bay et al. 2006); and some species with high dispersal abilities can still show limited dispersal (Barber et al. 2000).

Studies explicitly testing the Panama–Colombia gyre as a genetically isolating factor for high-dispersive species are scarce. Large-scale studies across the Caribbean suggest an overall lack of genetic differentiation for the bluehead wrasse, *Thalassoma bifasciatum* (Purcell et al. 2006; Haney et al. 2007). Similarly, the stoplight parrotfish, *Sparisoma viride*, shows high overall gene flow and weak genetic differentiation along the SE Caribbean (Geertjes et al. 2004). However, neither of those studies sampled the Panama–Colombia region, perhaps missing an important area of isolation. A microsatellite marker study in the hamlets (*Hypoplectrus nigricans* and *H. puella*) sampled from Panama, Bahamas and the MBRS demonstrated that both species manifest highly significant genetic differences among sites (Puebla et al. 2008). In those cases, the pattern of divergence seemed to reflect an "ecological speciation" similar to that of the wrasse *Halichoeres bivittatus* (Rocha et al. 2005). A study using microsatellite markers in *Stegastes partitus* sampled within the Panama–Colombia gyre found high genetic connectivity at sites separated by up to 800 km (Ospina-Guerrero et al. 2008), while Hepburn et al. (2009) found a similar pattern of limited genetic divergence in *S. partitus* among sites across the MBRS. However, no study has directly tested the possibility that the Panama–Colombia coastal region may constitute a genetically isolated region from other sites in the Caribbean.

Cowen et al.'s (2006) model predicts a meridional biogeographical break at the northern edge of the Nicaraguan rise. Therefore, we can hypothesize that any larva originating in the Panama–Colombia gyre that escapes the gyre and move away, would likely go toward Nicaragua, San Andres Island and/or Jamaica, but would not go toward the Mesoamerican reef. A critical test of the predicted isolation between the MBRS and the Panama–Colombia region is clearly needed.

Here we use microsatellite marker data for the bicolor damselfish (*S. partitus)* to assess genetic divergence within and between two regions: (a) the Mesoamerican barrier reef system (MBRS) and (b) reefs in Costa Rica–Panama (CR–PAN), located within the area of the Panama–Colombia gyre. This study has two main objectives: (1) to test for large-scale genetic isolation of the CR–PAN region from the MBRS, as predicted by bio-physical oceanographic models (e.g., Cowen et al. 2006) and (2) to test for small spatial scale genetic structure within the CR–PAN and MBRS regions and relate such structure to potential local barriers to gene flow.

Materials and methods

Study sites

Two Caribbean regions, the CR–PAN, extending ~120 km along the southeastern Central American coast, and a 220 km stretch of the MBRS, comprise the study area (Fig. 1). The CR–PAN region falls within the oceanographic regime of the Panama–Colombian gyre, and we use the CR–PAN region as a proxy representative of the gyre region defined by Cowen et al. (2006). The two regions are separated by ~1,100 km, with intervening reefs in Honduras, Guatemala, Nicaragua and Colombian islands (San Andres, Providencia, etc.). There is a ~120 km reef gap separating the two regions, made up of high-energy sandy beaches in northern Costa Rica (Cortés and Jiménez 2003). Populations from each region are affected by two separate oceanographic gyres along the Central American coast that may contribute to genetic isolation.

CR–PAN

The Costa Rica fringing reefs are small and patchy (Fig. 1) and continue their development in Panama (Cortés and Jiménez 2003). The main near-shore current flows from northwest to southeast, creating small eddies in the opposite

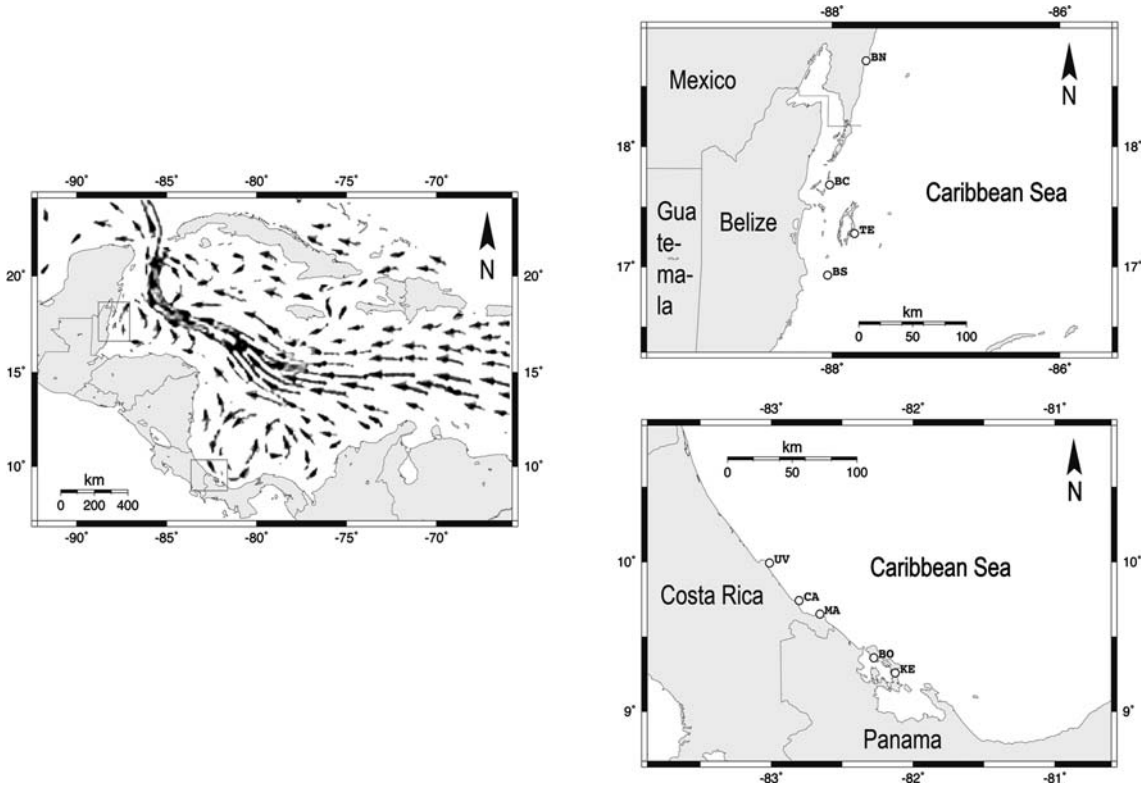

Fig. 1 Maps showing bicolor damselfish sampling locations in the Mesoamerican Barrier Reef System (MBRS) and Costa Rica–Panama (CR–PAN) regions. *Left*: Caribbean Sea and main current patterns (Roberts 1997), MBRS is shown in the top frame and CR–PAN in the low frame. *Lower right*: MBRS sites (*BN* Barrier North; *BC* Central Barrier; *BS* Barrier South). *Upper right*: CR–PAN sites (*UV* Uvita Island; *CA* Cahuita; *MA* Manzanillo; *BO* Bocas; *KE* Coral key). BO and KE are within the Bocas del Toro province in Panama. Maps made using online map creation http://www.aquarius.geomar.de/

direction (Cortés and Jiménez 2003). Local rivers, such as La Estrella, Sixaola and Changuinola, develop freshwater plumes and sediment input that may create unsuitable conditions for migrants. Fish diversity of this region is low relative to other Caribbean areas, possibly due to the poor reef development and low structural complexity (Phillips and Pérez-Cruet 1984; Fonseca et al. 2006). The effect of these habitat conditions on reef fish connectivity is unknown, but it may reduce opportunities for emigration out of the environment, increasing self-recruitment and also may select against incoming migrants. The two Panama sites (BO, KE, see Fig. 1) are fringing and patch reefs within the Archipelago of Bocas del Toro and are located within a semi-enclosed lagoon. Waves and currents have a strong effect outside of the archipelago, but the major islands act as barriers, decreasing wave action and moderating tides (Collins et al. 1996). Therefore, population isolation inside the lagoon may be expected. The three sample sites located in Costa Rica are located near the coast and in reef patches.

MBRS

The barrier reef of the MBRS is located approximately 20–40 km from the mainland, separated by a shallow lagoon. The barrier reef extends approximately 220 km with well-developed reefs (Fig. 1). Since barrier and atoll reefs are separated from the mainland, they may be less directly exposed to the effects of rivers and runoff than in CR–PAN. The MBRS region is strongly affected by severe weather events, and it is located in the Caribbean hurricane belt (Wells 1988). Water circulation has a predominant northwestward flow, influenced by the Caribbean Current (Sheng and Tang 2004; Tang et al. 2006). However, MBRS displays highly variable flow patterns driven by propagation of Caribbean eddies, creating strong southward or westward flow depending on eddy direction (Ezer et al. 2005). Three of the four MBRS sampling sites are within the barrier reef, and the remaining one is on the east coast of Turneffe atoll (Fig. 1).

Study species

We chose the bicolor damselfish, *Stegastes partitus*, as our study species because they are territorial and show strong site fidelity as adults (Nemeth 2005), but also have a larval phase that can disperse in the ocean. They are a very abundant species in the Caribbean basin, so it is easy to collect representative samples from many locations. Adults are

territorial, demersal spawners and reproduce year-round on a lunar cycle, with seasonal reproductive peaks from April to November (Robertson et al. 1988). The male defends nests (Knapp and Warner 1991), and eggs hatch after 2–5 days (Robertson et al. 1988) to produce pelagic larvae with a pelagic larval duration of about a month (Wellington and Robertson 2001). Although *S. partitus* is not of particular economic or conservation interest, it is abundant and shares life history traits with other coastal marine organisms.

Sampling design

We sampled adult damselfishes from five CR–PAN sites and four MBRS sites (Fig. 1). Between May and November 2006, a total of 595 adult *Stegastes partitus* were captured from the coral reefs in Central America, 285 samples from CR–PAN and 310 from MBRS. Sample sizes ranged from 42 to 96 fishes per site (Table 1). Fish were fin clipped, and the tissue was stored in a salt preservation solution (0.020 M EDTA, 0.025 M sodium citrate trisodium salt dehydrate, 5.3 M ammonium sulfate: "RNAlater"), for DNA analysis. Individuals over 40-mm fork length were considered adults, since they showed developed gonads above 38 mm (Thiessen 2007). To test for genetic stability across cohorts at one site in CR–PAN region [Manzanillo (MA)], we sampled 121 recently settled juvenile bicolor damselfish (Fig. 1), collected at the same time as the adults in MA.

DNA extraction and microsatellite analysis

Genomic DNA was extracted from fin clips following the silica-based 96-well plate extraction protocol (Elphinstone et al. 2003). DNA quality was verified using electrophoresis with 1.8% agarose gels. Each fish was genotyped at a total of twelve microsatellite loci (developed by Williams et al. 2003; Thiessen and Heath 2007; Table 1). PCR amplification was performed in 12-µl reactions consisting of approximately 100 ng template DNA, 1× PCR buffer (500 mM potassium chloride and 100 mM Tris–HCl, pH 8.3 at room temperature), locus-specific concentrations of $MgCl_2$ (Table 1), 200 µM of each dNTP, 32 µM of dye-labeled forward primer, 0.5 µM of reverse primer and 0.1 U Taq polymerase (Applied Biosystems, Foster City, USA). PCR conditions were as follows: initial denature at 94°C for 2 min, followed by 30–35 cycles of denaturing at 94°C for 15 s, locus-specific annealing temperature (Table 1) for 15 s, extension at 72°C for 30 s and a final extension of 72°C for 90 s. The microsatellite allele sizes were determined using a LiCor 4300 DNA analyzer and scored using GeneImagIR 4.05 (Scanalytics, Inc).

Genetic analysis

Allelic richness was calculated using FSTAT v2.9.3.2 software (Goudet 1995). Exact tests for Hardy–Weinberg equilibrium using the Markov chain method (1,000 permutation burn-in followed by 100,000 permutations; Raymond and Rousset 1995) for each locus within each population were performed in ARLEQUIN v3.11 (Excoffier et al. 2005). Linkage disequilibrium between all pairs of loci was examined with FSTAT, and tests for pairs of linked loci per population were performed with ARLEQUIN. Microchecker v2.2.3 was used to detect the presence of null alleles and large allele dropout (Van Oosterhout et al. 2004).

Genetic structure

Global and pairwise exact tests for differences in allele frequency distributions among populations were performed with TOOLS FOR POPULATION GENETIC ANALYSIS (TFPGA) v1.3 (Miller 1997), with 100 batches and 20,000 permutations per batch. Global and pairwise F_{ST} values were estimated to quantify the extent of differentiation between populations, using MSA v4.05 (Dieringer and Schlötterer 2003). Within- and between-region (MBRS and CR–PAN) global F_{ST} values were also calculated. An analysis of molecular variance (AMOVA) between regions and among-populations within regions was implemented in ARLEQUIN.

Measures of genetic distance ($F_{ST}/(1-F_{ST})$) were assessed for correlation with shortest water distance (km) to detect patterns of isolation-by-distance in adult populations, applying Mantel tests in GENALEX software (Peakall and Smouse 2006). Isolation-by-distance patterns were examined across all data and also within each region. Sequential Bonferroni corrections were applied to all multiple tests (Rice 1989).

Genetic clustering was assessed using STRUCTURE v2.2.3 (Pritchard et al. 2000), with one million Markov chain Monte Carlo (MCMC) repetitions and 500,000 burn-in runs. Since *S. partitus* larvae can remain in the plankton for extended periods of time, the admixture ancestry model was used with correlated allele frequencies to improve clustering in populations with high gene flow.

To evaluate the stability of the genetic structure across cohorts, we used the sample of recently settled juveniles from MA in comparisons with the adult population samples. First, we tested for genetic divergence between the adult and juvenile fish from MA. We then plotted pairwise D_C values between the juvenile MA sample and all other sampled sites against pairwise D_C values between the adult MA sample and all other sampled sites. If the pairwise genetic structure was similar for the adult and juvenile MA fish, such a plot should fit the 1:1 line. We tested this using a Pearson correlation. D_C values were calculated in POPULATIONS v1.2.28 software (Langella 2002).

Table 1 Number of individuals (*N*), allelic richness (*A*), observed heterozygosity (H_O) and expected heterozygosity (H_E) for adult populations of *Stegastes partitus* collected from Costa Rica, Panama and the Mesoamerican Barrier Reef System

Site		*Sp*GATA$_{40}$	*Sp*AAT$_{40}$	*Sp*AAC$_{44}$	*Sp*AAC$_{33}$	*Sp*AAC$_{41}$	*Sp*TG$_{10}$	*Sp*TG$_{16}$	*Sp*GG$_{47}$	*Sp*TG$_{8}$	*Sp*TG$_{53}$	*Sp*TG$_{13}$	*Sp*GT$_{10}$
T_A		56	49	48	60	56	55	52	48	48	48	49	56
BN (n = 88)	n	83	85	81	88	83	87	75	84	77	88	77	87
	H_O	**0.82**	0.82	0.37	0.78	**0.35**	**0.70**	**0.88**	0.50	**0.64**	0.85	0.68	0.84
	H_E	0.96	0.90	0.41	0.87	0.93	0.97	0.96	0.56	0.94	0.93	0.78	0.86
	A	26.29	14.17	8.86	12.11	18.42	37.03	25.52	4.28	20.14	25.12	7.33	13.25
BC (n = 65)	n	58	64	65	61	63	62	59	61	62	61	61	61
	H_O	**0.78**	0.91	0.32	0.77	**0.52**	**0.37**	0.80	0.64	**0.71**	0.84	0.62	0.80
	H_E	0.96	0.89	0.36	0.89	0.94	0.96	0.95	0.60	0.94	0.95	0.77	0.90
	A	25.78	15.00	8.06	13.20	20.80	30.33	23.47	4.84	21.29	25.91	6.87	12.16
BS (n = 78)	n	67	67	78	78	73	69	73	73	74	78	78	69
	H_O	0.82	0.90	0.28	0.81	**0.55**	0.64	0.89	0.63	**0.65**	0.87	0.77	0.81
	H_E	0.96	0.91	0.29	0.89	0.94	0.97	0.95	0.60	0.95	0.94	0.72	0.89
	A	25.42	16.49	7.63	12.53	22.14	37.49	23.22	5.80	21.80	25.25	7.55	14.20
TE (n = 79)	n	73	71	79	79	65	77	75	67	74	79	79	74
	H_O	0.78	0.87	0.35	0.86	**0.34**	**0.73**	0.89	0.45	0.69	0.92	0.62	0.86
	H_E	0.95	0.90	0.47	0.89	0.94	0.97	0.96	0.61	0.94	0.94	0.71	0.90
	A	23.38	13.88	9.84	13.64	21.71	37.15	26.67	5.38	22.45	27.53	6.43	12.61
UV (n = 47)	n	47	40	47	47	47	46	46	47	47	47	47	46
	H_O	**0.89**	0.88	0.40	0.77	0.89	**0.61**	0.89	0.68	0.66	0.81	0.60	0.78
	H_E	0.96	0.91	0.46	0.89	0.96	0.97	0.93	0.67	0.93	0.93	0.74	0.90
	A	27.89	13.90	10.16	14.35	27.75	33.91	19.55	4.97	20.43	26.59	6.77	15.10
CA (n = 48)	n	48	38	48	47	48	47	48	45	48	48	48	48
	H_O	0.88	0.87	0.23	0.77	0.88	0.60	0.88	0.49	0.69	0.83	0.60	0.77
	H_E	0.95	0.88	0.29	0.87	0.96	0.97	0.95	0.56	0.92	0.90	0.77	0.90
	A	21.67	13.00	9.13	12.39	26.45	35.95	24.51	5.53	18.31	24.26	7.73	14.32
MA (n = 96)	n	96	92	96	96	93	93	96	95	93	93	96	86
	H_O	0.95	0.84	0.59	0.77	0.95	0.68	0.97	0.57	**0.73**	0.95	0.59	0.87
	H_E	0.96	0.91	0.60	0.87	0.96	0.98	0.96	0.65	0.94	0.94	0.65	0.90
	A	27.37	15.01	8.99	12.22	26.11	37.83	26.67	5.07	20.91	26.15	6.03	15.04
BO (n = 52)	n	50	52	52	51	52	51	52	52	51	52	52	52
	Ho	0.90	0.88	0.42	0.82	**0.79**	**0.57**	**0.77**	0.47	**0.75**	0.96	0.56	0.85
	He	0.96	0.90	0.41	0.86	0.96	0.97	0.95	0.56	0.95	0.94	0.67	0.89
	A	26.16	14.53	10.12	13.47	24.64	31.97	24.05	4.73	25.35	26.68	6.59	12.10
KE (n = 42)	n	42	42	42	42	41	42	42	42	41	42	42	41
	Ho	0.88	0.86	0.24	0.81	0.88	**0.55**	0.83	0.45	0.78	0.95	0.71	0.78
	He	0.96	0.91	0.27	0.88	0.95	0.97	0.96	0.68	0.95	0.94	0.71	0.92
	A	25.21	14.71	8.43	12.61	19.90	32.78	27.01	5.90	26.71	26.71	8.71	14.77

Significant departures from Hardy–Weinberg equilibrium after Bonferroni corrections are bolded. Locus names same as in genebank, with the initials Sp before the names in the table. [$MgCl_2$] is 1.1 mM for all loci except SpTG10, SpTG16 and SpTG53, with 0.66 mM. Annealing temperature is indicated as T_A. Initial names are indicated in Fig. 1

Results

Genetic analysis

Deviations from Hardy–Weinberg Equilibrium (HWE) were found at 5 of the 12 loci following sequential Bonferroni corrections (Table 1). Locus *Sp*TG$_{10}$ displayed deviations in 88% of the populations, while loci *Sp*AAC$_{41}$ and *Sp*TG$_{8}$ both presented deviations in 66% of the populations. *Sp*GATA$_{40}$ and *Sp*TG$_{16}$ also showed deviations from HWE, but only in 33% of the populations. In all cases, departures from HWE were due to homozygote excess, but

they were not attributable to genotyping errors (based on Microchecker results). However, Microchecker identified null alleles in all populations for loci $Sp\text{TG}_{10}$ and $Sp\text{TG}_{8}$. Null alleles were present in only a few populations for all other loci, but they could not be distinguished from Wahlund effects. Linkage disequilibrium analyses concluded no significant global linkage between pairs of loci, after sequential Bonferroni correction. Approximately, 2.3% (14/594) of exact tests showed significant linkage disequilibrium within each population, after sequential Bonferroni correction. Due to HWE deviations, loci $Sp\text{TG}_{10}$, $Sp\text{AAC}_{41}$ and $Sp\text{TG}_{8}$, were removed from all genetic structure analysis.

Genetic structure

Large-scale (between regions) analyses

There were highly significant differences in the allelic frequencies, as shown by global exact test ($x^2 = 85.98$, d$f = 18$, $P < 0.0001$). Six out of 20 (33%) between-region pairwise comparisons were significant (Table 2). The exact test between MBRS and CR–PAN regions was also significant ($x^2 = 74.34$, $df = 18$, $P < 0.0001$). Global F_{ST} across all populations from both regions was low, yet significant ($F_{ST} = 0.006$; $P < 0.005$). The F_{ST} between the MBRS and the CR–PAN regions was 0.0030 and significant ($P < 0.005$). However, AMOVA detected no significant between-region component ($F = 0.0004$, d$f = 1$, $P > 0.30$, 0.05% variance), there was a significant among-site, within-region effect ($F = 0.0046$, d$f = 7$, $P < 0.0001$, 0. 46% variance), as well as among-individuals, within-sites ($F = 0.0464$, d$f = 586$, $P < 0.0001$, 4.62% variance). The populations sampled did not follow a pattern of isolation-by-distance ($r^2 = 0.002$, $P = 0.380$). Analysis using STRUCTURE indicates the presence of only 1 cluster ($K = 1$); however, STRUCTURE is not a powerful tool for detecting weak genetic structure.

Within-MBRS analysis

The global F_{ST} within the MBRS was 0.0004 and non-significant ($P > 0.25$). However, the global exact test for allele frequency distribution variation among populations within the MBRS was significant ($P < 0.0001$). Pairwise tests of allele frequency differences resulted in 2 out of 6 (33.3%) being significant within the MBRS after sequential Bonferroni corrections (Table 2). All pairwise F_{ST} values between MBRS populations were low and non-significant (Table 2). The populations within MBRS did not follow an isolation-by-distance pattern, the relationship was significant but negative, contrary to the expected pattern ($r^2 = 0.591$, $P = 0.030$).

Within-CR–PAN analysis

Within CR–PAN, the global F_{ST} was 0.010 and statistically significant ($P < 0.0001$), and the global exact test of allele frequency distribution variation was significant ($P < 0.0001$). Five out of ten (50%) of the pairwise exact test comparisons were significantly different within the CR–PAN (Table 2). Four out of ten pairwise F_{ST} values among CR–PAN populations were significant after sequential Bonferroni corrections (Table 2). The populations within CR–PAN do not follow an isolation-by-distance pattern ($r^2 = 0.113$, $P = 0.080$).

MA juvenile and adult comparison

The juvenile and adult samples at MA were genetically differentiated, with a pairwise $F_{ST} = 0.010$ ($P < 0.005$) and a highly significant pairwise exact test of allele frequency distribution ($P < 0.005$). We further found that the genetic relationship between the juvenile and adult MA samples with the other samples sites differed substantially (Fig. 2) where there was a weak correlation between the adult and juvenile genetic distances ($r^2 = 0.6964$).

Table 2 F_{ST} values (below diagonal) and D_C values with significance of exact tests (above diagonal) for adult populations of *Stegastes partitus* in the MBRS and CR–PAN, within-region comparisons are italic and bold italic

Significant values after sequential Bonferroni correction indicated in bold

	MBRS				CR–PAN				
	BN	BC	BS	TE	UV	CA	MA	BO	KE
BN	–	*0.212*	*0.189*	*0.200*	0.217	0.222	**0.214**	0.214	0.223
BC	*0.001*	–	*0.222*	***0.224***	0.239	0.232	**0.247**	0.241	**0.257**
BS	*0.000*	*0.000*	–	***0.230***	0.246	0.237	**0.238**	0.236	0.252
TE	*0.000*	*0.001*	*0.001*	–	0.240	0.246	**0.243**	**0.252**	0.252
UV	0.002	0.005	0.005	0.003	–	*0.256*	***0.238***	***0.258***	*0.262*
CA	0.000	0.000	0.001	0.003	*0.005*	–	***0.253***	*0.255*	*0.253*
MA	**0.013**	**0.016**	**0.019**	**0.018**	*0.005*	***0.014***	–	***0.266***	***0.247***
BO	0.002	0.001	0.000	0.002	***0.010***	*0.004*	***0.026***	–	*0.263*
KE	0.002	0.003	0.003	0.003	*−0.001*	*0.003*	***0.009***	*0.006*	–

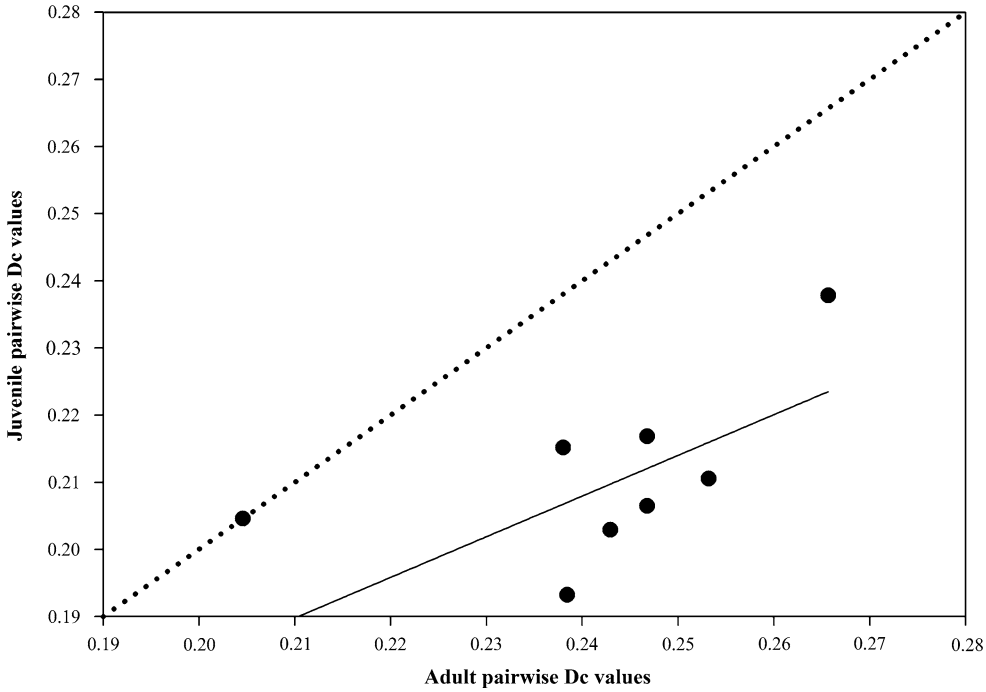

Fig. 2 Relationship of D_C values between Manzanillo juveniles and adults with all the other locations. *Dashed line* represents the 1:1 relationship

Discussion

We found evidence of weak but significant genetic differentiation between MBRS and CR–PAN regions. Our field data thus support bio-physical modeling predictions that CR–PAN populations would be isolated from the MBRS. Low F_{ST} values among regions are common in marine fishes (O'Reilly et al. 2004) and may reflect large effective population sizes (DeWoody and Avise 2000). It is likely that *S. partitus* does have very large effective population sizes in both the MBRS and the CR–PAN regions, and hence our low F_{ST} values may reflect limited genetic drift, rather than substantial gene flow between these regions. It is unlikely that the low level of regional genetic structure in our study was due to lack of statistical power, since our analyses detected local genetic structure. Our results are in concordance with other genetic studies of bicolor damselfish that report limited genetic structure in regions such as Puerto Rico and Jamaica (Lacson 1992), in Colombia (Ospina-Guerrero et al. 2008) and across the MBRS (Hepburn et al. 2009). The genetic structure observed at the regional scale did not follow an isolation-by-distance model of divergence, despite a relatively linear, but not continuous, distribution of suitable habitat along the coast. Other researchers have reported similar negative results in the Caribbean (e.g., Shulman and Bermingham 1995; Geertjes et al. 2004; Purcell et al. 2009; Hepburn et al. 2009), although Purcell et al. (2009) did report a significant IBD relationship for eastern Caribbean bicolor damselfishes, a pattern also seen in *Haemulon flavolineatum* in that same region (Purcell et al. 2006). Although bicolor damselfish life history traits and temporal stochasticity clearly play a role in the pattern of connectivity in the western Caribbean, our data indicate that marine current patterns, habitat availability and larval behavior of coral reef fish contribute to limit connectivity across the sampled regions.

We found higher among-site genetic structure in the CR–PAN region (relative to the MBRS and between regions), suggesting higher self-recruitment within the CR–PAN in comparison with the MBRS. The pattern of genetic structure within the CR–PAN region was not explained by geographic factors, nor did it conform to an isolation-by-distance model of divergence. In addition, notable geographic barriers by themselves do not seem to explain the patterns of genetic structure. For example, we would have expected fish populations from Bocas del Toro inner lagoon in Panama to be isolated from the other sites in the study area, due to the effects of restricted water circulation and the Sixaola River (on the Costa Rica–Panama border) runoff. However, those populations did not show substantial genetic isolation from the Costa Rica sites. Although rivers such as the Amazon and Orinoco have been shown to act as freshwater barriers to marine fish dispersal (Floeter et al. 2008), they create very large-scale freshwater plumes and substantial coastline modification (2,300 km; Rocha et al. 2005), and yet there is still some degree of connectivity across those rivers (Floeter et al. 2008). In contrast, the Sixaola River has only a ~35-km plume, thus it is perhaps not surprising that the river does not contribute to the genetic structure observed in the CR–PAN region.

The patterns we observed in genetic structure at the local scale may be explained by differences in habitat quality and

availability between the MBRS and CR–PAN sites. Habitat variability directly affects the reproductive biology and recruitment success of the bicolor damselfish. Baums et al. (2006) showed that larval competency affects recruitment success and thus connectivity more in fragmented reefs than in continuous ones because larvae have a broader settlement window if suitable habitat is abundant. This is supported by oceanographic modeling showing that for continuous reefs in the Mesoamerican region, connectivity patterns were not affected by variation in the duration of the pre-competent period (Paris et al. 2007). Since the CR–PAN reefs are patchily distributed and the reef system is less developed than that in MBRS, connectivity may be locally limited. However, the substantial genetic divergence between the adult and recently settled juveniles sampled at Manzanillo (MA), Costa Rica, highlights the fact that processes driving the genetic structure in CR–PAN are not only related to predictable habitat conditions. The observed genetic structure among the CR–PAN sites (as well as the limited structure identified at the MBRS sites) is likely affected by stochastic processes. Temporal genetic instability of bicolor damselfish populations has been reported in previous studies (Lacson and Morizot 1991; Thiessen 2007; Hepburn et al. 2009) and may be a fundamental property of such systems (Hepburn et al. 2009).

The current study showed limited connectivity among our two sampled regions (e.g., MBRS vs. CR–PAN), with substantially higher genetic divergence within one of our sampled region (CR–PAN). These results support published bio-physical model predictions and highlight the Panama–Colombia gyre as a possible isolating mechanism within the western Caribbean. However, additional research comparing this region with other locations in the Caribbean would allow a more general test of the bio-physical model predictions. Our data further suggest that habitat fragmentation may play a role in the widely reported local-scale genetic structure in coral reef fishes; although a lock of across-cohort stability in that structure indicates that additional factors must be contributing to the structure. Our results indicate that the coral reefs of the CR–PAN region may be of particular conservation concern due to its relative isolation, high levels of degradation and its limited extent.

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