

Microsatellite variation reveals high levels of genetic variability and population structure in the gorgonian coral *Pseudopterogorgia elisabethae* across the Bahamas

CARLA GUTIÉRREZ-RODRÍGUEZ and HOWARD R. LASKER

Department of Biological Sciences, University at Buffalo, State University of New York, Buffalo, NY 14260, USA

Abstract

The primary mechanism of gene flow in marine sessile invertebrates is larval dispersal. In *Pseudopterogorgia elisabethae*, a commercially important Caribbean gorgonian coral, a proportion of the larvae drop to the substratum within close proximity to the maternal colony, and most matings occur between individuals in close proximity to each other. Such limited dispersal of reproductive propagules suggests that gene flow is limited in this gorgonian. In this study, we characterized the population genetic structure of *P. elisabethae* across the Bahamas using six microsatellite loci. *P. elisabethae* was collected from 18 sites across the Bahamas. Significant deviations from Hardy–Weinberg equilibrium due to deficits of heterozygotes within populations were detected for all 18 populations in at least one of the six screened loci. Levels of genetic structure among populations of *P. elisabethae* were high and significant. A distance analysis placed populations within three groups, one formed by populations located within Exuma Sound, a semi-isolated basin, another consisting of populations located outside the basin and a third group comprising two populations from San Salvador Island. The patterns of genetic variation found in this study are concordant with the life-history traits of the species and in part with the geography of the Bahamas. Conservation and management plans developed for *P. elisabethae* should consider the high degree of genetic structure observed among populations of the species, as well as the high genetic diversity found in the San Salvador and the Exuma Sound populations.

Keywords: conservation, gene flow, gorgonian coral, microsatellites, population genetics, *Pseudopterogorgia elisabethae*

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Introduction

Gene flow is restricted to larval and gamete dispersal in marine sessile invertebrates (Hedgecock 1986), and is determined primarily by the amount of time these propagules spend in the water column, as well as by the speed and direction of water currents (Strathmann 1985; Graham & Sebens 1996). Thus, species with short-lived larvae often have lower levels of gene flow and higher degrees of genetic differentiation than species with long-lived larvae due to the short periods of time their larvae spend in the water column (e.g. Marcus 1977; Crisp 1978; Burton &

Feldman 1982; Burton 1983; Ayre & Dufty 1994; Hellberg 1994; Ayre *et al.* 1997a; Beiring 1997; McFadden 1997; Bastidas *et al.* 2001). Understanding patterns of genetic variation is becoming increasingly important for conservation, management and remediation efforts as marine communities in general, and coral reefs in particular, are under increasing adverse pressures from short and long-term anthropogenic effects, ranging from over-fishing to global warming.

Gorgonian corals are sessile marine invertebrates that constitute a large and diverse group of species of the benthic fauna of Caribbean coral reefs. The gorgonian coral *Pseudopterogorgia elisabethae* (Bayer 1961) is moderately common and widely distributed on coral reefs throughout the Caribbean (Bayer 1961; Kinzie 1970; Lasker & Coffroth 1983). *P. elisabethae* also has economic significance as it produces and is the sole source of pseudopterins, a class

Correspondence: Carla Gutiérrez-Rodríguez, Department of Biological Sciences, Ohio University, Athens, OH 45701, USA. E-mail: gutierre@ohio.edu

of natural products that has anti-inflammatory properties (Fenical 1987). Pseudopterosins extracted from *P. elisabethae* are used as a topical agent in cosmetic products (Mayer *et al.* 1998), and although this species has been harvested commercially in the Bahamas since 1994, knowledge about its population structure is lacking. Information regarding population structure, levels of gene flow and genetic diversity within and among populations of species harvested for commercial purposes are crucial for developing conservation and management plans.

The population structure of *P. elisabethae* is influenced, to a large extent, by its relatively unusual reproductive system. *P. elisabethae*, unlike most Caribbean gorgonians, is a surface brooder. Eggs are released by the polyps of female colonies and remain on the surface of the colony. Fertilization takes place either in or on the maternal colony and the development of the embryos occurs on the surface of the colony (Gutiérrez-Rodríguez & Lasker 2004a). After detaching from the maternal colony, most of the larvae remain in the water column and can potentially be dispersed to reefs other than the one of origin (Gutiérrez-Rodríguez & Lasker 2004a). However, 14% of the larvae sink to the substratum, and 83% of those 'land' within 5 m of the maternal colony (Gutiérrez-Rodríguez & Lasker 2004a). Additionally, paternity analyses of *P. elisabethae* larvae suggest that most matings are local, with the majority occurring between colonies located within 11 m of each other (Gutiérrez-Rodríguez 2003). The combination of somewhat limited larval and sperm dispersal in *P. elisabethae* may result in populations that are genetically differentiated. The purpose of this study was to characterize the genetic structure of *P. elisabethae* across a range of reefs in the

Bahamas with varying degrees of isolation suggested by their geographical distance and current regimes. Information about the population genetic structure of this gorgonian is particularly important, given that this species is being harvested in the Bahamas for economic purposes and levels of isolation may be important in determining the resilience of populations to harvesting.

Materials and methods

Sample collections

P. elisabethae collections from 18 sites in the Bahamas were made using SCUBA (Table 1, Fig. 1). Samples from Sweetings Cay near Grand Bahama Island were collected in 1995, and those from the other sites were collected between June 1999 and August 2000 (Table 1, Fig. 1). We collected samples from individuals of approximately the same size. However, in most colonial invertebrates, size is a poor indication of age (Hughes & Jackson 1980). Thus, populations consist of many overlapping generations and the sampled colonies undoubtedly represent many recruitment events. In order to investigate population structure at multiple geographical scales, we collected samples at sites located in different islands (separated by 12.54–443.65 km), as well as at sites situated in the same island (separated by 0.1–7.52 km).

Tissue samples were obtained from 38–58 *P. elisabethae* individuals along a 20-m transect at each of the locations by clipping ~10 cm of tissue from each individual found within 1 m of the transect line. *P. elisabethae* was not abundant at Rum Cay, Gorda Rock, East End Point of Eleuthera, Cat Island and Mores Island, and at those locations the

Island	Site	Abbreviation	Depth of site (m)	<i>n</i>
Near Grand Bahama	Sweetings Cay	SC	21.6	41
Mores Island	Mores Island	MI	11.0	50
Gorda Rock	Gorda Rock	GR	16.7	49
Near Abaco	Sandy Point Shallow	SPS	8.7	50
Near Abaco	Sandy Point Deep	SPD	16.7	48
Abaco	Cross Harbor	CH	12.8	50
Eleuthera	South Hampton Reef	SHR	16.0	50
Conception	Wedge Rock	CWR	20.0	50
Conception	The Notch	CTN	18.3	38
Conception	Out of Bounds	COB	21.0	40
San Salvador	Riding Rock	SS-RR	17.0	40
San Salvador	Pillar Reef	SS-PR	11.0	50
Rum Cay	RC	RC	21.6	48
Eleuthera	East End Point of Eleuthera	EEPE	12.7	49
Little San Salvador	Little San Salvador	LSS	10.3	58
Cat Island	Cat Island	CI	26.0	50
Long Island	Hog Cay-1	HC-1	12.3	49
Long Island	Hog Cay-2	HC-2	9.5	50

Table 1 Island, site, abbreviation, depth of collection site and number of samples collected per site (*n*). The populations are ordered geographically (North to South), with the populations located outside Exuma Sound listed first and then, starting with East End Point of Eleuthera, those located within Exuma Sound

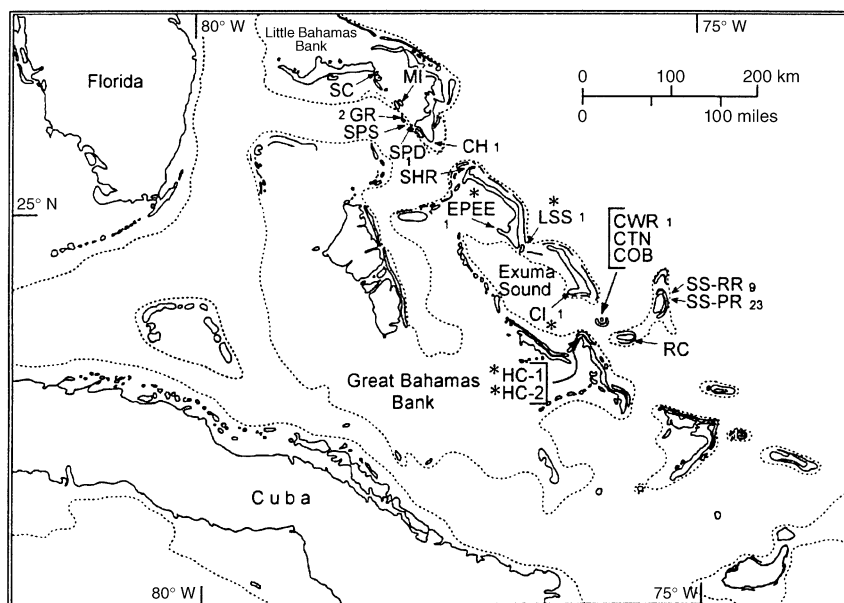


Fig. 1 Collection sites of *Pseudopterogorgia elisabethae* in the Bahamas (see Table 1 for name abbreviations). Stars by the abbreviations depict the Exuma Sound populations, and the numbers indicate the total number of private alleles (if any) per population. Dotted lines indicate approximately 100 m isobath. Modified from UNEP/IUCN (1988).

reefs were surveyed by two groups of divers swimming along an imaginary line in opposite directions. This strategy ensured that the same colony was not sampled more than once. All colonies found along the swimming path were sampled until a minimum of 35 samples was obtained. Samples were preserved in 95% ethanol or in a salt (NaCl) saturated 20% dimethyl sulphoxide (DMSO) solution (Seutin *et al.* 1991) until genetic analysis was conducted.

Microsatellite analyses

DNA was isolated from 3 cm of *P. elisabethae* tissue following a modification (Shearer *et al.* 2004) of the manufacturer's protocol for the Prep-A-Gene DNA extraction kit (Bio-Rad Laboratories, Hercules, CA, USA). The microsatellite loci and primers used in this study, with the exception of locus Pel1, have been described elsewhere (Gutiérrez-Rodríguez & Lasker 2004b). Pel1 was found in the ITS-2 region of the ITS-rDNA of aligned *P. elisabethae* sequences from nine different populations. Forward Pel1FOR (5'-GGACGCACGCTAGGCAAGC-3') and reverse Pel1REV (5'-GATGGCGCTCTGGCTATAC-3') primers flanking the microsatellite were designed and tested in AMPLIFY version 1.2 (Engels 1993) to determine the initial amplification conditions. Data analyses were conducted both with and without the data from the Pel1 locus because it has been suggested that the microsatellites present in the internal transcribed spacer (ITS)-1 and ITS-2 regions may not behave as codominant Mendelian markers (Harris & Crandall 2000). The results of both sets of analyses (data not shown) yielded similar patterns of genetic variation. We ensured that the designed primers for all six loci were specific to

the host DNA and not to their symbiotic zooxanthellae by amplifying the DNA of the hosts and zooxanthellae cultures as described in Shearer *et al.* (2004).

Total genomic DNA was amplified in 10 µL reactions using polymerase chain reaction (PCR) and the conditions described in Gutiérrez-Rodríguez & Lasker (2004b). Reaction conditions for Pel1 were performed as described for the other primers (Gutiérrez-Rodríguez & Lasker 2004b) using an annealing temperature of 60 °C, a 1.5 mM concentration of MgCl₂ and 30 cycles. Electrophoresis of the PCR products was performed in 7% polyacrylamide denaturing gels, and was visualized on a LI-COR NEN® Global IR2 DNA Sequencer System using fluorescent-labelled primers (Gutiérrez-Rodríguez & Lasker 2004b). The PCR product size was determined based on a standard 50–350 bp DNA ladder (LI-COR Biotechnology Division, Lincoln, NE, USA). The genotype of each individual was determined by comparing the migration of the alleles to the migration of the size standard using the computer program Gene Imager version 4.03 (Scanalytics Inc., Fairfax, VA, USA).

Statistical analysis

Descriptive statistics were determined for each of the six loci using GENEPOP version 3.1d (Raymond & Rousset 1995) as implemented for online use by E. Morgan (<http://wbiomed.curtin.edu.au/genepop/>). The program was also used to calculate f , an estimator of F_{IS} (inbreeding within each population; Weir & Cockerham 1984), and to determine heterozygote deficiencies for each population by calculating and comparing the observed and expected heterozygosities for deviations from Hardy–Weinberg equilibrium.

Table 2 Description of six polymorphic *Pseudopterogorgia elisabethae* microsatellite loci for 18 populations, including number of alleles in each population, total number of alleles per locus (TAL) and per population (TAP) as well as the total number of private alleles per locus (PAL) and per population (PAP). For name abbreviations of the populations refer to Table 1

Locus	Number of alleles																			
	SC	MI	GR	SPS	SPD	CH	SHR	CWR	CTN	COB	SS-RR	SS-PR	RC	EEPE	LSS	CI	HC1	HC2	TAL	PAL
Pel1	1	3	4	1	3	3	3	3	3	2	3	2	2	2	3	2	2	2	5	1
Pel56	3	1	3	1	3	3	1	5	5	7	14	24	1	10	9	8	9	10	28	12
Pel34	6	6	6	6	6	4	4	9	7	7	12	8	6	8	2	7	7	3	18	5
Pel74	3	2	2	1	2	4	2	5	5	6	14	23	2	12	11	9	6	10	29	12
Pel32	2	1	3	3	1	2	1	7	5	7	12	20	2	12	10	8	10	9	27	7
Pel19	6	5	7	5	7	5	3	10	9	8	9	11	6	8	4	5	7	5	15	3
TAP	21	18	25	17	22	21	14	39	34	37	64	88	19	52	39	39	41	39	—	—
PAP	0	0	2	0	1	1	0	1	0	0	9	23	0	1	1	1	0	0	—	—

GENEPOP calculates Fisher's exact tests using the Markov chain algorithm described by Guo & Thompson (1992) to estimate the unbiased exact P -values. In order to determine whether the populations were subdivided within the gorgonian gene pools, we tested the presence of genotypic linkage disequilibrium between all pairs of loci using GENEPOP. All results were adjusted for multiple comparisons using Bonferroni tests (Rice 1989).

We assessed population structure among populations by calculating Weir & Cockerham's (1984) estimators of Wright's (1978) F -statistics in the computer program FSTAT version 2.9.3.2 Goudet (2001). The estimators F and θ correspond to F_{IT} (total inbreeding coefficient) and F_{ST} (subdivision among sampled populations), respectively. We also calculated ρ (Goodman 1997), an estimator of R_{ST} using the computer program R_{ST} Calc (Goodman 1997). R_{ST} incorporates the stepwise mutation model believed to operate typically at microsatellite loci (Slatkin 1995). The significance of the estimators was determined using jack-knife and bootstrap resampling procedures with 1000 permutations, and the results were adjusted for multiple comparisons using Bonferroni corrections. We used Wright's (1943) equation ($Nm = 1/4[1/\theta] - 1$) to estimate gene flow as the number of migrants per generation. The computer program GENETIX version 4.03 (Belkhir *et al.* 1996–2002) was used to calculate Cavalli-Sforza & Edward's (1967) chord distance among populations, and a neighbour-joining tree was constructed using PAUP* 4.0b10 (Swofford 2000). Mean gene diversity (expected heterozygosity), allelic richness (number of alleles independent of sample size) per population, as well as tests to compare gene diversity and allelic richness between genetic groups were calculated as described in Goudet (2001). The computer program TFPGA version 1.3 Miller (1997) was used to perform a Mantel test with 1000 replicates to test for a significant relationship between geographical and genetic distance.

Results

Microsatellite variation

All *P. elisabethae* loci showed considerable variation in the number of alleles found across the populations. Locus Pel1 exhibited the lowest variation with only five alleles, while Pel74 had the largest number of alleles with 29 (Table 2). The number of private alleles, i.e. alleles present in one of the populations but absent in the others, varied among loci and populations: Pel1 had only one private allele, but Pel56 and Pel74 each exhibited 12 (Table 2). Most of the private alleles were from the Pillar Reef population on San Salvador (Fig. 1, Table 2). Overall mean gene diversity, excluding Pel19 (see next section) was greatest for the Pillar Reef and Riding Rock populations of San Salvador (0.777 and 0.752, respectively) and least for the Cross Harbor population of Abaco (0.09; Table 3).

Hardy–Weinberg equilibrium (HWE)

Significant deviations from Hardy–Weinberg expectations (HWE) within populations were observed in all 18 populations for at least one locus. Most of the populations exhibited HWE disequilibrium for more than one of the six screened loci, and some deviated from HWE at the majority of the loci (Table 3). For example, at Hog Cay-2 and Conception-Wedge Rock, five of the six markers exhibited a deficit of heterozygotes (Table 3). Populations that deviated significantly from HWE always had positive F -values and a test of heterozygote deficiency confirmed that those populations had a significant deficit of heterozygotes (Table 3). Pel19 was the only locus that significantly deviated from HWE in most (16 of 18) of the populations, suggesting the presence of null alleles at this particular locus. Because of this, we excluded Pel19 from further analyses. The F -values, when all populations were

Table 3 F_{IS} (f) and observed/expected heterozygosities for the different populations at each locus, as well as the mean observed and expected (gene diversity) heterozygosities per population. Fixed refers to populations with only one allele. F_{IS} values in bold type indicate significant deviations from HWE due to heterozygote deficiencies at the 0.05 level after strict Bonferroni corrections. Markov chain Monte Carlo tests were performed with 2500 dememorization steps and 300 batches of 2000 iterations per batch. For name abbreviations of the populations refer to Table 1

Pop	F_{IS} (f)						Heterozygosity (observed/expected)						Mean heterozygosity	
	Pel1	Pel56	Pel34	Pel74	Pel32	Pel19	Pel1	Pel56	Pel34	Pel74	Pel32	Pel19	Observed	Expected
SC	Fixed	−0.006	+0.462	+0.954	0.00	+0.683	Fixed	0.05/0.05	0.26/0.49	0.03/0.62	0.03/0.03	0.18/0.55	0.074	0.239
MI	−0.082	Fixed	+0.671	0.00	Fixed	+0.855	0.22/0.20	Fixed	0.20/0.62	0.02/0.02	Fixed	0.10/0.66	0.088	0.169
GR	+0.866	+1.0	+0.650	−0.011	−0.055	+0.556	0.06/0.44	0.00/0.56	0.24/0.68	0.04/0.04	0.14/0.13	0.20/0.46	0.096	0.372
SPS	Fixed	Fixed	+0.398	Fixed	+1.0	+0.540	Fixed	Fixed	0.35/0.59	Fixed	0.00/0.66	0.27/0.57	0.07	0.250
SPD	0.015	−0.025	+0.101	+0.901	Fixed	+0.313	0.07/0.06	0.08/0.08	0.49/0.54	0.04/0.42	Fixed	0.41/0.59	0.136	0.222
CH	−0.078	−0.005	+0.315	+0.386	0.00	+0.589	0.18/0.17	0.04/0.04	0.08/0.12	0.07/0.11	0.02/0.02	0.28/0.68	0.078	0.09
SHR	−0.100	Fixed	+0.648	+0.907	Fixed	+0.670	0.26/0.24	Fixed	0.25/0.70	0.04/0.42	Fixed	0.17/0.51	0.11	0.275
CWR	+0.782	+0.657	+0.265	+0.366	+0.169	+0.166	0.09/0.40	0.17/0.48	0.62/0.84	0.38/0.59	0.40/0.48	0.67/0.80	0.332	0.558
CTN	+0.793	+0.498	+0.598	+0.412	−0.258	+0.395	0.03/0.13	0.32/0.63	0.32/0.79	0.37/0.62	0.86/0.69	0.49/0.80	0.38	0.575
COB	−0.029	+0.687	+0.404	+0.369	+0.359	+0.448	0.08/0.08	0.18/0.58	0.40/0.67	0.35/0.55	0.42/0.65	0.42/0.76	0.286	0.511
SS-RR	−0.264	+0.284	+0.093	+0.079	+0.229	+0.370	0.46/0.36	0.63/0.88	0.76/0.83	0.81/0.88	0.61/0.79	0.51/0.81	0.654	0.752
SS-PR	−0.324	+0.124	+0.053	+0.150	+0.137	+0.276	0.50/0.38	0.80/0.91	0.78/0.82	0.78/0.92	0.74/0.86	0.60/0.83	0.72	0.777
RC	−0.032	Fixed	+0.576	0.00	0.00	+0.456	0.08/0.08	Fixed	0.33/0.78	0.02/0.02	0.02/0.02	0.36/0.65	0.162	0.181
EEPE	−0.056	+0.284	+0.501	+0.189	+0.055	+0.351	0.13/0.12	0.58/0.81	0.29/0.57	0.66/0.81	0.80/0.85	0.25/0.38	0.492	0.634
LSS	+0.112	+0.313	0.00	+0.119	+0.108	+0.954	0.19/0.22	0.53/0.77	0.02/0.02	0.60/0.68	0.61/0.69	0.02/0.40	0.39	0.475
CI	−0.021	+0.217	−0.036	−0.038	+0.055	+0.523	0.06/0.06	0.62/0.79	0.36/0.35	0.82/0.79	0.71/0.75	0.20/0.42	0.514	0.547
HC-1	−0.040	+0.210	+0.012	+0.187	+0.353	+0.697	0.10/0.10	0.63/0.80	0.71/0.72	0.60/0.74	0.53/0.82	0.20/0.64	0.514	0.635
HC-2	+0.675	+0.340	−0.063	+0.450	+0.296	+0.858	0.12/0.37	0.52/0.79	0.18/0.17	0.47/0.84	0.58/0.83	0.11/0.73	0.374	0.601

Table 4 Values of f , F , θ , Nm (θ), ρ , and Nm (ρ) for each locus across all populations and combined over loci. Significance of the estimators per locus and over all loci was determined by jackknifing and bootstrapping with 1000 iterations

Locus	f	F	θ	Nm (θ)	ρ	Nm (ρ)
Pel1	0.240*	0.740*	0.658*	0.129	0.512*	0.238
Pel56	0.373*	0.596*	0.355*	0.454	0.295*	0.597
Pel34	0.348*	0.541*	0.295*	0.597	0.591*	0.173
Pel74	0.327*	0.526*	0.296*	0.595	0.268*	0.683
Pel32	0.216*	0.460*	0.311*	0.554	0.191*	1.06
Overall	0.311*	0.566*	0.371*	0.424	0.371*	0.424

* $P < 0.001$ level.

combined, were high and significantly different from zero ($P < 0.001$) for each locus and across all loci (Table 4).

Significant genotypic linkage disequilibrium was found in 18 of 180 tests locus/site combinations (10% of the performed tests, $P < 0.05$). In general, the loci involved in most of the statistically significant genotypic linkage disequilibrium tests were those with the higher number of populations in HWE disequilibrium. For example, for loci Pel74 and Pel56, seven populations had heterozygote deficiencies, and these loci were involved in 13 and 10 of the significant genotypic linkage disequilibrium tests, respectively. On the other hand, at Pel1, only three populations were observed to have heterozygote deficiencies, and this locus was involved in only one of the significant tests for linkage disequilibrium.

Population genetic structure and gene flow

The tests of genetic differentiation indicated significant differences in allele frequencies among populations for all five loci. Estimates of population differentiation, θ and ρ , indicated high degrees of genetic differentiation between populations as both were significantly different from zero ($P < 0.001$) for each separate locus as well as when all loci were combined (Table 4). θ -values ranged from 0.311 to 0.658, whereas ρ -values varied between 0.191 and 0.591 (Table 4). When all loci were combined both estimates had the same value (0.371, Table 4). Estimates of gene flow (Nm) for each locus ranged between 0.129 and 0.597 for θ , and from 0.173 to 1.06 for the ρ estimates. Nm was 0.424 when all loci were combined (Table 4). Values of total inbreeding (F) were also high and significantly different from zero ($P < 0.001$) for each independent locus and when all loci were combined (Table 4). Pairwise comparisons between populations resulted in 142 significant differences ($P < 0.01$) when calculated with θ and in 146 when calculated with ρ ($P < 0.01$).

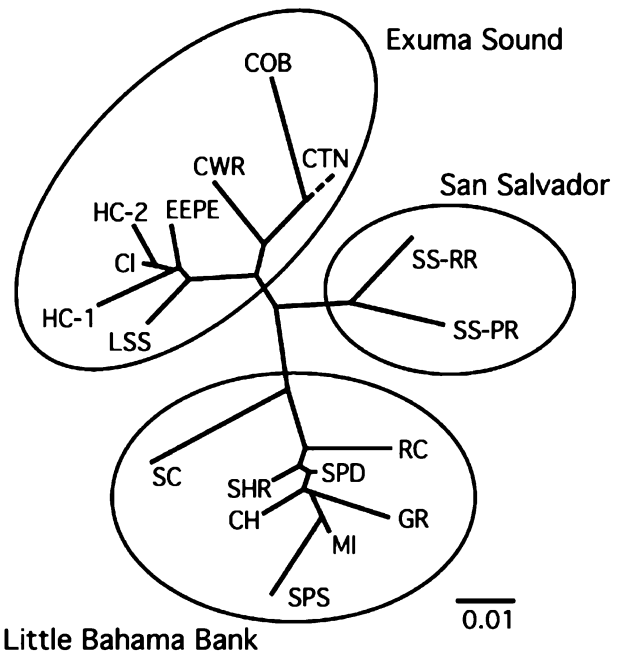


Fig. 2 Unrooted neighbour joining dendrogram depicting the relationships between different *Pseudopterogorgia elisabethae* populations in the Bahamas. The tree was calculated using Cavalli-Sforza and Edward's chord distance (1967).

Genetic distance

The dendrogram estimated with Cavalli-Sforza & Edward's (1967) chord distance suggests the presence of three groups (Fig. 2). The first group, 'Little Bahama Bank', is formed by all the islands sampled in this geographical area plus Rum Cay. The second group, 'Exuma Sound', encompasses the populations sampled within the basin plus the Conception sites, which are located near the deepwater entry to Exuma Sound (Fig. 1). The third group, 'San Salvador' clusters the two populations sampled in this island.

The tests of mean gene diversity and allelic richness among the three genetic groups showed that the San Salvador group had a significantly higher mean gene diversity (0.767, $P = 0.001$) and higher allelic richness (12.067, $P = 0.01$) than the other two genetic groups. The Exuma Sound and the Little Bahama Bank groups had a mean gene diversity of 0.562 and 0.222 and an allelic richness of 6.199 and 2.623, respectively.

A Mantel test showed a significant positive relationship ($r = 0.540$, $P = 0.001$) between genetic and geographical distances of *P. elisabethae* populations (Fig. 3). However, no significant correlation among the distances was found among populations in the Exuma Sound group ($r = 0.003$, $P = 0.44$), or among populations within the Little Bahama Bank group ($r = 0.065$, $P = 0.30$).

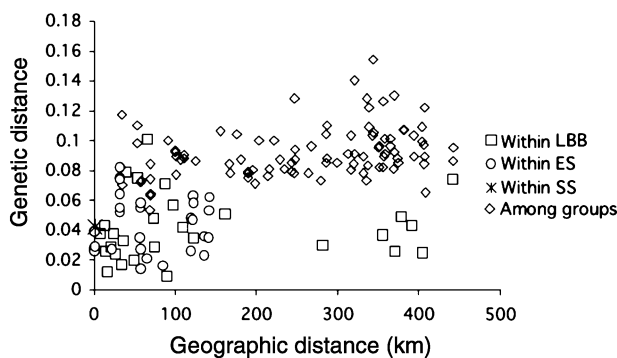


Fig. 3 Cavalli-Sforza and Edward's chord distance (1967) plotted against geographical distance (km) for the different populations of the Bahamas ($r = 0.540$, $P = 0.001$). The symbols represent the comparisons among populations within each of the genetic groups (Within LBB = among populations within the Little Bahama Bank genetic group, Within ES = among populations within the Exuma Sound genetic group, Within SS = between populations in the San Salvador genetic group, Among groups = among populations belonging to a different genetic group).

Discussion

Bahamian *P. elisabethae* populations exhibited a complex pattern of genetic structure both within single sites and between sites. Overall, we found significant deviations from HWE equilibrium due to heterozygote deficiencies and high levels of genetic differentiation among populations of *P. elisabethae*. These patterns can be a consequence of the reproductive traits of the species, hydrographic patterns in the Bahamas and historic events.

HWE equilibrium

Allozyme studies of marine invertebrates with low dispersal capabilities have often found heterozygote deficits (e.g. Grosberg 1991; Ayre & Dufty 1994; Brazeau & Harvell 1994; Hellberg 1995; Ayre *et al.* 1997a; Ayre *et al.* 1997b; Miller 1998). Those deficits have been attributed mainly to inbreeding and Wahlund effects. The observed heterozygote deficits among *P. elisabethae* populations could be due to the Wahlund effect and/or inbreeding. The presence of the Wahlund effect and inbreeding are supported by the significant linkage disequilibrium, which was confined mainly to loci in HWE disequilibrium. Associations between linkage disequilibrium and the loci that are not in HWE are expected when sampling members from different populations, i.e. a Wahlund effect. However, a Wahlund effect could explain the heterozygote deficiencies within individual samples in this study only if the populations were structured on scales of tens of meters. The potential for some philopatry in *P. elisabethae* makes such a pattern possible, but the observation that most planulae travel a

minimum of tens of metres (Gutiérrez-Rodríguez & Lasker 2004a) makes a Wahlund effect on this spatial scale less likely. Definitive testing of a Wahlund effect will require even finer scale sampling.

Inbreeding also can generate disequilibria among unlinked loci (Hartl & Clark 1989), and inbreeding is likely among *P. elisabethae* because both the larvae and sperm of the species have low dispersal capabilities. Some of the larvae of *P. elisabethae* settle within 5 m of the maternal colony (Gutiérrez-Rodríguez & Lasker 2004a), and paternity analyses suggest that most of the matings are local, occurring between individuals within an approximately 400 m² area (Gutiérrez-Rodríguez 2003). In combination, these traits should increase the likelihood of matings between close relatives.

In microsatellite data heterozygote deficiencies can also be generated by the presence of null alleles (Callen *et al.* 1993). The frequency of null alleles among loci (with the exception of locus Pel19) ranged from 0.02 to 0.04. Although low, these values could lead to a substantial number of heterozygotes containing a null allele, which would then be scored as homozygotes and create an apparent deficit in heterozygotes. While null alleles probably contributed to the heterozygote deficits, the association with linkage disequilibria and the observation that null alleles are locus specific (Lehmann *et al.* 1999) suggest that inbreeding or Wahlund effects were also evident. All the loci exhibited some cases of heterozygote deficits (Table 2), but with the exception of Pel19, which had heterozygote deficits at almost all sites, site was a significant predictor of whether there was a heterozygote deficit while locus was excluded in a stepwise log-linear analysis of the distribution of heterozygote deficits across sites and loci (site \times presence/absence of a deficit, $P = 0.004$). Differences between sites and not between loci are more consistent with the presence of inbreeding and/or a Wahlund effect.

Population genetic structure, gene flow and causes of the observed patterns

P. elisabethae showed high and significant genetic differentiation over all loci when estimated with both θ and ρ (Table 4), indicating high degrees of genetic differentiation between populations. The patterns of genetic similarities between populations depicted by the neighbour-joining tree were supported by the F_{ST} and R_{ST} pairwise comparisons. The few pairwise comparisons that did not yield significant population structure were between populations located within the Exuma Sound genetic group (seven and four of 46 with θ and ρ , respectively), among populations of the Little Bahamas Bank group (four and two of 28 with θ and ρ , respectively) and between the San Salvador populations (one of one with both estimators) (Table 5). In the same way, the rest of the F_{ST} and R_{ST} pairwise

Table 5 Matrix of pairwise estimates of $R_{ST}(\rho)$ above the diagonal and $F_{ST}(\theta)$ below the diagonal between *Pseudopterogorgia elisabethae* populations. For name abbreviations of the populations refer to Table 1

	SC	MI	GR	SPS	SPD	CH	SHR	CWR	CTN	COB	SS-RR	SS-PR	RC	EEPE	LSS	CI	HC-1	HC-2
SC		0.262	0.314	0.487	0.200	0.197	0.104	0.220	0.681	0.585	0.398	0.339	0.301	0.536	0.550	0.692	0.584	0.657
MI	0.636		0.122	0.127	0.079	0.004	0.129	0.348	0.649	0.583	0.446	0.372	0.472	0.403	0.367	0.492	0.465	0.518
GR	0.466	0.270		0.294	0.221	0.079	0.133	0.284	0.417	0.419	0.427	0.373	0.369	0.214	0.196	0.238	0.288	0.330
SPS	0.649	0.293	0.362		0.316	0.124	0.346	0.477	0.811	0.708	0.497	0.413	0.707	0.597	0.581	0.748	0.670	0.697
SPD	0.522	0.231	0.228	0.376		0.06	0.090	0.322	0.743	0.631	0.438	0.369	0.506	0.529	0.526	0.694	0.597	0.653
CH	0.710	0.388	0.318	0.483	0.152		0.079	0.292	0.559	0.519	0.422	0.356	0.368	0.329	0.292	0.388	0.388	0.444
SHR	0.457	0.379	0.351	0.468	0.151	0.441		0.207	0.564	0.509	0.405	0.351	0.256	0.412	0.418	0.535	0.469	0.549
CWR	0.358	0.508	0.388	0.457	0.464	0.564	0.465		0.236	0.223	0.214	0.221	0.095	0.335	0.378	0.483	0.376	0.450
CTN	0.466	0.493	0.382	0.477	0.465	0.562	0.452	0.216		0.062	0.311	0.295	0.614	0.329	0.444	0.526	0.412	0.465
COB	0.546	0.637	0.528	0.602	0.605	0.695	0.572	0.205	0.117		0.227	0.235	0.509	0.282	0.383	0.423	0.348	0.373
SS-RR	0.328	0.360	0.267	0.328	0.315	0.427	0.302	0.114	0.184	0.239		0.013	0.343	0.364	0.392	0.421	0.396	0.404
SS-PR	0.289	0.355	0.278	0.322	0.304	0.412	0.269	0.149	0.184	0.243	0.019		0.315	0.301	0.317	0.342	0.323	0.323
RC	0.611	0.224	0.294	0.399	0.247	0.456	0.334	0.482	0.468	0.619	0.319	0.309		0.565	0.603	0.739	0.637	0.689
EEPE	0.456	0.484	0.352	0.453	0.429	0.499	0.433	0.260	0.091	0.191	0.205	0.197	0.498		0.015	0.008	0.057	0.026
LSS	0.556	0.565	0.424	0.539	0.492	0.540	0.511	0.298	0.207	0.259	0.272	0.279	0.583	0.059		0.007	0.103	0.052
CI	0.493	0.513	0.386	0.493	0.439	0.502	0.441	0.292	0.128	0.229	0.248	0.241	0.524	0.014	0.044		0.093	0.062
HC-1	0.461	0.499	0.394	0.483	0.459	0.548	0.422	0.286	0.136	0.195	0.210	0.184	0.487	0.088	0.183	0.104		0.086
HC-2	0.494	0.487	0.352	0.457	0.400	0.455	0.405	0.317	0.180	0.278	0.234	0.225	0.504	0.040	0.067	0.024	0.118	

Numbers in bold type indicate significantly different from zero at the 0.01 level after Bonferroni corrections. Significance for both estimates was evaluated with 1000 permutations.

comparisons were consistently lower between populations clustered in the same genetic group than were comparisons between populations belonging to a different genetic group (Table 5). Estimations of the number of migrants per generation support these results. Average gene flow between populations in different genetic groups was lower [Exuma Sound vs. Little Bahama Bank ($Nm = 0.38$), Exuma Sound vs. San Salvador ($Nm = 0.98$), Little Bahama Bank vs. San Salvador ($Nm = 0.53$)] than average gene flow among populations from the same genetic group [among Exuma Sound populations ($Nm = 2.62$), among Little Bahama Bank populations ($Nm = 0.50$), between San Salvador populations ($Nm = 12.70$)]. This pattern of structure was again suggested when the data were analysed discriminating sites within islands (subpopulations) from sites between islands. θ -values among subpopulations (data not shown) were always lower than the θ -values in Table 3.

Because genotypic linkage disequilibrium could overestimate the genetic differentiation between populations, we repeated the population differentiation analyses excluding those loci (Pel74 and Pel56) that were consistently involved in the linkage disequilibrium tests that were significant. This analysis yielded significant ($P < 0.001$) θ and ρ -values, thus not changing the conclusions of the study.

Studies of marine invertebrates with limited larval dispersal using allozymes have found patterns of genetic differentiation similar to those found in this investigation, consistent with the hypothesis that species that brood larvae are more likely to exhibit lower levels of larvae

dispersal (e.g. Ayre & Dufty 1994; Hellberg 1994; Ayre *et al.* 1997a; McFadden 1997). However, other studies have reported low or no significant genetic differentiation among populations of other marine invertebrates with limited larval dispersal (e.g. Ayre *et al.* 1997a; Ayre & Hughes 2000), and such patterns have been attributed to physiological and behavioural characteristics of the planulae (Ayre *et al.* 1997a). Thus, levels of dispersal cannot always be predicted on the basis of the mode of sexual reproduction alone.

While limited dispersal may have led to differentiation among populations, the patterns of genetic similarity between reefs appear to be related to ocean currents within the Bahamas. The populations belonging to the 'Exuma Sound' group (Fig. 2) are located within or just outside of an active semi-enclosed basin (Fig. 1; Colin 1995). The similarity between these populations, and their dissimilarity to populations outside the basin parallels the presence of gyres within Exuma Sound, which could transport larvae within the basin, and limit the exchange with waters outside the basin (Hickey *et al.* 2000). There was no discernible isolation by distance effect among the Exuma Sound populations, which probably reflects both the relatively small distances between the sampled locations and the complexity of currents within Exuma Sound.

The Little Bahama Bank group is composed of those populations found along the southern edge of the Little Bahama Bank and also includes the population from Rum Cay, which is geographically closer to both the Exuma Sound, and San Salvador populations. These populations

are linked by long shore drift along the edge of the Little Bahama Bank and by the Antilles Current, which runs in a northwesterly direction east of the Bahamas (Wust 1964), and could carry larvae from Rum Cay into the New Providence Channel, Abaco and the Little Bahamas Bank. As among the Exuma Sound populations there was no evidence of isolation by distance among the populations within the Little Bahama Bank group.

Although the microsatellite data suggest that the two San Salvador populations are different from both the Little Bahama Bank and the Exuma Sound genetic groups, sequences of the internal transcribed spacer of the ribosomal DNA, which included many of the populations of this investigation, suggest that the San Salvador populations cluster with some of the populations within the Little Bahama Bank group (Gutiérrez-Rodríguez 2003). This could be due to the lower genetic variation that ITS-rDNA sequences have compared to microsatellites. Affinity of the San Salvador population with the Little Bahama Bank populations would also be consistent with the Rum Cay population's affinity with that group.

Differences between the San Salvador and the Little Bahama Bank populations are not simply a function of isolation-by-distance, as Rum Cay is as distant from those populations as San Salvador, yet Rum Cay was more similar to populations on the Little Bahama Bank than to those on San Salvador. Much of the difference between the San Salvador *P. elisabethae* populations and those from the Little Bahama Bank group is due to the higher gene diversity and allelic richness of the San Salvador populations. A potential explanation for that pattern is that the populations forming the Little Bahama Bank genetic group are relatively younger, and composed of colonists from San Salvador or from a pool of populations, of which San Salvador is a member. Alternatively, the lower genetic diversity observed in the Little Bahama Bank genetic group could be the result of recent bottlenecks or reductions in population size, following colonization of a limited number of individuals. Because of the deviations from HWE observed in this study it is not possible to do specific analysis to test for bottleneck events without violating the assumptions of these tests (i.e. Cornuet & Luikart 1996).

The Quaternary history of the Bahamas is characterized by drastic changes in sea level (e.g. Wilson *et al.* 1998; Hearty & Neumann 2001). During continental glaciations lower sea level left the Bahamian banks completely exposed, and the islands were platforms with near-vertical sides (Hearty & Neumann 2001). The low-stand period is likely to have been associated with the mobilization and deposition of sediments in the ocean, the loss of bank barrier reefs and habitat degradation of reef environments. Drastic changes in surface water temperature, characteristic of glaciations, could have also contributed to the degradation of the reef communities. Although *P. elisabethae* has a large

environmental range, our observations suggest that it is not found in areas of high sedimentation and turbidity. Thus, populations along the Little Bahamas Bank are likely to have undergone bottlenecks or local extinctions followed by recolonization events. Because San Salvador is a small, isolated island, surrounded by oceanic waters, its reefs may have been subject to less habitat degradation than reefs along the Little Bahamas Bank. The proximity of Rum Cay to the larger islands, that would have existed during a sea level low-stand, may have led to population decline similar to that seen along the Little Bahamas Bank.

The higher gene diversity and allelic richness in the San Salvador and Exuma Sound groups may reflect ancient populations with a combination of the original diversity and new evolved alleles, whereas the Little Bahama Bank group may have never had or may have lost gene diversity and allelic richness.

Studies of organisms with similar dispersal characteristics to *P. elisabethae* in the Bahamas, detailed analyses of circulation patterns through the Bahamas, as well as detailed phylogeographical studies of other marine organisms in the Bahamas and adjacent Caribbean islands are necessary to corroborate the hypotheses that we have suggested have led to the patterns of genetic variation found in this study.

Consequences for conservation

The results of this study have important consequences for the conservation of *P. elisabethae*. The high degree of population structure, low levels of gene flow and the differences in genetic diversity among populations are important factors to consider in the development of management plans for the species. In concert with the results of this study, the ITS-rDNA sequence data (Gutiérrez-Rodríguez 2003) and the pseudopterosin content in *P. elisabethae* colonies (W. Fenical, pers. comm.) indicate that Exuma Sound, Little Bahama Bank and San Salvador are distinct genetic groups. Therefore, management and conservation plans should consider the populations within each of these groups as unconnected and independent. The Exuma Sound and the two San Salvador populations exhibited the greatest genetic diversity of the sampled populations. Neither of these areas is currently subject to harvesting, but any future management plans should conserve the genetic variation found in those populations.

The limited gene interchange between populations in the Bahamas also implies that populations with reduced genetic diversity could not be expected to recover their genetic variation by the replenishment of alleles from other populations via natural gene flow. Furthermore, gene flow is not equivalent to demographic connectivity. Low migration rates on an evolutionary scale suggest that levels of larval dispersal between populations are

not enough to provide sufficient recruitment for sustainable populations. Thus, the conservation of local populations is crucial.

The results of this study have shown that the commercially important gorgonian *P. elisabethae* displays considerable genetic variation between the different Bahamian populations. The high and significant heterozygote deficiencies and population structure are concordant with the life-history traits of the species and in part with the geography and hydrographic conditions of the Bahamas. It is essential that future management and conservation plans for the species take into consideration these patterns.

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Carla Gutiérrez-Rodríguez is an evolutionary biologist interested in questions regarding population genetics, gene flow and phylogeography of freshwater and marine organisms, especially gorgonian and scleractinian corals. She is also interested in applying the results her studies to the conservation of species. Howard R. Lasker is a population ecologist interested in the ecology of coral reef organisms, especially gorgonian and scleractinian corals. Recent work in his laboratory has focused on patterns of colony growth of colonial organisms, the fertilization ecology of Caribbean gorgonians and the ecology and management of *Pseudopterogorgia elisabethae*.
