

# Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation

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## Abstract

DNA microsatellite markers were used to characterize the population genetic structure of the lemon shark, *Negaprion brevirostris*, in the western Atlantic. This study demonstrates for the first time the usefulness of microsatellites to study population genetic structure and mating systems in the Chondrichthyes. Lemon sharks (mostly juveniles) were sampled non-destructively from four locations, Gullivan Bay and Marquesas Key in Florida, Bimini, Bahamas, and Atol das Rocas, Brazil. At least 545 individuals were genotyped at each of four dinucleotide loci. The number of alleles per locus ranged from 19 to 43, and expected heterozygosities ranged from 0.69 to 0.90. Relatively little genetic structure was found in the western Atlantic, with small but significant values for estimators of  $F_{ST}$  and  $R_{ST}$  among populations,  $\theta$  (0.016) and  $\rho$  (0.026), respectively. No sharp discontinuities were found between the Caribbean sites and Brazil, and most alleles were found at all four sites, indicating that gene flow occurs throughout the western Atlantic with no evidence for distinct stocks.

**Keywords:** elasmobranchs, genetic variability, microsatellites, population genetic structure, sharks

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## Introduction

Despite a worldwide distribution, growing conservation concerns and a prevalent human fascination with sharks, surprisingly little is known about many aspects of the biology and life history of elasmobranchs. Little information is available on breeding patterns, demographics or population genetic structure of most elasmobranch species. Sharks clearly differ in many aspects of their population biology and life history from typical, long-lived teleost fishes; most sharks exhibit slow growth rates, produce relatively few young, and exhibit long inter-birth intervals. There is growing concern regarding the decline of many shark species (Manire & Gruber 1990; Musick *et al.* 2000). Clearly, management plans designed for teleost fish will be inappropriate for sharks, and better knowledge of their ecology and population biology is needed for successful management.

Variable genetic markers have been used to infer the popu-

lation genetic structure of several large, mobile marine organisms (Baker *et al.* 1990; Amos *et al.* 1993; Berube *et al.* 1998), but studies of genetic structure and stock assessment in sharks have been few (for review, see Heist 1999). Such studies have generally been hampered by lack of genetic variability at allozyme loci (Smith 1986; MacDonald 1988; Lavery & Shaklee 1989) and in mitochondrial DNA (Heist *et al.* 1995; 1996a,b), perhaps because sharks have a lower rate of molecular evolution than other vertebrates (Martin *et al.* 1992; Martin 1995). Migration rates, dispersal movements and patterns of gene flow have therefore been poorly characterized in most sharks. Microsatellites have been shown to have high allelic diversity and heterozygosity (Ashley & Dow 1994), even in species with low levels of allozyme variation (Hughes & Queller 1993; Jarne *et al.* 1994), and thus may provide resolution of population genetic structure in sharks. However, the only published population study on sharks utilizing microsatellites was limited by the low microsatellite variability as well as low microsatellite frequency in the species studied (sandbar sharks, *Carcharhinus plumbeus*) (Heist & Gold 1999), thus bringing into question their utility for shark population studies.

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The current study uses microsatellite loci to assess population genetic structure in the lemon shark, *Negaprion brevirostris*. The lemon shark is a large coastal species belonging to the family Carcharhinidae (the Requiem sharks). The family includes more than 50 species of large, active sharks. Lemon sharks occur in three widely separated regions: the western Atlantic from New Jersey to Brazil, the west African coast, and the eastern Pacific from Baja California (including the Sea of Cortez) to coastal Columbia. Here we report the results of genetic surveys from populations in the western Atlantic.

Successful recruitment of many coastal sharks, including lemon sharks, seems to involve critical 'nursery grounds', specific coastal areas where females travel to give birth and where juvenile sharks may remain for several years. We took advantage of such nursery grounds to sample large numbers of juvenile sharks non-destructively from four sites. We had two primary objectives: (i) to characterize the genetic variability at lemon shark microsatellite loci and compare microsatellite variability to that found in other vertebrates; and (ii) to assess population structure in the western Atlantic. We report highly variable microsatellite loci in an elasmobranch species and provide the most detailed characterization of the population genetic structure for any shark species to date.

## Materials and methods

### Study sites

We sampled lemon sharks from four areas (Fig. 1). Bimini, Bahamas, our main study site, is a small chain of mangrove-

fringed islands found approximately 85 km east of Miami, Florida. The Bimini islands surround a shallow lagoon of approximately 21 km<sup>2</sup> (Morrissey & Gruber 1993), which serves as a nursery area for a relatively unexploited population of approximately 500 juvenile and sub-adult lemon sharks. The Bimini site has been sampled for five years (1995–1999), with approximately 1000 samples collected to date. Sampling is concentrated in the North Sound of Bimini lagoon, where we estimate that 80–90% of the juveniles present are sampled each year.

Marquesas Key, Florida (Marquesas) is part of the Florida Keys and is located approximately 25 km west of Key West Florida. Marquesas Key is also mangrove-fringed and forms a circular lagoon approximately 5 km in diameter. Approximately 30–40 juveniles use these mangroves as a feeding ground (unpublished data). Sub-adult and adult sharks are plentiful throughout the surrounding keys. Although Marquesas is a bird sanctuary, recreational fishing for lemon sharks is legal in the area and occurs throughout the year.

Twenty-two lemon shark samples were obtained from Gullivan Bay (GB), located on the west coast of Florida approximately 5 km south of Marco Island. Gullivan Bay lies approximately 130 km north of Marquesas Key.

The Atol das Rocas (Rocas) site is in the southern Atlantic Ocean. It is a biological reserve located 200 km north-east of Brazil. This atoll rests on an extinct underwater volcano that rises 3600 m above the sea floor. The reef has an area of 7.2 km<sup>2</sup> and forms a shallow lagoon devoid of mangroves. Rocas is the southern-most known breeding area for lemon sharks. Rocas is protected by the Brazilian government so the population is undisturbed. In this lagoon, 60–70 juvenile and sub-adult lemon sharks

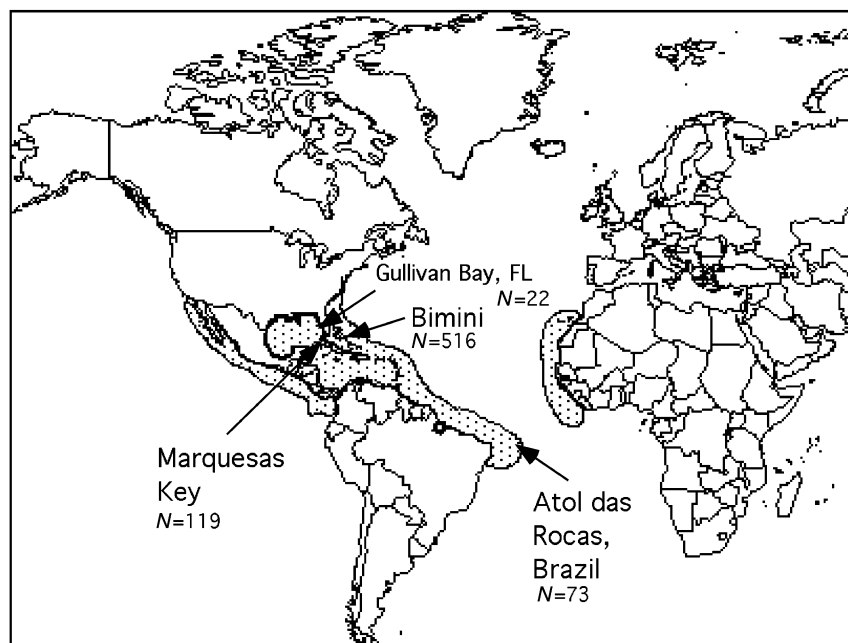


Fig. 1 Sampled populations of western Atlantic lemon sharks (*Negaprion brevirostris*). Stippled areas indicate the range of the species. *N* represents the number of individual lemon sharks sampled from each site.

can be found (S. H. Gruber, personal observation) and a total of 73 samples were obtained during collecting trips in 1999 and 2000.

### Sampling techniques

Juvenile sharks were caught using 180 m long gill nets and employing a 'remove and hold' technique (Manire & Gruber 1991). As part of a long-term growth study, all sharks were weighed, measured, sexed, and marked with an electronic passive integrated transponder (PIT) tag. Sub-adult and adult sharks were captured using either long-line fishing gear or rod and reel. These sharks were also PIT-tagged, measured and sexed. A small piece of fin was taken from all sharks and stored in 20% DMSO buffer (Seutin *et al.* 1991) until DNA extraction was performed. As sampling was conducted over multiple years, sample sizes exceed estimated population sizes due to mortality and recruitment.

### Genetic techniques

DNA was extracted from fin samples following a salting-out protocol (Sunnucks & Hales 1996). Lemon shark microsatellite primer pairs were developed following a standard screening protocol (Dow *et al.* 1995). Briefly, DNA from a sub-adult lemon shark caught at Bimini, Bahamas, was extracted and used to construct a genomic library of small (200–1500 bp) fragments. Size-selected *Sau3AI* fragments were ligated into pUC18 vector and transformed into *Escherichia coli* competent cells. Bacterial colonies were lifted onto nylon membranes and screened with a cocktail of chemoluminescently labelled di- and trinucleotides following the manufacturer's protocol (Amersham ECL 3'-oligolabelling and detection system). Plasmid DNA from positive colonies was extracted and used for sequencing (Amersham ThermoSequenase radiolabelled cycle sequencing kit). Sequencing reaction products were run on polyacrylamide gels and exposed to autoradiographic film. Primers were developed from sequences flanking the core repeat with the aid of Mac Vector software (IBI).

A total of 15 primer pairs flanking microsatellite repeats was scored for variability using a subset of at least 23 individuals (Table 1). Of these, only four loci were polymorphic. These four loci were used for genotyping a larger sample of lemon sharks. The forward primer from each locus was labelled fluorescently with one of three dyes (6-FAM, HEX or TET; PE Biosystems). Polymerase chain reaction (PCR) amplifications were carried out in a final volume of 7.5  $\mu$ L and contained 40–100 ng genomic DNA, 0.25 mM dNTPs (0.4 mM for LS11 and LS15), 0.2  $\mu$ M primers (0.23  $\mu$ M for LS11 and LS15), 0.17  $\mu$ g BSA, 1  $\times$  PCR buffer, a variable amount of  $MgCl_2$  (1 mM for LS11 and LS15, 2.5 mM for LS22, and 2 mM for LS30), and

0.3 units Amplitaq Gold DNA polymerase (PE Biosystems). PCR reactions began with a hot start at 95  $^{\circ}C$  for 12 min and continued as follows: LS11: 12 cycles of 94  $^{\circ}C$  for 30 s, 58  $^{\circ}C$  for 30 s, and 72  $^{\circ}C$  for 60 s, followed by 20 cycles of 89  $^{\circ}C$  for 30 s, 60  $^{\circ}C$  for 30 s, and 72  $^{\circ}C$  for 60 s; LS15: 13 cycles of 94  $^{\circ}C$  for 30 s, 58  $^{\circ}C$  for 15 s, and 72  $^{\circ}C$  for 60 s, followed by 20 cycles of 89  $^{\circ}C$  for 30 s, 60  $^{\circ}C$  for 15 s, and 72  $^{\circ}C$  for 60 s; LS22: 12 cycles of 94  $^{\circ}C$  for 5 s, 56  $^{\circ}C$  for 15 s, and 72  $^{\circ}C$  for 45 s, followed by 20 cycles of 89  $^{\circ}C$  for 15 s, 57  $^{\circ}C$  for 15 s, and 72  $^{\circ}C$  for 45 s; LS30: 15 cycles of 94  $^{\circ}C$  for 5 s, 53  $^{\circ}C$  for 15 s, and 72  $^{\circ}C$  for 45 s, followed by 20 cycles of 89  $^{\circ}C$  for 15 s, 55  $^{\circ}C$  for 15 s, and 72  $^{\circ}C$  for 45 s. A 10 min extension at 72  $^{\circ}C$  concluded each reaction.

The fluorescently labelled PCR products were electrophoresed on an ABI 373A automated sequencer along with a fluorescently labelled size standard (TAMRA-350, PE Biosystems). Labelling with three separate dyes enabled us to run three loci per lane, and a 66-well comb was used. Genescan software (PE Biosystems) was used to score PCR products. Recent evidence indicates that the size of alleles assigned by Genescan may not necessarily reflect the actual difference in nucleotide number between alleles (Haberl & Tautz 1999), especially at loci where there is a large allelic distribution (K. A. Feldheim, personal observation). Therefore, we constructed an allelic ladder for each locus by pooling several previously scored individuals on one electropherogram. The smallest allele was then scored, based on Genescan sizing, and two base pairs were added to the next physical peak. New alleles were added until every unique allele was represented in the allelic ladder. We found the use of the allelic ladder to be essential for accurate and consistent scoring. Use of a 66-well comb was ideal as allelic ladders typically occupied 10–15 lanes.

### Data analyses

Individuals were included in analyses if they were scored for at least three of the four loci. As a result, sample sizes are not equal across loci (Table 2). Descriptive statistics (i.e. allele number and heterozygosities) for each locus were calculated using GDA version 1.0 (Lewis & Zaykin 2000). Global tests for deviation from Hardy–Weinberg equilibrium (heterozygote deficits) were conducted using GENEPOP version 3.1d (an updated version of 1.2 as described in Raymond & Rousset 1995), sub-option 4. This program calculates unbiased estimates of  $P$  values using a Markov chain method. Homogeneity of allele distributions between populations for each locus was tested using the genic differentiation test in GENEPOP version 3.1d.

GDA was used to calculate an estimator of Wright's  $F_{ST}$  ( $\theta$ , Weir & Cockerham 1984). Significance of  $\theta$  was tested by bootstrapping over all loci (1000 replicates). In addition to  $F$  statistic estimators, we also calculated  $R_{ST}$  (Slatkin

**Table 1** Microsatellite loci characterized from a *Negaprion brevirostris* genomic library

Locus name	Primer sequence (5' → 3')	Repeat type*	Number of individuals scored	Number of alleles
LS39	F: CAGTAAACATCCCAAGGTGCTTC R: AATCGCACTGACCCATTCCG	[AG] <sub>6</sub>	62	1
LS28	F: TGGTTGATGCTTAACTGCCCTC R: TCTCCTCACCCTTTGTCTC	[AG] <sub>6</sub> Ö[AG] <sub>4</sub>	49	1
LS32	F: TTAAGTCAGGCTATTTGTGGACTCGT R: GCTTGCTTTACACCTACCCATTT	[AC] <sub>4</sub> [AG] <sub>2</sub> [AC] <sub>7</sub>	63	1
LS34	F: AGTCCCACCAGGATTTTCATG R: GTGGCATTGAGTTACGGTTTTTGC	[GA] <sub>6</sub> TA[GA] <sub>4</sub>	46	1
LS23	F: GCCATCGCTCAGTTAGCAA R: CCTCTGACAGTGCAATGTATCCTC	[CA] <sub>5</sub> GA[CA] <sub>7</sub>	73	1
LS20	F: AAGTCTGGAGCACAGCCTTCAAG R: AACTCCCATGGGAATCGTGG	[AC] <sub>8</sub> GC[AC] <sub>3</sub>	68	1
LS37	F: ATGTGCTTGAATCTCCCAAATGG R: GACCCTTACTCTGATACCTCTCT	[AC] <sub>8</sub>	48	1
LS25	F: ATAACCGTGATATCTATCTCA R: CCCAAACTAGGCACAGATTAC	[AC] <sub>9</sub> [TC] <sub>7</sub>	23	1
LS27	F: GGTGAATTTTACTCTGGCTGGGG R: TGCTCGCTATGAACCATGTCAC	[AG] <sub>10</sub>	69	1
LS24	F: GGATGTGTTAGTGAGGTGGTGAGTG R: AGGGCAGAGACAGCAGGGAATATC	[AC] <sub>12</sub>	67	1
LS53	F: GCCTCATTCTGCTCCTGTGTTTT R: CACATAACCTCCTCCTGCTTCC	[AC] <sub>14</sub>	23	1
LS15	F: TGCCTGGGTTGTTGTTTTGG R: GCACCTTGGATAGTTTGAGCAGG	[AC] <sub>20</sub>	>500	30
LS22	F: TTTCTTGTAGCCAGTTGTGGTG R: TTTGGATACTGCTGGGGTCAGG	[AC] <sub>23</sub>	>500	22
LS30	F: ACGTTAAATATCTCAGGCTCAGAG R: CCTTATTAACAACCTCAAACCTCGCC	[AC] <sub>25</sub>	>500	19
LS11	F: CCAGGAGAGAAGCATCTCACAG R: TGTCATTAGGATTTGCAGCC	[AC] <sub>33</sub>	>500	43

\*Determined from sequence of cloned allele.

**Table 2** Description of four variable *Negaprion brevirostris* microsatellite loci at four populations, including number of alleles (*A*), observed ( $H_O$ ) and expected heterozygosities ( $H_E$ ), number of individuals scored (*N*) and number of private alleles (*PA*)

Locus	<i>A</i>	$H_O$	$H_E$	Sample size ( <i>N</i> )*					<i>PA</i>
				Bimini	Marquesas	Rocas	GB	Total	
LS11	43	0.679	0.693	414	56	54	21	545	16
LS15	30	0.791	0.804	477	118	71	22	688	7
LS22	22	0.870	0.896	464	118	70	21	673	6
LS30	19	0.734	0.748	513	119	73	21	726	3

\*Juvenile and subadult lemon sharks from each population.

1995), a statistic that incorporates the stepwise mutation model (SMM) thought to operate at microsatellite loci.  $R_{ST}$  is based on variances in allele size across all loci; therefore loci with smaller variances will contribute less to the final value of  $R_{ST}$ . The computer program  $R_{ST}$  Calc (Goodman 1997) uses standardized allele sizes to determine

variance components at each locus and calculate  $\rho$ , an estimator of  $R_{ST}$ . We calculated  $\rho$  between populations and over all populations, with significance determined by 1000 permutations of the data.  $R_{ST}$  Calc was also used to calculate a distance measure,  $(\delta\mu)^2$ , which accommodates the SMM process at microsatellite loci (Goldstein *et al.* 1995). Another distance measure, Nei's *D* (Nei 1972) was calculated between pairs of populations using the POPGENE program version 1.31 (Yeh & Boyle 1997). We also used GENEPOP to implement the private allele method (Slatkin 1985; Barton & Slatkin 1986) for estimating the number of migrants per generation.

## Results

### Microsatellite variation

Microsatellite repeats were relatively rare in the lemon shark genome, thus making traditional screening protocols extremely laborious (unpublished data). Sequencing of 70 positive clones yielded 48 with (AC)<sub>*n*</sub> motifs, 17 with

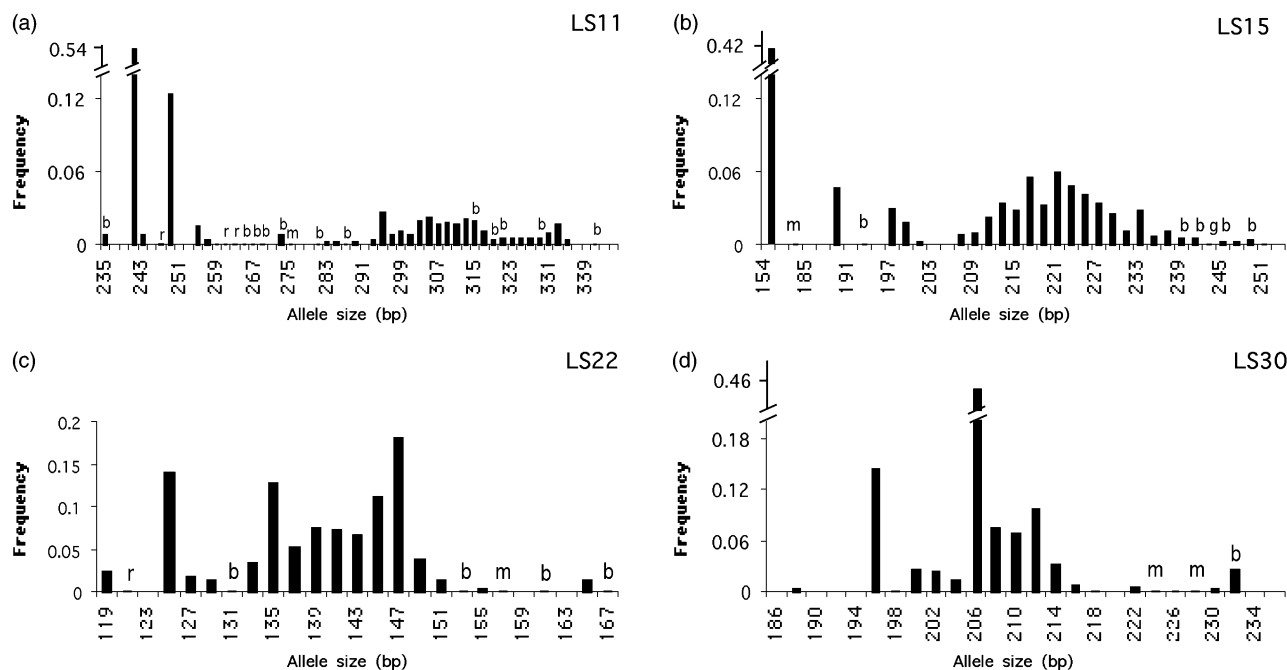


Fig. 2 Allele frequency distributions for four microsatellite loci across all sampled lemon shark populations. Allele sizes are given in base pairs of PCR products. The letters above the bars represent private alleles found in one population (b, Bimini, Bahamas; g, Gullivan Bay, Florida; m, Marquesas Key, Florida; r, Atol das Rocas, Brazil).

(AG)<sub>n</sub> motifs and five with compound repeats having two or more motifs in close proximity.

Fifteen loci were scored for at least 23 individual lemon sharks (Table 1). Remarkably, 11 of the loci were monomorphic. For the monomorphic loci, the cloned and sequenced allele had a dinucleotide repeat of 14 units or less, but typically such loci show variability in vertebrates, including teleost fish. Thus it appears that relatively short microsatellites may have extremely limited polymorphism in sharks. We did, however, find several long microsatellites with the cloned allele ranging in size from 20 to 33 repeats, and these were extremely polymorphic, with 19 (LS30) to 43 (LS11) alleles per locus and expected heterozygosities ranging from 0.69 to 0.90 (Table 2).

Global tests for heterozygote deficiencies revealed significant departures from Hardy–Weinberg expectations at LS11 and LS15 ( $P < 0.0001$  and  $P = 0.004$ , respectively). Although a deficiency of heterozygotes may be indicative of null alleles at microsatellite loci (Callen *et al.* 1993; Pemberton *et al.* 1995), we feel that in this case it is more likely related to the Bimini sample structure, as described below. Tests for deviations from Hardy–Weinberg equilibrium excluding Bimini were not significant (data not shown).

#### Population genetic structure

The genic differentiation test indicated significant differences in allele frequencies for all four loci in all pairwise

population comparisons, except for three comparisons involving Gullivan Bay. Non-significant results for Gullivan Bay may simply reflect its smaller sample size. Despite among-population differences in allele frequencies, each locus had one relatively common allele that was shared across populations. This is especially obvious at LS15, where allele 154 had a frequency of 0.419 over all populations (Fig. 2b), and was the most common allele in all four populations (Fig. 3). For LS11, allele 241 had a frequency of 0.536 over all populations (Fig. 2a), with frequencies at Bimini, Marquesas, Rocas and GB of 0.527, 0.544, 0.630 and 0.452, respectively. Similar common alleles are also present across populations at LS22 and LS30. Private alleles were found at each locus, but occurred at low frequencies (Fig. 2). LS11 had the largest number of alleles (43) and the highest proportion of private alleles (16).

The Weir and Cockerham's estimator of population subdivision,  $\theta$ , ranged from 0.006 to 0.028, while the  $R_{ST}$  estimator  $\rho$  ranged from  $-0.002$  to 0.067 for each locus. Both estimators had small but statistically significant values over all loci and populations ( $\theta = 0.016$ ,  $P < 0.05$  and  $\rho = 0.026$ ,  $P < 0.0001$ ). The statistical significance of such small values is apparently indicative of the high level of resolution achieved by our sample sizes and high variability at the microsatellite loci. Estimates of the number of migrants ( $Nm$ ) were 15.4 and 9.4 for  $\theta$  and  $\rho$ , respectively, and 8.9 using Slatkin's private allele method.

For pairwise calculations of  $\theta$  and  $\rho$  between populations

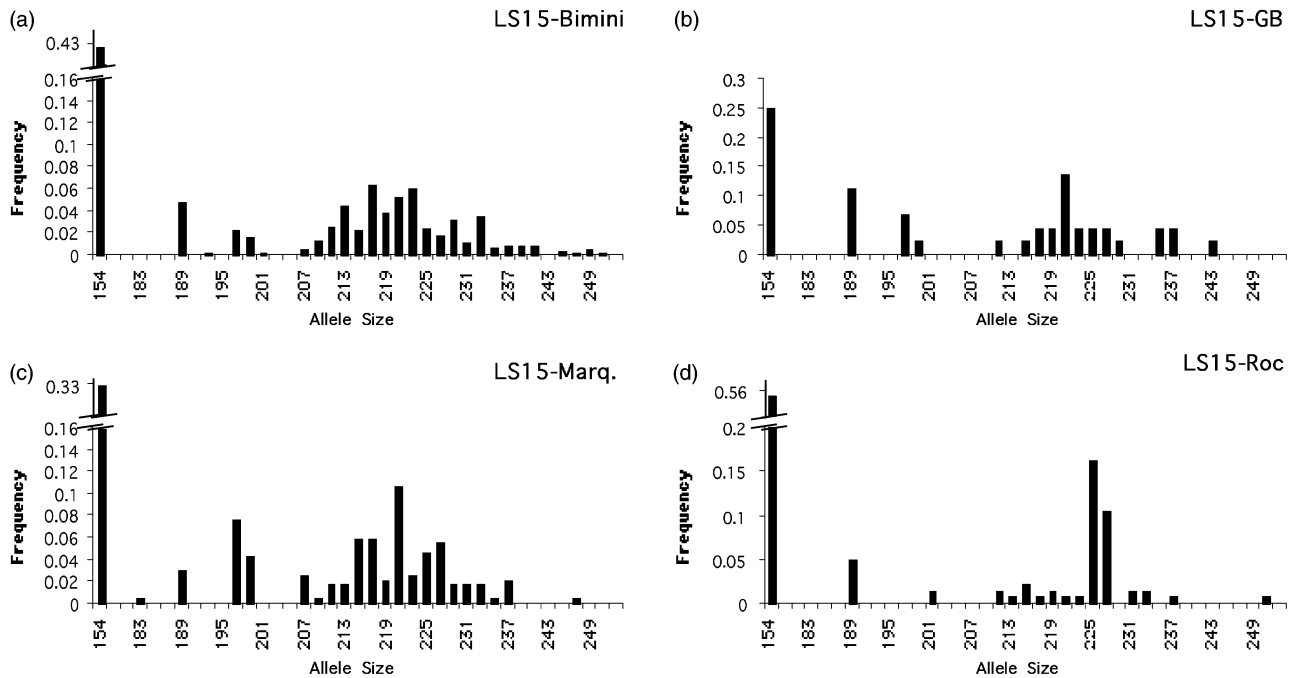


Fig. 3 Allele frequency distributions for locus LS15 for each population. Allele 154 is the most common allele across all four populations. Allele sizes are given in base pairs of PCR products.

**Table 3** Matrix of estimators of population subdivision ( $\theta$  and  $\rho$ ) and genetic distances ( $(\delta\mu)^2$  and Nei's  $D$ ) between pairs of populations

	Bimini	GB	Marquesas
GB	0.016*		
	0.027**		
	0.069		
	0.082		
Marquesas	0.011*	0.005	
	0.013†	0.049†	
	0.020	0.037	
	0.047	0.058	
Rocas	0.019*	0.034*	0.025*
	0.046‡	0.038**	0.062‡
	0.040	0.186	0.093
	0.062	0.118	0.078

Significant at the \* $P = 0.05$  level; \*\* $0.01 < P < 0.05$ ;

† $P < 0.005$ ;

‡ $P < 0.0001$ . Significance for  $\theta$  was determined by 1000 bootstraps. Significance for  $\rho$  was determined by 1000 permutations.  $\theta$  is the first value in each comparison,  $\rho$  is the second,  $(\delta\mu)^2$  is the third, and Nei's  $D$  is the fourth.

(Table 3), all comparisons were significant with the exception of  $\theta$  for Gullivan Bay versus Marquesas (0.005). Interestingly, the value of  $\rho$  for the Gullivan Bay/Marquesas comparison was relatively high (0.049). This may reflect inaccuracies associated with calculating these parameters

for the relatively small sample from Gullivan Bay (Ruzzante 1998). The values for the comparisons of northern populations versus Atol das Rocas are consistently higher than those among the Caribbean and Florida populations, but the values are not dramatically higher as might be expected given the distance of Rocas from the northern populations. All estimates are relatively low, below 0.070.

Pairwise distance measure between populations,  $(\delta\mu)^2$  and Nei's  $D$ , indicate greatest similarity between Marquesas and Bimini and the largest distance between Rocas and Gullivan Bay (Table 3). Although distances involving the widely separated Rocas population were relatively high, they overlap the range of values found for distances between the northern populations. Thus, distance measures do not support a sharp discontinuity between the northern sites and Atol das Rocas.

## Discussion

Our study is the first to use highly variable microsatellite markers to examine population structure in an elasmobranch. Heist & Gold (1999) found low levels of variability at three microsatellite loci for another carcharhinid shark, the sandbar shark (*Carcharhinus plumbeus*). As these authors point out, the core number of repeats of their loci was very small (ranging from 6 to 9), and core repeats of less than 10 tend to exhibit low variability (Weber 1990). A recent report of microsatellite loci in the white shark,

*Carcharodon carcharias*, found up to 10 alleles per locus when the cloned and sequenced allele had at least 22 repeats (Pardini *et al.* 2000). We found that lemon shark loci with long uninterrupted repeats (>20 repeats) were extremely variable (Tables 1 and 2, Fig. 2). Thus, the mutation rates for elasmobranch microsatellite loci appear to be as high as in other vertebrates but only once a particular threshold of repeat length is reached. If the type of data shown in Table 1 proves to be representative of sharks or elasmobranchs in general, application of microsatellite analysis to elasmobranchs will present unique challenges, given the relatively low frequency of microsatellite loci in their genomes and the low variability of all but the longest repeats. Considerable effort and resources, as well as the use of enrichment protocols (Kijas *et al.* 1994) will be required for the further development of microsatellite markers. Despite these problems, the four loci used in this study showed remarkably high levels of variability as measured by the number of alleles per locus and levels of heterozygosity. Thus, microsatellites will apparently allow relatively high levels of resolution in studies of population genetic structure in elasmobranchs.

A deficiency of heterozygotes at LS11 and LS15 may indicate null alleles at these loci. However, this finding is more likely due to the population structure and our sampling technique at Bimini. As part of a long-term life history study, we exhaustively sample juvenile lemon sharks in two areas of the Bimini lagoon, including 60–80 young of the year annually. The average litter size is 8–10, so young of the year sharks may represent the offspring of only 6–10 adult females. In addition, there is evidence that females return to Bimini to give birth on a biennial cycle (unpublished data). Given these observations, our Bimini sample probably includes groups of full- and half-sibs within and across sampling years. Once the Bimini sample was removed from the data set, there were no significant departures from Hardy–Weinberg equilibrium.

Barriers to gene flow for highly vagile marine species would seem to be minimal, and indeed several large pelagic fish species exhibit little structure worldwide. For example, mitochondrial DNA studies revealed little genetic divergence between Atlantic and Pacific populations of skipjack tuna (Graves *et al.* 1984), albacore (Graves & Dizon 1989) and yellowfin tuna (Scoles & Graves 1993). Other cosmopolitan marine species, however, exhibit varying patterns of differentiation within and between ocean basins (Graves 1998). Thus, simple generalizations that relate the distributions of marine species to gene flow patterns and stock structure are not possible. Microsatellite studies have revealed heterogeneity in some species such as cod (Bentzen *et al.* 1996) and whiting (Rico *et al.* 1997) that, based on other types of genetic markers, had previously exhibited little geographical variation in genetic structure.

Although there have been relatively few studies of

stock structure in sharks, some aspects of the biology of coastal species indicate that genetically distinct populations could exist even in large mobile species. Many coastal sharks, including lemon sharks, rely on shallow lagoons or 'nursery grounds' for successful recruitment. Females give birth in these nurseries and young sharks may remain there for extended periods. We have recently demonstrated that female lemon sharks at Bimini exhibit site fidelity to specific nurseries for parturition (unpublished data). Females at Bimini are often observed with fresh mating scars, suggesting that such nurseries may also serve as a breeding area. Fidelity for such breeding areas could result in genetic structure despite the high mobility of adults. To date, however, the relatively few population genetic studies have generally inferred relatively low levels of heterogeneity and population structure for sharks. A mitochondrial DNA survey of shortfin mako (*Isurus oxyrinchus*) found significant differences in haplotype frequencies between the North and South Atlantic, but little structure across the large Indo-Pacific and South Atlantic expanses of its range (Heist *et al.* 1996a). Little or no genetic divergence was found for sharpnose sharks (*Rhizoprionodon terraenovae*) or sandbar sharks (*C. plumbeus*) from US coastal Atlantic waters and the Gulf of Mexico (Heist *et al.* 1995; 1996b).

Our sampling of lemon sharks did not provide uniform coverage of the western Atlantic portion of the species range, with three of four collection sites clustered in the Caribbean and Gulf of Mexico. However, the samples from Atol das Rocas, which lies over 6000 km farther south, provide data over a much larger spatial scale. Our results indicate that lemon sharks in the western Atlantic do not represent a single panmictic population. However, the statistically significant differences detected among populations were small, indicating that there is substantial gene flow among sites. Use of highly polymorphic loci may reveal statistically significant heterogeneity that may not be biologically meaningful (Hedrick 1999), and the heterogeneity we detected does not warrant recognition of distinct stocks or management units of lemon shark in the western Atlantic. Many shark species are known to migrate long distances (Casey & Kohler 1990), although it is unknown whether lemon sharks make transoceanic journeys. A recent recapture suggests they are at least capable of such journeys—a telemetry transmitter implanted in a newborn lemon shark in 1988 at Bimini was recovered from the coelom of this shark when it was captured in 1998 in Apalachicola Bay, Florida. This shark had travelled a minimum distance of about 1000 km and the journey required crossing the Gulf Stream through open ocean.

Nevertheless, it is somewhat surprising that there was no marked genetic discontinuity at the larger geographical scale when we compared the three northern populations

to the Brazilian site of Atol das Rocas. Although the Rocas population shows a slightly higher degree of differentiation, it shares most alleles with the northern populations. Furthermore, the Rocas population has relatively few private alleles (Fig. 2) and shared most common alleles, at all loci, with northern populations (Fig. 3). This indicates that either western Atlantic populations diverged recently or there is some trans-equatorial migration between northern and southern populations. In their study on mako sharks, Heist *et al.* (1996a) hypothesized that the equator represents a barrier to gene flow between northern and southern Atlantic populations, perhaps due to temperature preference of these sharks. For lemon sharks, no such barrier is apparent.

Estimates of gene flow derived from genetic data must be viewed with caution because of the assumptions underlying such estimates (Waples 1998). With microsatellites, there is uncertainty regarding the relative accuracies of measures based on the variance in repeat numbers ( $R_{ST}$ ) compared to those based on variance in allele frequencies alone ( $\theta$ ) (Paetkau *et al.* 1997; Gaggiotti *et al.* 1999). Gaggiotti *et al.* (1999) used simulation studies to demonstrate that  $\theta$ -based estimates perform better when sample sizes are small and the number of loci scored is low, while  $\rho$  performs better under ideal conditions of large sample sizes and many loci. In our study, we have relatively large sample sizes but only four loci. We are, however, relatively confident in our conclusions regarding migration rates because both variance estimates ( $\theta$  and  $\rho$ ) and the private alleles method provide similar and consistently high estimates of  $Nm$  (9–15) between populations.

The microsatellite data presented here indicate that the lemon shark populations in coastal waters of the entire western Atlantic, Caribbean and Gulf of Mexico probably represent a single stock for management purposes, with gene flow connecting the northern and southern hemisphere. We have, however, been unable to sample two important parts of the species range that are entirely disjunct from the western Atlantic, the coastal waters of the eastern Pacific, primarily off Mexico, and coastal populations in the eastern Atlantic off Africa. It would seem that barriers to gene flow between these populations and the western Atlantic would be insurmountable, but this needs to be tested.

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Kevin Feldheim is working in Mary Ashley's laboratory towards completion of a PhD on gene flow patterns and breeding biology of the lemon shark. Ashley's group uses molecular genetic approaches to study genetic structure, dispersal, and mating systems in a wide variety of taxa, including several threatened species. The research on lemon sharks is being conducted in close collaboration with Samuel Gruber and builds on his 30-year research effort to elucidate many aspects of lemon shark biology.

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