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Genetic similarity of disjunct populations of the giant sea bass *Stereolepis gigas*

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Sequence variation in nuclear and mitochondrial genes of the giant sea bass *Stereolepis gigas* collected from the Pacific coast and the northern Sea of Cortez was examined. Restriction fragment length polymorphism analysis and direct sequencing showed extremely low mtDNA sequence diversity (13 closely related haplotypes with no evidence of geographical population subdivision). The mitochondrial haplotype mismatch distribution is consistent with a population expansion following the Last Pleistocene glaciation. Differences in single nucleotide polymorphism frequencies between Pacific and Sea of Cortez populations were detected at two of four nuclear loci, which may reflect natural selection or genetic drift in populations with low effective numbers of males. Although Pacific coast and Sea of Cortez populations of giant sea bass do not exhibit the mitochondrial phylogenetic break characteristic of many species with disjunct Pacific and Gulf populations, the possibility of genetic differentiation at nuclear loci suggests that a cautious approach to broodstock selection for captive breeding and restoration programmes be exercised.

Key words: genetics; giant sea bass; mitochondrial DNA; phylogeography; single nucleotide polymorphisms; *Stereolepis gigas*.

INTRODUCTION

Despite its status as California's largest marine teleost, the giant sea bass *Stereolepis gigas* (Ayres, 1859) is not biologically well characterized. Giant sea bass are found along the Pacific coast from Humboldt Bay to the tip of the Baja peninsula (primarily from Point Conception southward) and in the northern Sea of Cortez (Crooke, 1992). Adults are commonly found in rocky habitat near kelp beds, while juveniles are found in both kelp bed areas and on sandy bottoms. Spawning has not been observed directly, but is believed to occur between July and September, following the formation of large aggregations containing both sexes (Domeier, 2001). Larvae spend *c.* 1 month in the plankton before settlement. Movement patterns of juveniles and adults are poorly known.

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Because giant sea bass grow slowly and mature at a relatively old age, they are susceptible to overfishing. As a consequence, they have suffered a serious decline in numbers and are considered to be critically endangered with extinction according to the International Union for the Conservation of Nature and Natural Resources (Baillie & Goombridge, 1996). Historical catch statistics for giant sea bass (Domeier, 2001) show commercial landings from U.S. waters peaking in 1932 near 91 000 kg before declining. Mexican waters were more productive (peaking at >363 000 kg in 1932) and did not permanently sink <91 000 kg until 1964. Giant sea bass were also targeted by a few hook-and-line anglers, and caught incidentally by gillnets set for halibut and white sea bass.

Recreational landings, reported in numbers of fish rather than pounds, show a similar trend of peaking and permanent declining. The peak in California landings occurred in 1963, while Mexican landings peaked in 1973. That these recreational fisheries peaked after the commercial fishery is due to the later development of the recreational fishery rather than a reflection of the giant sea bass population. Commercial and sport fishing for this species were banned in California in 1982 but there are no restrictions on fishing this species in Mexico, and recreational and commercial fishing continues.

Captive giant sea bass have been successfully spawned and the resulting larvae were reared through settlement (Shane *et al.*, 1996). Captive breeding programmes aimed at restoration of natural populations must take into account the geographical distribution of genetic diversity when selecting broodstock. In the case of giant sea bass, which is found in both the Pacific coast (primarily from Point Conception, U.S.A. to south of Bahia Magdalena, Mexico) and the northern Sea of Cortez, it is possible that significant genetic differentiation might exist between Pacific and Sea of Cortez populations (Bernardi *et al.*, 2003), if the latter came into existence *via* an mid-peninsular seaway during the Middle Pleistocene (Walker, 1960; Riddle *et al.*, 2000). Alternatively, these disjunct populations may be genetically similar if gene flow is occasionally facilitated by deep-water migration of adults, as suggested for giant sea bass (Walker, 1960). Therefore, patterns of genetic variation in both mitochondrial and nuclear genes were assessed in *S. gigas* from both the Pacific coast and the northern Sea of Cortez to determine the level of genetic variability in this species and the extent of differentiation between Pacific and Sea of Cortez populations.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PROCESSING

Tissue samples (skeletal muscle or fin clips) were taken from 56 specimens of *S. gigas* collected from the Pacific coast and the northern Sea of Cortez (Table I and Fig. 1). Samples were stored in 70–95% ethanol prior to extraction. DNA was extracted from tissue samples using DNA easy kits (Qiagen, Valencia, CA, USA). Restriction enzyme digests were conducted according to the manufacturer's instructions (New England Biolabs, Beverly, MA, USA; MBI Fermentas, Hanover, MD, USA). Automated DNA sequencing was performed in a Spectrumedix SC2410 capillary sequencer using ABI Big Dye 3.1 chemistry. Sequences were deposited in GenBank (accession numbers DQ336168–DQ336173).

TABLE I. *Stereolepis gigas* collections

Location	Number of individuals
Pacific coast	
Palos Verdes	5
Catalina Island	3
Oceanside	26
Santa Rosalia, Baja California	8
Sea of Cortez	14

MITOCHONDRIAL DNA

Universal primers were used for polymerase chain reaction (PCR) amplification of mitochondrial fragments, including (1) a *c.* 1·2 kb fragment containing the 3′ end of the tRNA-Met gene and the 5′ end of the nicotinamide adenine dinucleotide (NAD) dehydrogenase subunit 2 (ND2) gene, (2) a *c.* 1·4 kb fragment containing the control region (D-loop) and part of the small subunit (12S) rRNA gene, (3) a *c.* 0·7 kb fragment of the cytochrome oxidase subunit I gene (COI), (4) a *c.* 2·2 kb fragment spanning several genes (tRNA-Gly/ND3/tRNA-Arg/ND4L/ND4/tRNA-His) and a *c.* 1·6 kb fragment containing part of the mitochondrial cytochrome oxidase *b* gene, two tRNA genes and part of the control region (Table II).

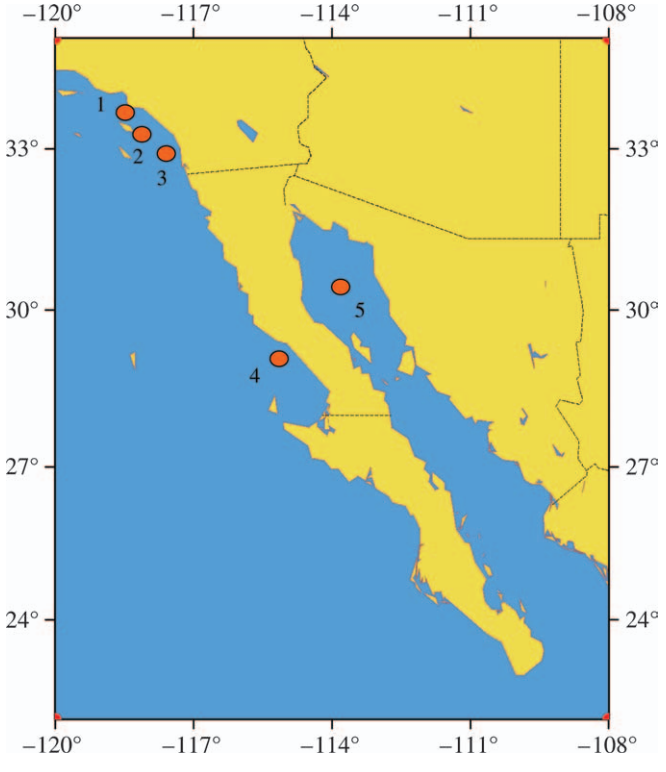

FIG. 1. Approximate locations of collection sites for *Stereolepis gigas*.

TABLE II. PCR primers used to amplify mitochondrial and nuclear genes in *Stereolepis gigas*

Region	Primer sequences	References
mtDNA		
ND2	AAGCTATCGGGCCCATACCC CCGCTTAGYGCTTTGAAGGC	Park *et al.* (1993); unpubl. data
Control region	AACTCTCACCCCTAGCTCCCAAAG ATAGTGGGGTATCTAATCCCAGTT	Meyer *et al.* (1994), Palumbi *et al.* (1991)
COI	CCTGCAGGAGGAGGAGAYCC AGTATAAGCGTCTGGGTAGTC	Palumbi *et al.* (1991)
Cyt *b*/D-loop	CATCCAACATCTCAGCATGATGAAA CCTGAAGTAGGAACCAGATG	Palumbi *et al.* (1991), Meyer *et al.* (1994)
ND3/4	GTACACGTGACTTCCAATCA AGAATCACAATCTAATGTTT	Park *et al.* (1993)
Nuclear		
Calmodulin intron 4	CTGACCATGATGGCCAGAAA GTTAGCTTCTCCCCCAGGTT	Chow (1988)
G6PD intron 4	GAGCAGACGTATTTTGTGGG GCCAGGTAGAAGAGGCGGTT	Chow & Nakadate (2004)
LDH-A intron 1	GCAGGAACAAGGTGACGGTGGT GATCCGTGCTGCAGGTCCATGAC	Unpubl. data
Rhodopsin	CNTATGAATAYCCTCAGTACTACC TGCTTGTTCATGCAGATGTAGA	Chen *et al.* (2003)
RPS7 intron 2	AGCGCCAAAATAGTGAAGCC GCCTTCAGGTCAGAGTTCAT	Chow & Hazama (1998)

All primers are presented in 5′→3′ orientation.

For four of the five mitochondrial amplicons, panels of 16–24 individuals were screened with a battery of restriction enzymes, with digestions performed as specified by the manufacturer (New England Biolabs) in 20 μl reactions containing 5 units of enzyme per reaction. Digestions were incubated for a minimum of 5 h before being stopped with loading dye (20% Ficoll 400, 0·1 M Na_2EDTA, pH 8, 1% sodium dodecyl sulphate, 0·25% bromophenol blue and 0·25% orange G). Digests were run on 2% agarose gels, stained with ethidium bromide and viewed with an ultraviolet transilluminator. The control region amplicon was screened with 15 restriction enzymes (*Acc*I, *Alu*I, *Ava*II, *BamH*I, *Bfa*I, *Dde*I, *Dpn*II, *Hae*III, *Hha*I, *Hpa*II, *Mse*I, *Msp*I, *Nla*III, *Rsa*I and *Sau96*I), the COI amplicon was screened with 16 restriction enzymes (*Alu*I, *Alw26*I, *Ava*II, *Bfa*I, *Bsr*I, *Dpn*II, *Hae*III, *Hha*I, *Hinf*I, *Hpa*II, *Mse*I, *Nla*III, *Rsa*I, *Sau96*I, *Taq*I and *Tsp509*I) and the cyt *b*/D-loop amplicon was screened with 15 restriction enzymes (*Alu*I, *Alw26*I, *BamH*I, *Bsr*I, *Dpn*II, *Fsp*I, *Hae*III, *Hha*I, *Hinf*I, *Hpa*II, *Mse*I, *Nla*III, *Rsa*I, *Taq*I and *Tsp509*I), while the ND3/4 amplicon was screened with only two restriction enzymes (*Alu*I and *Dpn*II).

In addition to restriction fragment length polymorphism (RFLP) analysis, two mitochondrial amplicons (ND2 and control region) from 53 individuals were directly sequenced. For the ND2 amplicon, only the forward PCR primer proved suitable as a sequencing primer, generating 739 bp of sequence, including the 3′ terminus of tRNA-Met (25 bp) and 714 bp of ND2 coding sequence. For the control region, sequence data were obtained for a 1351 bp fragment (GenBank DQ336169) spanning the 3′ end of tRNA-Pro (21 bp), the D-loop (846 bp), tRNA-Phe and the 5′ end of the small subunit rRNA gene (416 bp).

NUCLEAR DNA

Five nuclear regions were amplified using generic primers as presented in Table II: calmodulin intron 4, lactate dehydrogenase A intron 1 (LDH), glucose 6 phosphate dehydrogenase intron 4 (G6PD), rhodopsin (Rh) and ribosomal protein (RP) S7 intron 2.

STATISTICAL ANALYSES

Statistical analyses of genetic data were conducted using a number of software programmes. For nuclear genes containing more than one polymorphic nucleotide position, haplotypes were inferred using PHASE 2.1 (Stephens *et al.*, 2001; Stephens & Donnelly, 2003). All probabilities for phase assignments were >0·95, except for three cases (G6PD for the one Sea of Cortez individual and LDH for the two Pacific coast individuals), which were classified as haplotype unknown. Exact tests of fit to Hardy–Weinberg expectations (HWE) were performed using the Markov chain of Guo & Thompson (1992), implemented by the programme GENEPOP 3.4 (http://wbiomed.curtin.edu.au/genepop/index.html). This programme was also used to calculate heterozygote deficiencies/excesses as measured by F_{IS} as formulated by Weir & Cockerham (1984), and to estimate the number of migrants between populations using the private allele method of Barton & Slatkin (1986).

Arlequin 3.01 (Excoffier *et al.*, 2005) was used for analyses of population structure, including the analysis of molecular variance (AMOVA), inference of demographic history and the estimation of migration between populations using mitochondrial sequence data. StatXact 4.0.1 (Cytel) provided exact R×C tests for comparison of allelic frequencies. DnaSP 4.10.4 (Rozas *et al.*, 2003) was used to perform tests of neutrality (Tajima's *D*) and to analyse mismatch distributions (Slatkin & Hudson, 1991; Rogers & Harpending, 1992) for the inference of demographic history from mtDNA data. Relationships among mitochondrial haplotypes were depicted by a median-joining network (Bandelt *et al.*, 1999) calculated using NETWORK 4.1.1.1 (www.fluxus-engineering.com).

RESULTS

MITOCHONDRIAL DNA

Restriction digest analyses revealed no polymorphisms in the COI amplicon (screened with 16 enzymes), the control region amplicon (screened with 15 enzymes), the cyt *b*/D-loop amplicon (screened with 15 enzymes) or the ND3/4 amplicon (screened with two enzymes). Direct sequencing of the ND2 amplicon (739 bp, GenBank DQ336172) revealed three variable sites; two were synonymous third-position transitions and one was a transition causing a conservative amino acid replacement (Ala→Thr) in three individuals from California. Direct sequencing of the control region amplicon (1351 bp, GenBank DQ336169) revealed seven-variable nucleotide positions (six transitions, one transversion): four in the D-loop, one in the tRNA-Phe gene and two in the 12S rRNA gene.

Sequence data for the two mitochondrial regions were concatenated to yield a 2090 bp composite sequence, which exhibited 13 haplotypes in the 53 individuals sequenced. Of these, four (C, F, H and I) were common and were found in both the Pacific and the Sea of Cortez samples (Table III). The haplotype median-joining network shows little evidence of phylogeographic structure (Fig. 2). This is reflected in the AMOVA analysis: the comparison of the Pacific *v.* the Sea of Cortez samples showed $F_{ST} = 0$, with 100% variation occurring within subpopulations, *i.e.* haplotypes drawn randomly from the two regions were on average as similar to each other as haplotypes drawn from the same region. Overall sequence diversity was extremely low (0·00086 in the Pacific sample and 0·0010 in the Sea of Cortez sample), despite the inclusion of the entire D-loop region, which for most vertebrates is the most variable segment of the mitochondrial genome.

Because the Pacific and the Sea of Cortez samples showed homogeneity of mitochondrial haplotype frequencies, they were pooled for subsequent analyses. The observed frequency distribution of haplotypes was consistent with a historical population expansion, as shown by a negative (but not significant) value of Tajima's (1989) D (−0.64) and a significant value of Fu's (1997) F_s statistic (−4·83, $P = 0{\cdot}015$). Fu's F_s test is more powerful for detecting population

TABLE III. Geographical distribution of mitochondrial haplotypes in *Stereolepis gigas*

	A	B	C	D	E	F	G	H	I	J	K	L	M
Pacific coast													
Palos Verdes			1		1	1				1			
Santa Catalina Island								1	2				
Oceanside		2	3	1	2	6		5	2	1		2	
Santa Rosalia, Pacific Baja						2		3		1	1		1
Sea of Cortez													
Northern Gulf of California	2		3			3	1	2	1				2

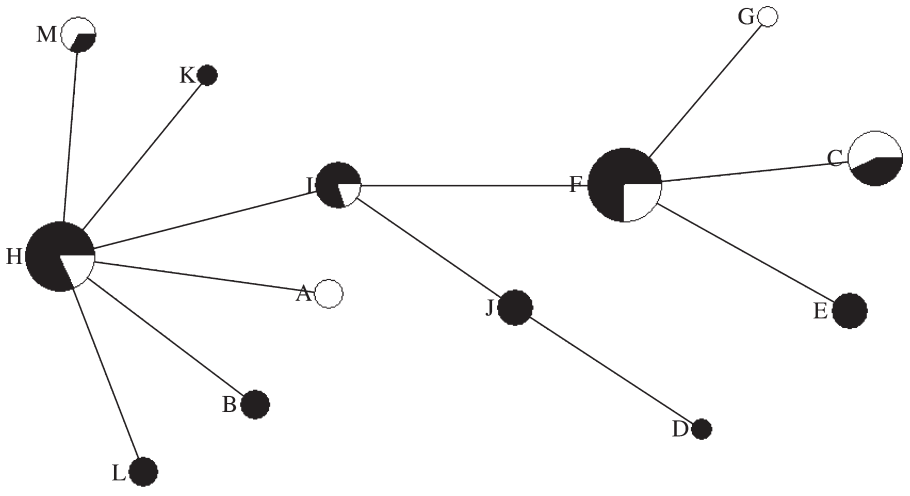

FIG. 2. Median-joining network depicting genealogical relationships among 13 mitochondrial DNA haplotypes in *Stereolepis gigas*. Size of circle is approximately scaled to haplotype frequency. Black, Pacific coast; white, northern Gulf of California.

expansion and selective sweeps (removal of linked neutral variants during the fixation of a selectively advantageous mutation) than that of Tajima's *D* (Ramos-Onsins & Rozas, 2002). Historical population growth is also indicated by unimodal wave of the mismatch distribution for mitochondrial sequences (Fig. 3).

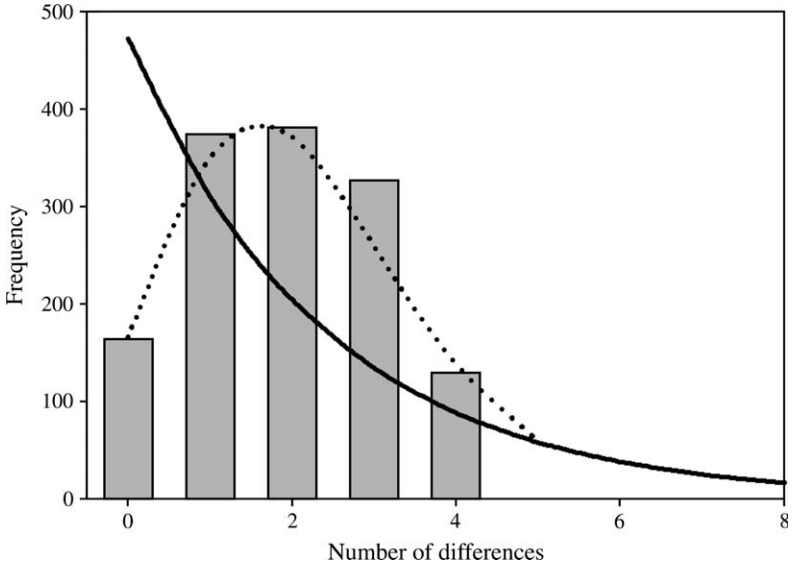

FIG. 3. Observed mitochondrial DNA haplotype mismatch distribution (▭) and theoretical cures for an historical population expansion (••••) and for constant population size (▬).

NUCLEAR GENES

Calmodulin

The calmodulin primers amplified a 421 bp product. PCR products directly sequenced with the forward primer exhibited poor quality (multiple overlapping peaks) in the region corresponding to nucleotides 1–231, after which clean sequence was obtained. Conversely, products sequenced with the reverse primer showed clear traces from positions 1–188 only. Alignment of the high-quality portions showed no polymorphism in 33 individuals sequenced. To obtain less ambiguous sequence data for the amplicon, multiple clones (total $n = 18$) were sequenced from four individuals. Amplicons were cloned using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) and sequenced using T3 and T7 primers. Of the cloned sequences, 13 were identical and matched the sequence obtained by direct cycle sequencing. Each of the remaining five clones differed from the consensus sequence at a different nucleotide. These variants may represent *Taq* polymerase error, or the amplification of very similar paralogues, or both. In support of the latter possibility, it is noteworthy that the calmodulin gene in the pufferfish *Tetraodon nigroviridis* Marion de Procé, 1822, most similar to that of *S. gigas*, has two chromosomal copies (GSTENT00014816001 and GSTENT00018439001) at the Tetraodon Genome Browser (http://www.genoscope.cns.fr/externe/tetranew/), which show 100% nucleotide identity over the region examined in *S. gigas*. The low-level co-amplification of a second nearly identical locus may have been responsible for the substandard quality of the cycle sequence data. This locus was not included in the data analysis.

G6PD intron 4

Direct sequencing of the G6PD amplicon from 50 specimens revealed three intronic polymorphisms, a G/T transversion at position 557 and two C/T polymorphisms at nucleotides 629 and 924 of GenBank DQ336170. For some chromatograms, single nucleotide polymorphism (SNP) 557 was difficult to score, so this polymorphism was scored by digesting the amplicon with *Pvu*II. RFLP profiles were consistent with the original chromatogram interpretation for 18 of 20 individuals; the two discrepancies were G/T heterozygotes originally scored as T/T homozygotes.

Individual SNPs exhibited similar frequencies in the Pacific and the Sea of Cortez samples, with HWE proportions in both (Table IV). Phase inference yielded four haplotypes (GCC, GCT, TCC and TTC), which showed similar frequencies in the Pacific and in the Sea of Cortez samples, with HWE proportions in both (Table V).

LDH-A intron 1

The LDH amplicon was sequenced in both directions with the original PCR primers to yield 739 bp of sequence data (686 bp intron, 53 bp exon; GenBank DQ336171). Sequences were obtained from 53 specimens, revealing three intronic polymorphisms at nucleotide positions 127 (A/G), 388 (C/T) and 467 (A/G) and four inferred haplotypes (ACA, GCA, GCG and GTG).

Two of three SNP loci exhibited frequency differences between Pacific and Sea of Cortez samples; one of these (LDH388, $P = 0{\cdot}002$) was significant after

TABLE IV. Allelic frequencies at individual SNP loci

Amplicon	SNP	Pacific coast	Sea of Cortez	Combined	*P*(Diff)
G6PD	557:G	0·750	0·850	0·770	0·554
	F_{IS}	0·212	0·640	0·275	
	P(HWE)	0·218	0·162	0·099	
	629:C	0·763	0·900	0·790	0·229
	F_{IS}	0·115	−0·059	0·106	
	P(HWE)	0·657	1·000	0·419	
	924:C	0·975	0·900	0·960	0·178
	F_{IS}	−0·013	−0·059	−0·032	
	P(HWE)	1·000	1·000	1·000	
LDH	127:G	0·814	0·800	0·811	1·00
	F_{IS}	−0·217	−0·200	−0·223	
	P(HWE)	0·311	1·000	0·177	
	388:C	0·933	0·650	0·882	0·002*
	F_{IS}	0·296	0·386	0·397	
	P(HWE)	0·175	0·144	0·017	
	467:A	0·900	0·650	0·855	0·009†
	F_{IS}	0·147	0·386	0·277	
	P(HWE)	0·390	0·137	0·068	
Rhodopsin	146:A	1·00	0·900	0·982	0·031
	F_{IS}	NA	−0·059	−0·009	
	P(HWE)	NA	1·000	1·000	
RPS7	525:C	0·944	0·900	0·954	0·610
	F_{IS}	−0·035	0·000	−0·039	
	P(HWE)	1·000	1·000	1·000	

P(HWE), exact test probability of fit to HWE; *P*(Diff), exact probability of difference in allele frequencies between Pacific and Sea of Cortez populations.

*Significant after sequential Bonferroni correction with tablewide $\alpha = 0{\cdot}07$.

†Significant after sequential Bonferroni correction with tablewide $\alpha = 0{\cdot}05$.

NA = Not applicable.

sequential Bonferroni correction, while the other (LDH467, $P = 0{\cdot}009$) was nearly so (Table IV). Differences in inferred haplotype frequencies were nearly significant ($P = 0{\cdot}071$).

Rhodopsin

Sequence data (GenBank DQ336173) were obtained for 813 bp of intronless coding sequence yielding 271 amino acids corresponding to residues 34–304 of the Rh gene from mullet (CAA77248). Sequences from 55 individuals showed almost no variation, except for the two Sea of Cortez individuals heterozygous (A/C) at nucleotide position 146, a synonymous transition. The rare C allele differs significantly in frequency between samples from the Pacific and the Sea of Cortez ($q = 0$ *v.*10%, Fisher's exact test $P = 0{\cdot}031$).

Ribosomal protein S7 intron 2

The RPS7 amplicon was sequenced in both directions with the original PCR primers to yield 716 bp of sequence (42 bp exon, 684 bp intron; GenBank

TABLE V. Inferred haplotype frequencies for two nuclear loci in *Stereolepis gigas*

Locus	Haplotype	Sea of Cortez	Pacific coast	Combined	*P*(Diff)
G6PD	GCC	0·900	0·718	0·755	0·262
	GCT	0·050	0·026	0·031	
	TCC	0·000	0·013	0·010	
	TTC	0·050	0·244	0·204	
	n	10	39	49	
	F_{IS}	−0·029	0·107	0·114	
	P(HWE)	1·00	0·291	0·222	
LDH-A	ACA	0·250	0·079	0·125	0·071
	GCA	0·071	0·013	0·029	
	GCG	0·536	0·724	0·673	
	GTG	0·143	0·184	0·173	
	n	14	38	52	
	F_{IS}	0·347	−0·013	0·126	
	P(HWE)	0·529	0·132	0·020	

P(HWE), exact test probability of fit to HWE; *P*(Diff), exact probability of difference in haplotype frequencies between Pacific and Sea of Cortez populations.

DQ336168). A single C/G intronic polymorphism was detected at position 525. Digestion of the amplicon with *Hinc*II confirmed the genotypes inferred from sequence chromatograms. The frequency of the G allele was similar in the Pacific and in the Sea of Cortez samples, with no departures from HWE observed.

Multilocus analyses

For the combined nuclear data set, AMOVA showed negligible differentiation between Pacific and Sea of Cortez populations, when inferred haplotypes or individual SNPs were analysed (F_{ST} = 0·00016 and 0, respectively), with virtually all variation occurring within populations (99·98 and 100%, respectively). However, the probability of genic differentiation across loci (probabilities from individual tests combined by Fisher's method) was nearly significant when the set of eight SNPs was considered ($P = 0{\cdot}063$), or when the four genes were considered ($P = 0{\cdot}0734$). The number of migrants (Nm) per generation between Pacific and Gulf populations estimated by the private allele method of Barton & Slatkin (1986) was likewise low for both the SNP data set (Nm = 1·09) and the four genes (Nm = 4·36). These estimates are clearly different from Nm = ∞ estimated from the mitochondrial sequence data using the approach of Slatkin (1995).

DISCUSSION

Two features emerged from both RFLP and direct sequence analysis of mitochondrial and nuclear genes in *S. gigas*: extremely low sequence diversity and the lack of clear genetic differentiation between Pacific and Sea of Cortez populations. *Stereolepis gigas* is one of a number of marine fishes that are

found in the northern Sea of Cortez and in the northern Pacific coast of the Baja California peninsula, but are rare or absent in the warmer waters of the southern peninsula (Walker, 1960; Bernardi *et al.*, 2003). The latter authors examined mtDNA variation in 12 such species and found that eight displayed reciprocal monophyly and high genetic divergence between populations, consistent with ancient vicariant events separating the Gulf and Pacific populations. These species also showed genetic differentiation in Pacific coast populations north and south of Punta Eugenia, a phylogeographic boundary (Briggs, 1974; Terry *et al.*, 2000). The remaining four species did not show a Pacific–Gulf break, suggesting high levels of current or recent gene flow. The giant sea bass falls into this latter category, with no indication of phylogeographic separation visible in mtDNA (Fig. 1). It seems probable that individual *S. gigas* may be able to circumnavigate the cape region frequently enough to prevent substantial genetic divergence, as Walker (1960) suggested.

For mtDNA, the pattern of low nucleotide diversity ($\pi = 0{\cdot}09\%$) and high haplotype diversity ($h = 0{\cdot}88$) places the giant sea bass in category 2 of Grant & Bowen (1998), typical of species that appear to have undergone a demographic expansion after a period of low effective population size. Another species in category 2, the red grouper *Epinephelus morio* (Valenciennes, 1828), is thought to have experienced a population bottleneck during the Late Pleistocene, followed by recovery to its present abundance (Richardson & Gold, 1997).

The mismatch distribution of mitochondrial haplotypes in *S. gigas* is smooth and unimodal (Fig. 2), consistent with historical population expansion and clearly different from the 'ragged' multimodal pattern expected from an equilibrium population of constant size (Rogers & Harpending, 1992). This is underscored by the significant result of Fu's F_s test, where the high negative value indicates an excess of rare haplotypes – a pattern associated with either historical population growth or a selective sweep (Fu, 1997). While the inference of a historical population expansion seems incompatible with the well-known recent decline in abundance of this species, it is probable that the genetic signature observed here reflects a more substantial earlier expansion event rather than the very recent (*c.* 10 generations) population decline. This counter-intuitive pattern has also been noted in the coconut crab, which has experienced a dramatic decline in abundance over the past several 100 years (Lavery *et al.*, 1996).

The time of the inferred expansion can be estimated by analysing the mismatch distribution to provide an estimate of $\tau = 2\ \mu t$ (where μ is the mutation rate and t is the generation time in years), with confidence intervals (Schneider & Excoffier, 1999). Using a generation time of $t = 10$ years and a mutation rate of 10^{-8} per nucleotide, the time of the population expansion may be estimated as *c.* 10 200 years ago, with 95% confidence limits of *c.* 5900 and 13 500 years. This suggests a population expansion following the end of the last glaciation in the eastern Pacific, *c.* 10 000–11 000 years ago, a scenario opposite to that suggested by Graham *et al.* (2003), in which populations of large fish such as *S. gigas* declined as expanses of Ice Age kelp forest gave way to sandy habitats.

In contrast to the picture of genetic uniformity among Pacific coast and Sea of Cortez populations presented by mtDNA, nuclear genes showed evidence of potential differentiation in two of the four genes examined (LDH-A and Rh).

The homogeneity of mtDNA haplotypes implies contemporary or recent gene flow, which should act to prevent differentiation at neutral nuclear loci. Explanations for this disparity include natural selection acting in the fact of gene flow and random genetic drift. In the first case, selected loci may show differences in allelic frequencies despite moderate gene flow. Random genetic drift, on the contrary, is generally expected to be more pronounced for mtDNA genes than for nuclear genes, since the effective population size for mitochondrial genes is one quarter that of nuclear genes in the ideal population. Therefore, one would have expected to see greater geographic differentiation in mitochondrial genes than in nuclear genes, a common observation reflected in the 'three-times rule' (Palumbi *et al.*, 2001). However, this is only true for species with equal effective numbers of males and females; species with sex ratios skewed in favour of females or high variance in reproductive success in males may show more rapid genetic drift in nuclear genes. For example, when $N_f/N_m > 7$, the effective population size of nuclear genes is actually smaller than that of mitochondrial genes (Storz *et al.*, 2001).

Little is known about the reproductive biology of *S. gigas*. In related species that are protogynous hermaphrodites, overfishing has been associated with extremely skewed female:male sex ratios. For example, the frequency of males in Gulf coast gag [*Mycteroperca microlepis* (Goode & Bean, 1879)] dropped from 17 to 1%, from the 1970s to the 1990s (Coleman *et al.*, 1996). Available data on *S. gigas* (unpubl. data) show size distributions of males and females to be similar and sex ratios to be roughly 1:1, suggesting that this species is not a sequential hermaphrodite. It is possible, however, that reproductive competition among males may lead to a higher variance in reproductive success compared with females, enabling nuclear loci to drift more rapidly than mitochondrial genes. Sampling additional individuals and additional nuclear loci should determine whether this apparent disparity between mitochondrial and nuclear genes is real.

The goal of this study was to determine whether populations of giant sea bass from the Pacific and the Sea of Cortez are genetically homogeneous, or similar enough that stock restoration programmes might safely use broodstock from various geographic sources for captive breeding. While the mitochondrial sequence data are consistent with high levels of gene flow between disjunct populations of *S. gigas* along the Pacific coast and the northern Sea of Cortez, nuclear SNP frequencies suggest very limited exchange. The allelic frequency differences observed at two of four nuclear loci examined may reflect the action of natural selection, genetic drift, or both, but do not necessarily indicate a major phylogeographic break that would distinguish evolutionarily significant units. Sampling additional nuclear genes will clarify the nature and extent of genetic differentiation between Pacific coast and Sea of Cortez giant sea bass populations. In the interim, a conservative approach using locally or regionally derived broodstock for restoration programmes is recommended.

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