SCI. MAR., 67 (4): 451-460 SCIENTIA MARINA 2003

Microsatellite variation in the Mexican rockfish *Sebastes macdonaldi**

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SUMMARY: The Mexican rockfish *Sebastes macdonaldi* is the Northeast Pacific rockfish with the southernmost distribution, featuring isolated populations in the Gulf of California. We analysed seven microsatellite loci in 111 organisms collected throughout most of its geographical range to test long-standing hypotheses regarding its disjunct distribution. One locus was fixed and the number of alleles in polymorphic loci ranged from 2 to 24 (average 13.5). We found very high levels of polymorphism (overall He = 0.75) comparable to other congeneric species but no significant differences in genetic diversity among localities or between Pacific and Gulf of California populations ($p > 0.1$). Significant shifts in allelic and genotypic frequencies were detected at three loci, which resulted in a small but significant partitioning of genetic variance among California, Baja California and Gulf of California populations ($F_{ST} = 0.007$, $p = 0.03$) and between Gulf and Pacific populations ($F_{ST} = 0.01$, $p = 0.004$). The latter but not the former result was corroborated by analyses of molecular variance (AMOVA) using the number of distinct alleles (F_{ST}) and the sum of square differences of allele sizes (R_{ST}) as Euclidean distances. The evidence argues against contemporary gene flow between the gulf and the Pacific ocean and against an ancient invasion of the gulf with a founder effect. The small level of divergence favours a recent dispersal but a larger data set including DNA sequences amenable to phylogenetic analyses will help to test alternative hypotheses of dispersal versus vicariance.

Key words: Mexican rockfish, disjunct distribution, microsatellites, genetic structure, Baja California, México.

RESUMEN: VARIACIÓN DE MICROSATÉLITES EN EL ROCKOT MEXICANO *SEBASTES MACDONALDI*. – El rockot mexicano *Sebastes macdonaldi* es la especie de *Sebastes* del Pacífico nororiental con la distribución geográfica más sureña e incluye poblaciones aisladas dentro del Golfo de California. Analizamos siete loci microsatelitales en 111 organismos que se colectaron a lo largo de gran parte de su distribución geográfica para probar hipótesis acerca de su distribución discontinua. Un locus se encontró fijado y el número de alelos en loci polimórficos varió entre 2 y 24 (media 13.5). Encontramos altos niveles de polimorfismo (en general He = 0.75) comparables con otras especies congenéricas, sin embargo no se encontraron diferencias en los niveles de diversidad entre las localidades ni entre las poblaciones del Pacífico y del Golfo de California ($p > 0.1$). Se detectaron cambios significativos en las frecuencias alélicas y genotípicas en tres loci, que resultaron en una división pequeña pero significativa de la varianza genética entre California, Baja California y el Golfo de California ($F_{ST} = 0.007$, $p = 0.03$) y entre las poblaciones del golfo y del Pacífico ($F_{ST} = 0.01$, $p = 0.004$). Solamente esta última diferenciación fue corroborada por un análisis de variancia molecular (AMOVA) en el que se utilizaron el número de alelos distintos (F_{ST}) y la suma de diferencias cuadráticas en tamaños alélicos (R_{ST}) como distancias Euclideanas. La evidencia contradice la presencia de flujo genético contemporáneo entre el golfo y el Pacífico así como una invasión antigua del golfo mediante un evento fundador. El bajo nivel de diferenciación favorece la posibilidad de una dispersión reciente dentro del golfo pero se necesitan datos adicionales que incluyan secuencias de ADN que permitan análisis filogenéticos para probar hipótesis más precisas sobre escenarios de dispersión y vicariancia.

Palabras clave: rockot mexicano, distribución discontinua, microsatélites, estructura genética, Baja California, México.

*Received January 7, 2003. Accepted June 3, 2003.

INTRODUCTION

Rockfishes of the genus *Sebastes* represent a challenge for the study of marine speciation and evolution. Among their most relevant biological features are their mode of reproduction, their large number of species and their large morphological and ecological diversities. Rockfishes all have internal fertilisation, an unusual feature among marine teleosts, and matrotrophic viviparity has been shown in some species (e.g. Boehlert *et al.*, 1991). The very large number of species is undoubtedly their best known attribute, as *Sebastes* represents the most numerous genus in the family Scorpaenidae, with more than 110 species worldwide (Eitner *et al.*, 1999; Nelson, 1994). In concert with their species-rich nature, rockfishes are also well known for their conspicuous morphological and ecological diversity. This is reflected in a variety of forms and colorations ranging from small and slender pelagic planktivores, like *Sebastes goodei*, to bulky and heavily spined sit-and-wait predators, like *Sebastes nebulosus* (Eschmeyer and Herald, 1983). Finally, the antitropical distribution (Fig. 1) of this group of cold-temperate fish has been considered one of the most interesting anomalies among scorpaenids (Eschmeyer and Hureau, 1971). Even though the origin of the genus appears to be among subtropical western Pacific scorpaenids (Washington *et al.*, 1984), their present centre of distribution is on the western coast of North America, where more than 70% of the species are found in very high levels of sympatry (Chen, 1971; Rocha-Olivares *et al.*, 1999c).

Of the seven species of *Sebastes* found in the Gulf of California (Chen, 1975), only *S. macdonaldi* is also distributed in the Pacific coast of Baja California (Chen, 1975; Moser, 1971). *S. macdonaldi* is the Northeast Pacific rockfish with the southernmost distribution, ranging from Point Sur, Central California, to offshore banks off Bahía Magdalena, Baja California (Fig. 1). The species distribution is discontinuous since it has not been reported further south at the entrance to the Gulf of California. Within the gulf, Mexican rockfish is found near Guaymas and in Bahía de Los Angeles (Chen, 1975; Moser, 1971; Thomson *et al.*, 2000).

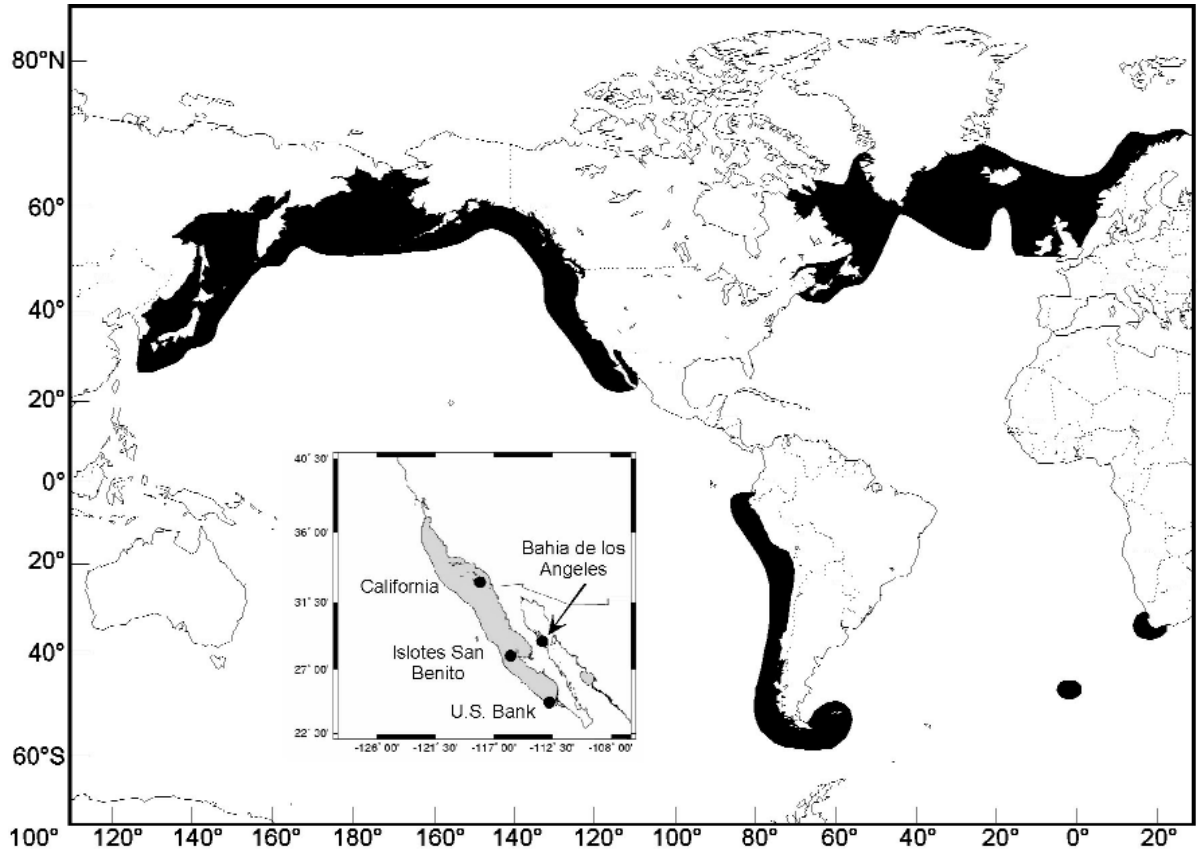

FIG. 1. – World wide distribution of the genus *Sebastes*. Insert: geographic range of *S. macdonaldi* and sampling localities (modified from Rocha-Olivares *et al.*, 1999a).

TABLE 1. – Sampling localities of *Sebastes macdonaldi*; n = sample size.

Locality	Collection date	n	Abbreviation[1]
Los Angeles, California, U.S.	March-2000/March-2002	14	CAL
Islotes San Benito, México	August-1996	8	BAJA
Uncle Sam Bank, México	August-1996	45	BAJA
Bahía de los Angeles, México	March-1995/August-1995	44	BLA

[1] refers to the name identifying hitherto these samples. See text.

Populations in the gulf are thus isolated from those in the Pacific coast by the Baja California peninsula and by the influence, throughout most of the gulf, of warm tropical waters flowing North from the Costa Rica Coastal Current. This disjunct distribution raises a couple of fundamental biogeographical and evolutionary questions: (1) What is the level of differentiation of the populations residing in the Gulf of California? (2) Are populations within the Gulf of California vicariant relicts or are they descendants of a founder population that invaded from the Pacific? These questions have remained unanswered for a long time (Chen, 1975) and can be addressed using genetics.

Microsatellites are co-dominant molecular genetic markers inherited following Mendelian rules and consist of DNA tandem repeats dispersed throughout most eukaryotic nuclear genomes. Polymorphisms in these loci are manifested as variations in the number of tandem repeats, e.g. (AC)n, introduced during DNA replication, resulting from their insertions or deletions following mechanisms not entirely understood (Colson and Goldstein, 1999; Falush and Iwasa, 1999). Repeating motifs of microsatellites range from 1-10 base pairs (bp), but most loci frequently used range from 2-6 bp (Hancock, 1999). Due to their high mutation rates and levels of polymorphism, microsatellites are powerful markers to address a variety of genetic, ecological, and evolutionary questions (e.g. Baker, 2000; Dowling *et al.*, 1996; Machugh *et al.*, 1996).

Here we use microsatellites to study Mexican rockfish from Gulf of California and Pacific populations in order to test null hypotheses derived from the questions above: (1) the levels of molecular genetic diversity are the same in the gulf and in the Pacific, and a smaller genetic diversity would be expected if Gulf of California populations were derived from a small set of founder individuals that invaded from the Pacific; and (2) Gulf of California populations of Mexican rockfish are not genetically differentiated from those in the Pacific.

MATERIALS AND METHODS

Sampling

Fish were obtained by hook and line from depths of ca. 200 m between March 1995 and March 2002. Samples were taken from three localities off the Pacific coast of North America and one from the Gulf of California (Fig. 1). Because of the small sample size, data from Islotes San Benito (n = 8) were pooled for analyses with those from Uncle Sam Bank (henceforth referred to as sample BAJA, see Table 1). Tissue samples (muscle and liver) were dissected from fish and preserved in 95% ethanol (EtOH). Samples were maintained at room temperature in the field and at 4 °C in the laboratory until molecular analyses.

DNA extraction and purification

Total genomic DNA was extracted from 25-100 mg of preserved tissue digested overnight at room temperature in 500 µl DNAzol (Molecular Research Center, Inc.) and 100 µg proteinase K (Gibco BRL). DNA purification was carried out according to the manufacturer's protocol and purified DNA was precipitated in 250 µl 100% EtOH and rinsed in 800 µl 75% EtOH. Purified DNA was finally re-dissolved in 50 µl TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 8.0). The quality and quantity of extracted DNA was visually assessed in a 1% agarose minigel (0.5X TBE) stained with ethidium bromide (0.5 µg/ml).

DNA amplification

Seven microsatellite alleles were PCR amplified with fluorescently marked primers designed for *Sebastes rastrelliger* (cf. GenBank AF269052-AF269061). Five PCR master mixes were prepared (BRL PCR Reagent System, Gibco BRL): primers for loci *Sra*5-9+*Sra*7-2 and *Sra*7-7+*Sra*11-103 were multiplexed whereas loci *Sra*7-25, *Sra*16-5 and

*Sra*15-8 were prepared separately. The thermal cycling profile was: initial 2.5 min at 90°C, followed by 35 cycles of 45 sec at 95°C, 1 min at 53 or 57°C, and 1 min at 72°C; with a final extension of 5 min at 72°C (PTC-200 ABI thermal cycler). The presence of amplified product was checked with a 1.5% agarose minigel stained with ethidium bromide (0.5 µg/ml).

Microsatellite genotyping

Diluted PCR products (1:2 or 1:10) were electrophoresed in Long-Ranger denaturing 6% polyacrylamide gels (FMC Bioproducts). Amplified alleles were detected with a DNA automated sequencer (Gene Analyzer ABI 377) and their sizes were determined with the program GeneScan 3.3, using a fluorescent ladder (Rox 500) loaded in each experimental lane.

Data analyses

Genetic diversity

Based on a large dataset of *Sebastes* spp genotypes for these seven loci (R.D. Vetter, C.A. Kimbrell and E.A. Lynn, unpublished data), allele size (bp) was converted into number of tandem repeats. Individual diploid genotypes were represented as the combination of both alleles, expressed as their size in repeat numbers, to compute genotypic and allelic frequencies per locus for each locality. Expected heterozygosity (He) and the average squared difference in allele size (Rho) were also computed for each locus (Robertson and Hill, 1984; Weir, 1996). Goodness of fit to Hardy-Weinberg (H-W) expectations was calculated for genotypic frequencies and significance was determined using a Markov chain approach (Guo and Thompson, 1992) and linkage disequilibrium tests were performed for all loci pairs (Ohta, 1982). The previous analyses were performed with GENEPOP 3.3 (Raymond and Rousset, 1995). Levels of genetic diversity (He) among localities were tested non-parametrically with STATISTICA 5.5 (StatSoft Inc., 1999).

Genetic differentiation

Genetic structure was quantified as the level of heterogeneity in allelic and genotypic frequencies with the program GENEPOP 3.3 (Raymond and Rousset, 1995). The test of allelic differentiation provides an unbiased estimate of the level of significance of the test described in Raymond and Rousset (1995), whereas the genotypic differentiation test is based on the G exact test of Goudet *et al.* (1996). We also computed fixation indices as F_{ST}, under the approximation of an analysis of variance (Cockerham, 1973; Weir and Cockerham, 1984), and its analogous R_{ST}, for population pairwise comparisons. The latter incorporates the correlation of the weighted mean allele size expressed as the number of tandem repeats (Michalakis and Excoffier, 1996). Sequential Bonferroni correction (Rice, 1989) was used to adjust α-levels for multiple testing. Finally, we performed an analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) using values of the pairwise number of distinct alleles (F_{ST}) or the sum of square differences of allele sizes (R_{ST}) as Euclidean distances with the program ARLEQUIN 2.0 (Schneider *et al.*, 1999).

RESULTS

Genetic diversity

Of the seven microsatellite loci, the repeating motifs of four were dinucleotides, one was a trinu-

TABLE 2. – Levels of microsatellite polymorphism in *S. macdonaldi*.

Locus	Bp[1]	A[2]	*n*[3]	Mean allele size[4]	s_	C.V. (%)	He			Rho		
							CAL	BAJA	BLA	CAL	BAJA	BLA
Sra5-9	2	1	109	96.1	n.d.	n.d.	0	0	0	0	0	0
Sra7-2	2	24	110	167.5	114.7	6.39	0.904	0.914	0.912	74.49	57.87	63.92
Sra7-7	2	12	111	191.9	25.3	2.62	0.651	0.748	0.782	9.52	13.35	13.12
Sra7-25	2	8	103	173.5	54.8	4.27	0.591	0.502	0.654	29.71	23.38	33.87
Sra11-103	3	2	110	269.6	2.0	0.53	0.506	0.469	0.473	0.51	0.47	0.47
Sra16-5	4	19	99	236.7	20.7	1.92	0.930	0.910	0.916	24.72	22.78	33.08
Sra15-8	4	16	108	326.4	201.0	4.34	0.920	0.904	0.914	16.81	22.64	28.46
Average[5]		13.5					0.750	0.741	0.775	25.96	23.42	28.32

[1] length of repetitive DNA motif in bp; [2] number of alleles; [3] number of individual genotypes included in analyses; [4] in bp; [5] for polymorphic loci; n.d. not defined.

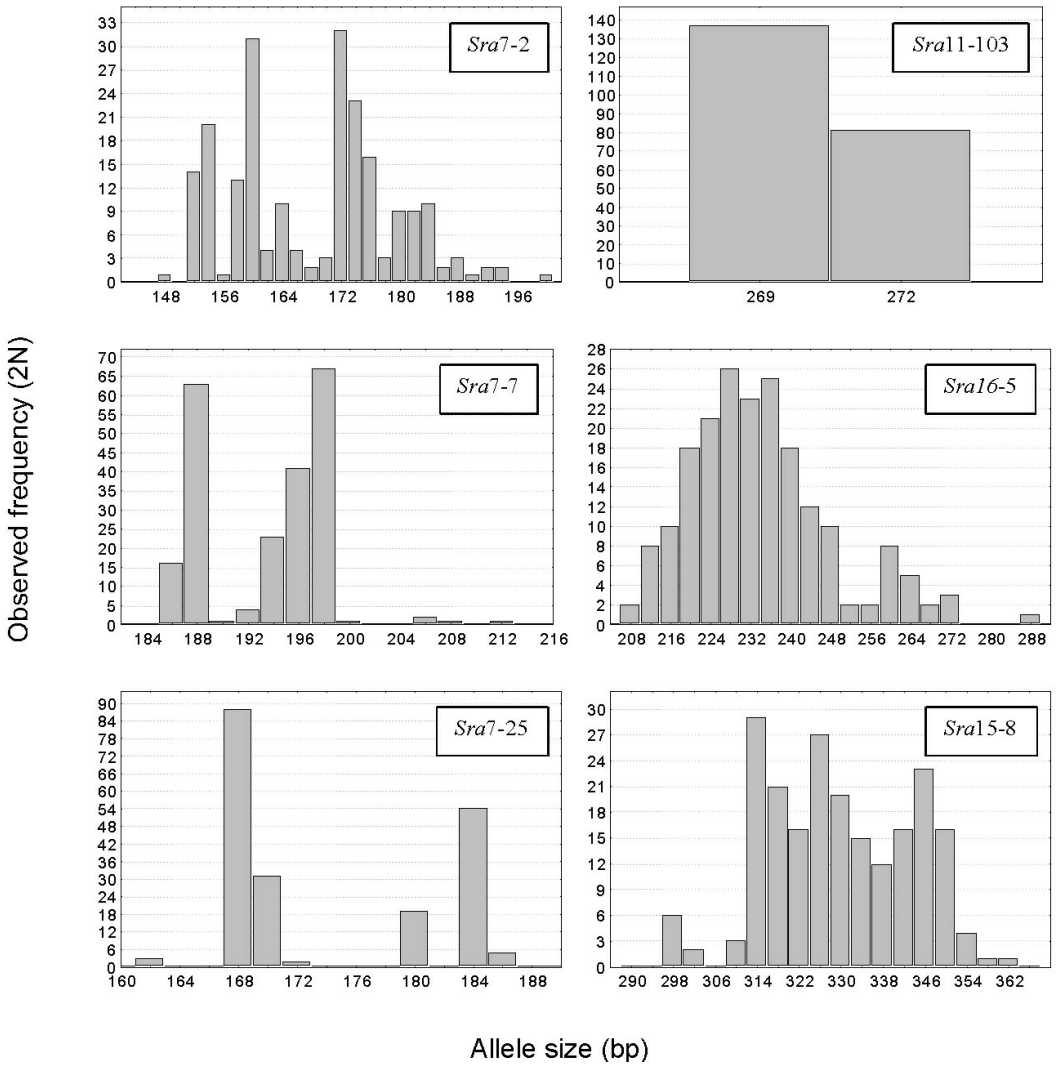

FIG. 2. – Allele size frequency distributions of the seven polymorphic microsatellite loci of *Sebastes macdonaldi* used in this study.

cleotide and two were tetranucleotides. They were all polymorphic in *Sebastes macdonaldi* with the exception of locus *Sra*5-9 (Table 2). The number of alleles in polymorphic loci ranged from 2 to 24 (average 13.5). The degree of polymorphism (numbers and frequencies of alleles) was variable (Fig. 2, Table 2). Locus *Sra*11-103 was bi-allelic and hence the least variable. The rest of allelic distributions were multimodal and bimodal for three of the loci (Fig. 2). Coefficients of variation (CV) in allele size reflect the genic diversity of microsatellite loci. For example, in the case of locus *Sra*7-2 CV it is the highest (> 6%) due to the wide range of allele sizes, whereas for locus *Sra*11-103 CV it is an order of magnitude smaller (< 0.6%) (Table 2).

Genotypic frequencies of all loci were found to conform to H-W and linkage equilibria within all localities ($p > 0.05$). Levels of expected heterozygosity were variable among loci and among localities (Table 2) and very high (He > 0.9) for loci *Sra*7-2, *Sra*16-5 and *Sra*15-8, whereas the locus with the smallest He was *Sra*11-103 (He < 0.51) at all sampled localities. Rho was largest for loci *Sra*7-2, *Sra*7-25, and *Sra*15-8 (Rho > 16.8). Levels of molecular genetic diversity (He) were not significantly different among the three localities (Kruskall-Wallis H, $p = 0.67$) or between the Pacific and the Gulf of California (Mann-Whitney U, $p = 0.11$).

Population differentiation

The degree of allelic differentiation was tested among the three localities (BLA, BAJA and CAL) as well as between the Gulf of California and the Pacific (BLA and BAJA+CAL). Allelic and genotypic frequencies of all polymorphic loci were not signif-

TABLE 3. – Significance levels (S.E.) in test of allelic and genotypic differentiation for microsatellite loci of *S. macdonaldi.*

Locus	3 localities (BLA, BAJA, CAL) Allelic	Genotypic	Gulf vs. Pacific (BLA, BAJA+CAL) Allelic	Genotypic
Sra7-2	0.629 (0.019)	0.455 (0.018)	0.577 (0.015)	0.590 (0.013)
Sra7-7	0.059 (0.008)	0.067 (0.007)	**0.001 (0.001)**	**0.003 (0.001)**
Sra7-25	0.213 (0.012)	0.158 (0.009)	**0.046 (0.005)**	0.076 (0.004)
Sra11-103	0.825 (0.005)	0.872 (0.003)	1.000 (0.000)	0.895 (0.002)
Sra16-5	0.265 (0.014)	0.279 (0.018)	0.133 (0.009)	0.138 (0.008)
Sra15-8	0.206 (0.012)	0.308 (0.019)	**0.030 (0.004)**	**0.039 (0.004)**
Overall		0.198		**0.005**

TABLE 4. – Significance levels in test of pairwise allelic and genotypic differentiation for microsatellite loci of *S. macdonaldi*.

Comparison	Type	Locus Sra7-2	Sra7-7	Sra7-25	Sra11-103	Sra16-5	Sra15-8	Overall
BLA-BAJA	Allelic	0.512	0.011	0.044	1.000	0.124	0.023	
	Genotypic	0.405	0.018	0.058	1.000	0.094	0.041	0.009
BAJA-CAL	Allelic	0.586	0.968	0.572	0.663	0.575	0.868	
	Genotypic	0.293	0.905	0.586	0.671	0.574	0.909	0.925
BLA-CAL	Allelic	0.610	0.074	0.891	0.656	0.663	0.550	
	Genotypic	0.584	0.043	0.561	0.678	0.734	0.523	0.512

icantly heterogeneous among the three localities ($p > 0.059$). However, significant frequency shifts were found between the gulf and the Pacific in allele frequencies of loci *Sra*7-7, *Sra*7-25 and *Sra*15-8 ($p < 0.05$) and in genotypic frequencies of loci *Sra*7-7, *Sra*15-8 and all loci pooled together ($p < 0.04$) (Table 3). Pairwise comparisons showed significant allelic differentiation (individual $p < 0.05$) at loci *Sra*7-7, *Sra*7-25 and *Sra*15-8 between BLA and BAJA. The same was true for genotypic frequencies, except that for locus *Sra*7-25 the differentiation was marginally non-significant (individual $p = 0.058$). After sequential Bonferroni correction for multiple tests, none of the pairwise comparisons of allelic and genotypic frequencies was significant (Table 4).

Fixation indices based on allelic frequencies (F_{ST}) were very small for all loci and localities (< 3.94%) but significant for loci *Sra*7-7, *Sra*7-25 and over all loci among the three localities. Comparison between Gulf and Pacific fish resulted in significant fixation indices for loci *Sra*7-7 and over all loci, whereas loci *Sra*7-25 and *Sra*15-8 were marginally

TABLE 5. – Fixation indices (F_{ST}) of microsatellite loci of *S. macdonaldi*. Significance (in parenthesis) is shown only for values < 0.055.

Locality[1]	Locus Sra7-2	Sra7-7	Sra7-25	Sra11-103	Sra16-5	Sra15-8	Overall
3	-0.0038	**0.0283 (0.0068)**	0.0230 **(0.0440)**	-0.0145	0.0014	0.0041	**0.0066 (0.0312)**
2	0.0005	**0.0394 (0.0000)**	0.0204 (0.0537)	-0.0108	0.0053	0.0089 (0.0518)	**0.0113 (0.0039)**

[1] 3 refers to BLA, BAJA and CAL and 2 to Gulf vs. Pacific

TABLE 6. – Fixation indices F_{ST} and R_{ST} (below the diagonal) and their respective significance values (above the diagonal) from pairwise comparisons of polymorphic microsatellite loci of *S. macdonaldi*.

F_{ST}	BAJA	BLA	CAL	RST	BAJA	BLA	CAL
BAJA	-	**0.0351**	0.8887		-	**0.0332**	0.6875
BLA	**0.0070**	-	0.1621		**0.0192**	-	0.4658
CAL	-0.0087	0.0059	-		-0.1170	-0.0050	-

TABLE 7. – Analysis of molecular variance (AMOVA) of microsatellite loci of *S. macdonaldi* using the number of distinct alleles (F_{ST}) and the sum of square differences of allele sizes (R_{ST}) as Euclidean distances.

Component	Partition Variance	% total	Φ_{ST}	p[1]
F_{ST} CAL, BAJA & BLA				
Between populations	0.0077	0.36	0.0034	0.1095
Within populations	2.0994	99.64		
F_{ST} Gulf vs Pacific				
Between populations	0.0177	0.84	0.0084	0.0088
Within populations	2.0956	99.16		
R_{ST} CAL, BAJA & BLA				
Between populations	0.6453	0.89	0.0089	0.1310
Within populations	71.7403	99.11		
R_{ST} Gulf vs Pacific				
Between populations	1.1987	1.65	0.0165	0.0303
Within populations	71.5580	98.35		

[1] p values calculated from random permutation tests, represent probability of obtaining by chance alone a more extreme variance component and Φ-statistic than the one observed (Excoffier *et al.,* 1992).

non-significant (Table 5). Pairwise F_{ST} and R_{ST} values were also small (F_{ST} <0.7% R_{ST} < 2.0%) and not significant after sequential Bonferroni correction (Table 6). Finally, AMOVA results corroborated that no significant molecular genetic variance (expressed as F_{ST} or R_{ST}) was associated with subdivision among the three localities ($p > 0.1$), though significant (R_{ST}, $p = 0.03$) and highly significant (F_{ST}, $p = 0.009$) Φ-statistics were obtained between Gulf and Pacific populations (Table 7).

DISCUSSION

Genetic diversity

Even though the loci and primers used in this study were originally described and designed for a congener (*Sebastes rastrelliger*), they all have worked satisfactorily in *S. macdonaldi* as well as in other Northeast Pacific species (R.D. Vetter, C.A. Kimbrell and E.A. Lynn, unpublished data, Buonaccorsi *et al.*, 2002). This remarkable level of cross-species microsatellite conservation is a clear indication of the close molecular relatedness of species of *Sebastes* that has surfaced in other microsatellite studies (e.g. Miller *et al.*, 2000; Roques *et al.*, 1999; Wimberger *et al.*, 1999) as well as other molecular investigations (Rocha-Olivares *et al.*, 1999a; Rocha-Olivares *et al.*, 2000; Rocha-Olivares *et al.*, 1999b; Rocha-Olivares *et al.*, 1999c). Most loci analysed were polymorphic, except for one. A total of 78 alleles were found in polymorphic loci (average per locus = 13.5), though all loci were not equally variable. Loci *Sra*7-2, *Sra*16-5, and *Sra*15-8 display greater allelic diversity as well as high levels of heterozygosity. All genotypic frequencies were in H-W equilibrium, suggesting that null alleles were unlikely to have been encountered, biasing genotypic frequencies. Heterozygosity levels were large (He > 0.50) for loci *Sra*7-2, *Sra*7-7, *Sra*7-25, *Sra*16-5, and *Sra*15-8.

Molecular genetic diversity in fish has been found to be associated with life history traits reflecting habitat types. Marine species generally possess significantly higher levels of genetic diversity (average He = 0.79) than freshwater (average He = 0.46) or anadromous (average He = 0.68) species (DeWoody and Avise, 2000). Therefore, the high heterozygosities found in *S. macdonaldi* ($0.74 \leq He \leq 0.78$) are not atypical. A very close value (mean He = 0.72 in a set of shared loci) was found among 400 grass rockfish *S. rastrelliger* sampled along the Pacific coasts of Oregon and California (Buonaccorsi *et al.*, unpublished data). Buonaccorsi *et al.* (2002) studied exactly the same loci analysed here (except for *Sra*7-2) in the copper rockfish *Sebastes caurinus* finding $0.63 \leq He \leq 0.71$ in fish sampled throughout its geographic range. Overlapping levels of microsatellite heterozygosity (0.5-0.87) were found at six loci of the Pacific ocean perch *Sebastes alutus* (Miller *et al.*, 2000). In contrast, only three of five polymorphic loci described for the quillback rockfish *S. maliger* possessed comparable levels of He (0.66-0.79), whereas the others showed low polymorphism (He < 0.5) (Wimberger *et al.*, 1999). Eight microsatellite loci described for the four North Atlantic redfish (*S. fasciatus, S. mentella, S. marinus,* and *S. viviparus*) were also highly polymorphic ($0.5 \leq He \leq 0.96$) and at least half the loci had He > 0.8 in all species (Roques *et al.*, 1999).

Germane to the zoogeographical hypothesis is the fact that no significant difference in molecular diversity was found between Pacific (mean He = 0.75) and Gulf of California populations (mean He = 0.78). A high genetic diversity in the Gulf of California is not consistent with a recent founder event. At face value, very similar levels of heterozygosity in the gulf and Pacific populations suggest that: (1) Gulf of California populations are not reproductively isolated from those in the Pacific; (2) there was an ancient dispersal event and invasion of the gulf from the Pacific that may have resulted in a founder

effect, but the signal has been eroded by the rapid mutation rate of microsatellite loci; (3) there was never a genetic founder effect and contemporary gulf populations were separated from the Pacific by vicariance; or finally (4) the invasion is too recent and/or the colonisers too numerous for random genetic drift to cause a detectable drop in genetic diversity. Admittedly, some of these hypotheses have specific predictions regarding the pattern and extent of genetic differentiation to be found among geographic regions.

Population structure

The patterns of allelic and genotypic differentiation suggest that significant differentiation is associated with comparisons between fish from the Pacific and the Gulf of California. On the other hand, the absence of differentiation between Pacific samples (i.e., CAL and BAJA) may reflect high levels of gene flow along the Pacific coast of California and Baja California, but the small sample size of CAL (n = 14) limits the statistical power. A larger sample size is required from California to corroborate this observation. Three of the seven loci (*Sra*7-7, *Sra*7-25 and *Sra*15-8) were mainly responsible for the observed significant differentiation (Tables 3 and 4). These significant allelic and genotypic frequency shifts were sufficient to translate into a weak but significant partitioning of the genetic variance among localities (overall F_{ST} = 0.0066, p = 0.03) and an even stronger differentiation between gulf and Pacific organisms (overall F_{ST} = 0.0113, p = 0.004). In a study that included the same loci used here, except for *Sra*11-103, Buonaccorsi *et al.* (unpublished data) also found a low and marginally significant partitioning (F_{ST} = 0.0013, p = 0.0497) in the grass rockfish *S. rastrelliger* distributed continuously along the coasts of California and Oregon. The same loci, however, were all significantly differentiated (overall F_{ST} = 0.036, $p < 0.001$) among populations of copper rockfish (Buonaccorsi *et al.*, 2002).

A small, albeit statistically significant, level of genetic differentiation is consistent with the *de facto* isolation of the Gulf of California populations from the Pacific. Ecological studies and direct observations suggest that bottom dwelling *Sebastes,* such as *Sebastes macdonaldi,* are unlikely to engage in long distance migrations but rather are relatively sedentary around rocky outcrops and other hard structures they inhabit (e.g., Love *et al.*, 1999; Love *et al.*, 2000), and even some midwater *Sebastes* species appear to have small radii of movement in short time scales (Pearcy, 1992). Thus, it seems unlikely that *S. macdonaldi* in particular would travel thousands of kilometres around the tip of the Baja California peninsula into the Gulf of California. This unlikely journey would have to entail some kind of southern submergence, as surface and subsurface waters are presumably too warm for the survival of the species larvae and juveniles, in order to reach regions marked by strong upwelling in the Gulf of California, where established populations are viable. Thus, hypothesis number one above lacks any positive evidence, is unparsimonious, and is not supported by the data. Hypothesis number two, involving an ancient founder event, is also inconsistent with the data. Presumably-isolated populations of Puget Sound copper rockfish *S. caurinus* were found to be highly divergent from nearby samples taken from the Gulf Islands (F_{ST} = 0.037) and from the Pacific coast (F_{ST} = 0.087) at the same loci studied here. The age of Puget Sound populations cannot be more than 11,500 years, a time at which the sound became marine after being filled with fresh water as the glaciers melted in the area (Buonaccorsi *et al.*, 2002). The degree of genetic partitioning between Gulf of California and Pacific populations of the Mexican rockfish is only one third to one seventh of that amount. The large genetic differentiation observed in the copper rockfish may be partly explained by natural selection (Buonaccorsi *et al.*, 2002), as Pacific and Puget Sound fish may face different selective pressures. However, it is unlikely that all loci would have been equally affected by genetic hitchhiking to a selected locus, and one can also argue that populations of the Gulf of California are subject to a very different physical environment than those in the Pacific (Alvarez-Borrego, 2001; Chen, 1975). Thus, an ancient invasion of the gulf is not consistent with the low level of genetic differentiation. Distinguishing between hypotheses three and four above may prove to be difficult with the data at hand. Both hypotheses may be consistent with the main findings of this study, namely comparable levels of genetic diversity and little differentiation between gulf and Pacific populations. The small level of genetic differentiation would favour a recent dispersal since vicariance would entail a separation that is at least a million years old, according to the hypothesised middle Pleistocene midpeninsular seaway connecting the gulf and the Pacific ocean (Riddle *et al.*, 2000). Analysis of another genetic marker amenable to phylogenetic analyses, such as mito-

chondrial or nuclear DNA sequences, may help to elucidate the historical scenario. In conclusion, we have found small but significant levels of genetic differentiation between allopatric and isolated populations of the Mexican rockfish in the Gulf of California from those in the Pacific coast of North America. The evidence argues against contemporary gene flow between the two regions and an ancient invasion of the gulf with a founder effect. A larger data set including DNA sequences amenable to phylogenetic analyses will help to test alternative hypotheses of dispersal versus vicariance.

ACKNOWLEDGMENTS

We gratefully acknowledge the help and support of the California Department of Fish and Game and the *R.V. Mako* crew and volunteers for sample collection. Funding was provided by CONACYT grant I36064-V and CICESE (Rocha-Olivares) and NOAA-NMFS-SWFSC (Vetter). This paper is partly derived from R. A. Leal-Navarro undergraduate Honors Thesis at the Universidad Autónoma de Baja California.

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Scient. ed.: G. Pequeño