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GENETIC STRUCTURE OF GREEN ABALONE *HALIOTIS FULGENS* POPULATION OFF BAJA CALIFORNIA, MEXICO

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ABSTRACT The design of appropriate management plans of the green abalone *Haliotis fulgens* fishery needs a better understanding of the present status of the genetic diversity of the wild stock, as well as its genetic structure. Samples from nine locations along the Baja California Peninsula, including one from an oceanic island (Isla Guadalupe), were obtained covering the areas where the commercial fishery is active. DNA was extracted from muscle tissue of 50 individuals from each location, and was used for PCR amplification of 4 microsatellites (*Hka28*, *Hka56*, *Hful260*, and *Hful603*). The number of alleles observed in all samples with *Hka28* and *Hka56* (23–35 and 15–19, respectively) was higher than that observed in *Hful260*, and *Hful603* (3 and 6 alleles, respectively). A relatively high mean heterozygosity was observed in all locations with the lowest value of 0.687 in Isla Guadalupe. A deviation from Hardy-Weinberg Equilibrium (HWE) caused by a heterozygote deficiency was only observed in 2 out of 36 tests, indicating that this disequilibrium is random. An AMOVA showed a significant F_{ST} ($P < 0.00196$) suggesting genetic differentiation among locations. Pairwise analyses using F_{ST} and allele frequencies showed that the significant difference was caused by Isla Guadalupe, which indicates a restricted gene flow between this and the other locations. Nevertheless, no significant differences were observed among sites along the Peninsula. The implications of these results on the management of the fishery are discussed.

KEY WORDS: abalone, *Haliotis fulgens*, genetic diversity, microsatellites

INTRODUCTION

Abalone is an economically important fishery resource in the Western coast of the Baja California Peninsula. Its actual annual production of 285 t is valued at US$26 million (Carreón-Palau et al. 2003). In spite of the high commercial value of the *Haliotis* spp. fishery in the world, there is a poor understanding of the population genetic structure of some abalone species (Withler 2000).

There are several studies of the genetic structure of haliotids along the eastern Pacific, which results are rather contrasting. The analyses of the genetic structure of red abalone *Haliotis rufescens* in California by means of allozymes (Gaffney et al. 1996), the mitochondrial gene COI (Burton & Tegner 2000), and one microsatellite (Kirby et al. 1998), suggested that this species represents an undifferentiated population. In California, Hamm and Burton (2000) found population differentiation in the *H. cracherodii* with three allozyme loci. Zúñiga et al. (2000) analyzing six allozyme loci found genetic homogeneity in green abalone *H. fulgens* at some locations of the central part of Baja California. In the same region but in island locations, Del Río Portilla and González Avilés (2001) observed population differentiation in the yellow abalone *H. corrugata*. The use of different genetic markers to determine the genetic structure is adequate to get a better picture on what happens in each haliotid species.

A proper knowledge of the genetic structure and diversity of abalone populations is an important task to aid fishery managers to define fishery stocks and analyze data and models in terms of the geographical and ecological limits of the populations.

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Because of their high variability, microsatellites have been widely used to determine the genetic structure of marine species. For haliotids, microsatellites have been characterized for *H. rufescens* (Kirby et al. 1998), *H. rubra* (Huang & Hanna 1998, Evans et al. 2000), *H. discus* (Sekino & Hara 2001), and *H. kamtschatkana* (Miller et al. 2001) to be used for natural and aquaculture population studies. Recently, Cruz et al. (2005) developed microsatellites for *H. fulgens* and Gutierrez-Gonzalez and Perez-Enriquez (2005) showed the use of *H. kamtschatkana* microsatellites in *H. fulgens* analyses.

Because at the moment few microsatellites has been isolated for green abalone, in this study we analyzed the allelic frequencies of four microsatellite loci of *H. fulgens* in several locations along the west coast of the Baja California Peninsula, Mexico, to determine the levels of genetic diversity and estimate the population differentiation.

MATERIAL AND METHODS

Sample Collection Sites and Study Area

Genetic diversity analyses were carried out at nine locations off Baja California: Isla Guadalupe, Isla Cedros, Punta Eugenia, Bahia Tortugas, Bahia Asuncion, Punta Abreojos, San Juanico, and Isla Magdalena (Fig. 1). The samples at each location came from single beds, with the exception of Bahia Tortugas where two beds were sampled: Bahia Tortugas and Corralito, which were treated as different locations. Sampling was done from the commercial catch during the fishing season from February to June 2000. An epipodium fragment of 50 individuals per site was fixed and stored in 95% ethanol. DNA extraction was carried out following Sweijd et al. (1998).

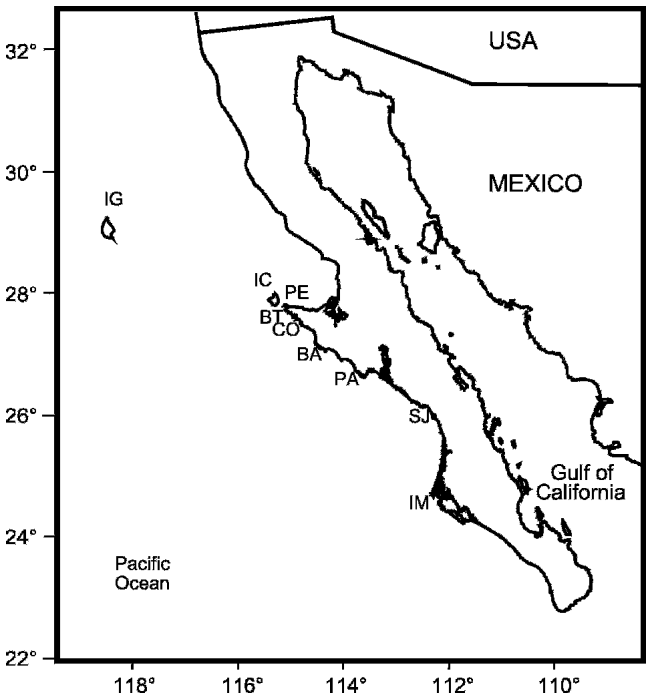

Figure 1. Nine sample sites of *Haliotis fulgens* along the west coast of Baja California Peninsula. IG: Isla Guadalupe, IC: Isla de Cedros, PE: Punta Eugenia, BT: Bahia Tortugas, CO: Corralito, BA: Bahia Asunción, PA: Punta Abreojos, SJ: San Juanico, and IM: Isla Magdalena.

Microsatellite Analysis

The microsatellites used for the genetic analysis were *Hka28, Hka56* (Miller et al. 2001), *Hful603* (Cruz et al. 2005), and *Hful260* (see below for description of isolation). The amplification criteria for PCR were the same as those described in Gutierrez-Gonzalez and Perez-Enriquez (2005), with annealing temperatures for *Hka28, Hka56, Hful603* and *Hful260* of 52°C, 52°C, 54°C, and 57°C, respectively.

To obtain *Hful260,* a microsatellite isolation procedure was performed following Sambrook et al. (1989) and Takagi et al. (1997). Briefly, using the DNA of two individuals, a *Hae* III partial genome library was constructed and 400–900 bp fragments were purified using a QIAquick Gel Extraction kit (QIAGEN). Fragments were ligated into a *Sma* I digested pBluescript II SK plasmid, which were used to transform *E. coli* DH5-α competent cells by thermal shock. The cells were grown on LB agar plates containing ampicillin, IPTG, and X-Gal; white colonies were isolated and transferred to nylon membranes, where the DNA was fixed and hybridized with a biotinylated $(GT)_{10}$ probe following a nonradioactive protocol (Bronstein et al. 1990). Chemiluminescence detection was carried out by means of Phototope-Star Detection Kit (NEB). DNA from positive clones was purified from plasmids with a QIAprep Spin Miniprep Kit (QIAGEN). DNA was sequenced using T3 and T7 primers in an ABI Prism 310 sequencer (Perkin Elmer). Sequences containing microsatellites were used for primer design and deposited in GenBank (Access nos. AF436390, AF436391, AF436392, and AF436393). Of the four microsatellites, the only useful locus was *Hful260,* because two others were monomorphic, and primers could not be designed for the remainder.

Data Analysis

Allele frequencies were calculated and used to estimate genetic diversity parameters, such as alleles number (NA) per locus, and observed (H_o) and expected heterozygosity (H_e). Departures from Hardy-Weinberg Equilibrium (HWE) at each locus and population were estimated by Fisher's exact test, employing the Markov chain method implemented in GENEPOP ver. 3.4 (Raymond & Rousset 1995).

Genetic differentiation among locations was carried out by an analysis of molecular variance (AMOVA) performed with ARLEQUIN ver. 2000 (Schneider et al. 2000), which calculated the Wright fixation index (F_{ST}). Pairwise F_{ST} values between all populations, with their significances, were also calculated with FSTAT ver. 2.9.3.2 (Goudet 1995). Critical significant levels for both tests were adjusted using a sequential Bonferroni approach (Rice 1989).

A regression analysis of F_{ST} versus the logarithm of the geographical distance for all pairs of locations was done to test isolation-by-distance using GENEPOP. A Mantel test with 10,000 permutations was used to test the significance of this correlation.

RESULTS

Genetic Diversity

The level of polymorphism of microsatellites widely varied among them, ranging from 4 alleles at *Hful260* to 36 alleles at *Hka28* (Table 1). The highest number of alleles (N_A) was observed in *Hka28* locus ranging from 21 in Punta Eugenia to 36 in San Juanico and Bahia Tortugas. The number of alleles was similar among populations with mean values per locus between 11.75 and 15. Despite the Isla Guadalupe sample having the highest number of alleles at *Hful260* (3) and *Hful603* (6) loci, this locality showed the least mean number of alleles (11.75).

Observed and expected heterozygosities were also similar across the nine locations (Table 1), with mean values ranging between 0.687 and 0.737. Isla Guadalupe showed lowest mean value ($H_o = 0.687$), in contrast to Isla Cedros, which had the highest ($H_o = 0.737$). Despite a smaller sample size, Corralito had a mean heterozygosity ($H_o = 0.721$) compared with the rest of the locations with high diversity values.

In only two of 36 possible tests, significant deviations from HWE were observed after the sequential Bonferroni procedure (Table 1), indicating that most locations conformed to HWE. Those two cases (i.e., Punta Eugenia at *Hka28*, and Bahia Tortugas at *Hka56*) were caused by heterozygous deficiencies. F_{IS} values did not show any trend to either homozygous or heterozygous excess (data not shown).

Allele Frequencies

Size frequency distribution was similar in *Hka28* and *Hka56* loci, in which the vast majority showed frequencies of less of 10% (Fig. 2 and Fig. 3), with no alleles surpassing 20%. The most notable differences in the *Hka28* locus (Fig. 2) were in allele *221* (16%) at Isla Guadalupe, and alleles *207* (12%) and *213* (15%) at Punta Eugenia. Allele *98* (12%) in *Hka56* locus (Fig. 3) at Isla Guadalupe, was absent at San Juanico, Punta Abreojos, and Bahía Tortugas; and at minor frequencies at the rest of the locations.

TABLE 1.

Genetic diversity at four microsatellites in 9 samples of *Haliotis fulgens*; N_A, allele number; n, sample size; H_o, observed heterozygosity; H_e, expected heterozygosity; P, probability.

Loci	I. Magdalena	S. Juanico	P. Abreojos	B. Asunción	B. Tortugas	Corralito	P. Eugenia	I. Cedros	I. Guadalupe
Hka28									
n	50	50	50	48	50	40	43	48	51
N_A	29	36	33	31	36	33	21	28	24
H_o	0.900	0.940	0.980	0.917	1.000	0.950	0.837	0.918	0.882
H_e	0.957	0.966	0.962	0.962	0.964	0.966	0.934	0.947	0.939
P	0.103	0.176	0.234	0.108	1.000	0.272	0.0027*	0.130	0.054
Hka56									
n	50	49	50	48	50	40	43	50	52
N_A	15	16	18	15	16	16	20	17	14
H_o	0.920	0.939	0.940	0.979	0.780	0.950	0.954	0.940	0.846
H_e	0.910	0.902	0.908	0.918	0.928	0.915	0.915	0.919	0.904
P	0.480	0.451	0.780	0.985	0.0002*	0.894	0.818	0.775	0.098
Hful260									
n	50	50	48	46	50	40	42	49	52
N_A	2	2	2	2	2	2	3	2	3
H_o	0.400	0.380	0.438	0.435	0.440	0.400	0.571	0.429	0.481
H_e	0.425	0.447	0.488	0.472	0.440	0.426	0.511	0.452	0.463
P	0.464	0.225	0.336	0.413	0.625	0.489	0.831	0.481	0.644
Hful603									
n	50	49	50	48	50	41	43	50	52
N_A	4	5	5	3	6	4	4	5	6
H_o	0.660	0.510	0.540	0.563	0.620	0.585	0.535	0.660	0.538
H_e	0.595	0.602	0.600	0.549	0.620	0.541	0.588	0.625	0.591
P	0.885	0.239	0.312	0.592	0.467	0.743	0.298	0.795	0.060
Mean									
n	50	49.50	49.50	47.50	50.00	40.25	42.75	49.25	51.75
N_A	12.50	14.75	14.50	12.75	15.00	13.75	12.00	13.00	11.75
H_o	0.720	0.692	0.724	0.723	0.710	0.721	0.724	0.737	0.687
H_e	0.722	0.729	0.739	0.725	0.738	0.712	0.737	0.736	0.724

* Significant departure from HWE ($P < 0.05$) after the Bonferroni adjustment for 36 pairwise tests.

The frequencies of the main alleles of the *Hful260* locus (Fig. 4a), *196* and *199*, were similar in all locations. For the *Hful603* locus (Fig. 4b), the allele *196* showed the highest value (56%) at Bahía Asunción. In contrast to the other loci, neither of these two loci showed evident genetic differences among the locations.

Population Genetic Structure

Although the analysis of molecular variance (AMOVA) showed that most of the variation was found within locations, the estimated F_{ST} of 0.00062 was significantly different from zero ($P = 0.00196$) (Table 2), indicating a population differentiation among locations. The pairwise F_{ST} analysis indicated that Isla Guadalupe was responsible for the difference. Few other differences among the locations situated along the Peninsula coast were also obtained (Table 3). Nevertheless, a hierarchical AMOVA left Isla Guadalupe as a separated group, with no significant differences among the rest of the locations.

Overall, the genetic differences were not explained by geographical distance, because the isolation-by-distance analysis showed no significant correlations (Fig. 5).

DISCUSSION

Even though the abalone fishery in Mexico is considered as depleted and in an incipient state of recovery (Muciño-Diaz & Sierra-Rodriguz 2005), the high genetic diversity observed across all sampling locations along Baja California Peninsula, indicates that *H. fulgens* population has not passed through a bottleneck as yet. This is similar to what has been observed in other "overexploited" abalone species, such as *H. kamtschatkana* populations from Canada in which an analysis with 12 microsatellites has also revealed high diversity parameters (mean number of alleles per locus = 25.5 and mean $H_o = 0.89$) (Withler et al. 2001). With the exception of two cases, all locations were in Hardy-Weinberg equilibrium showing that the population is randomly distributed. This is unlike with haliotids species in which heterozygote deficits, because of population mixing, inbreeding, or null alleles, are rather common (Huang et al. 2000; Zuñiga et al. 2000, Del Río-Portilla & González-Avilés 2001, Conod et al. 2002, Withler et al. 2001, Maynard et al. 2004). Heterozygous deficiency is also frequently observed in other marine invertebrates (Gaffney et al. 1990, Bierne et al. 1998). The disequilibrium we found (i.e., 2 of 36) is within the range of what is considered random.

The analysis of genetic differentiation based on allele frequencies showed that, with few exceptions, most alleles were present in all sites. However, in two loci (*Hka28* and *Hka56*) no dominant alleles were found. This could be either an indication of homogeneity among locations or a sample size effect. Although we cannot rule out the possibility of sample size,

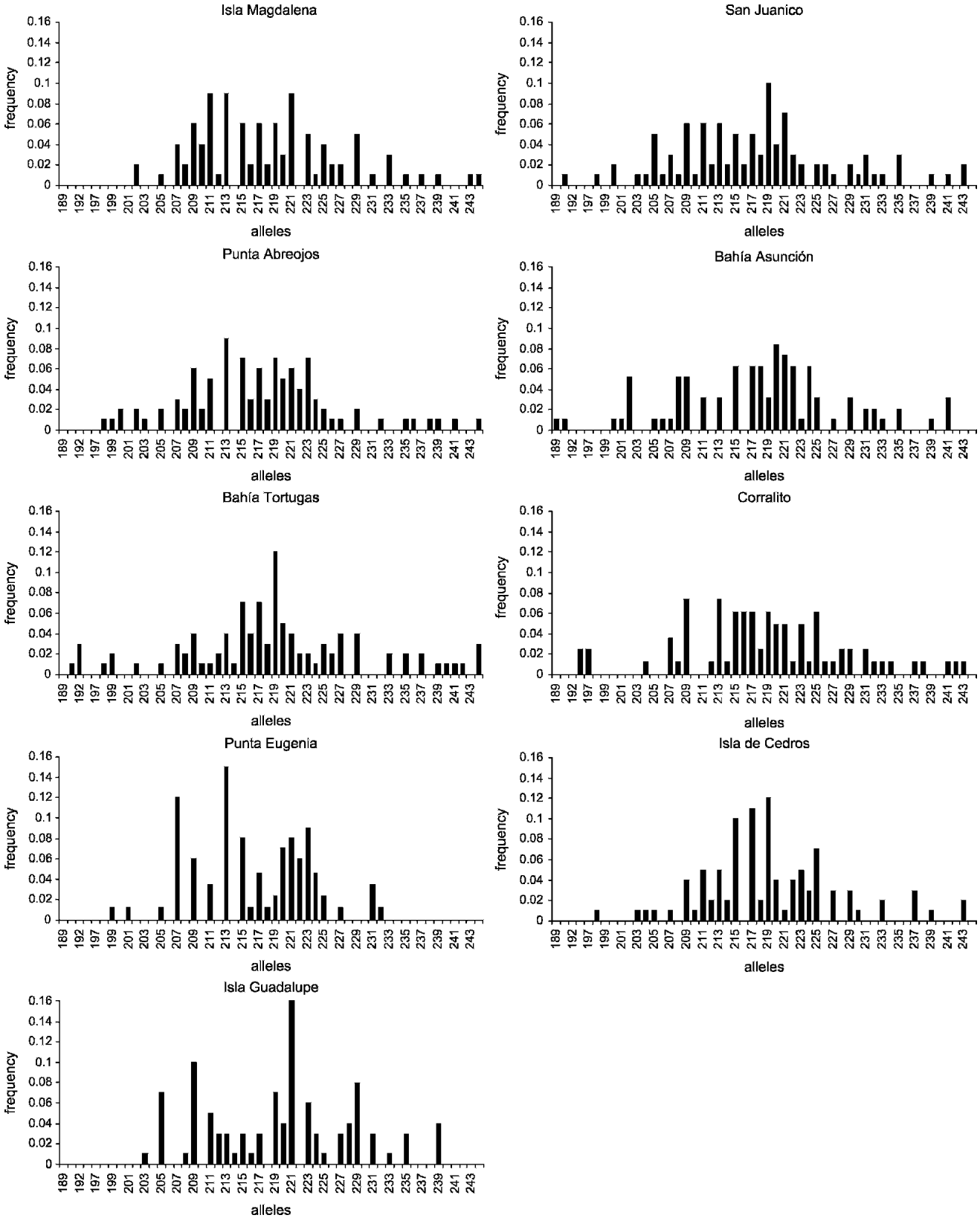

Figure 2. Allele frequencies of locus *Hka28* of *Haliotis fulgens* at each location from the west coast of Baja California Peninsula.

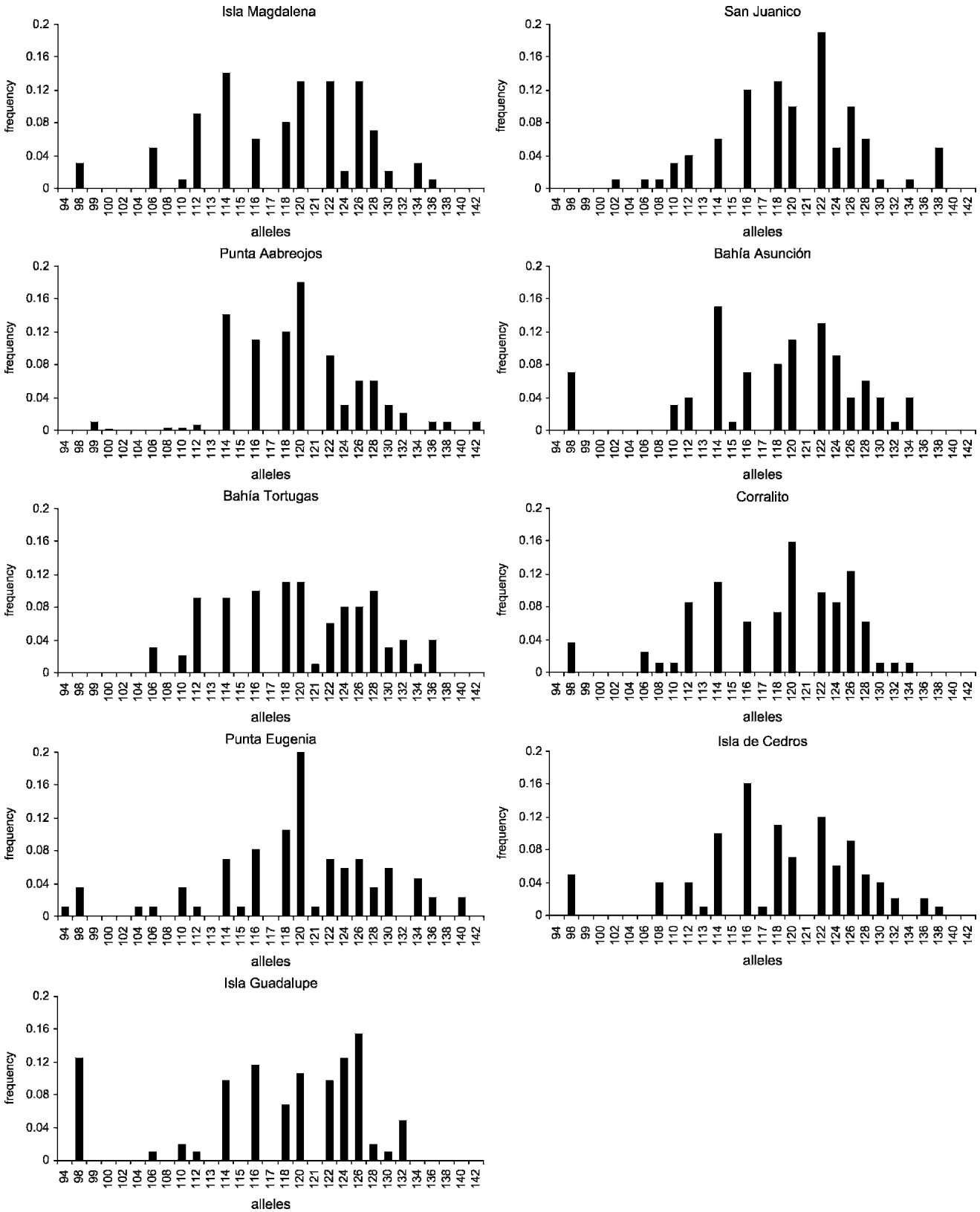

Figure 3. Allele frequencies of locus *Hka56* of *Haliotis fulgens* at each location from the west coast of Baja California Peninsula.

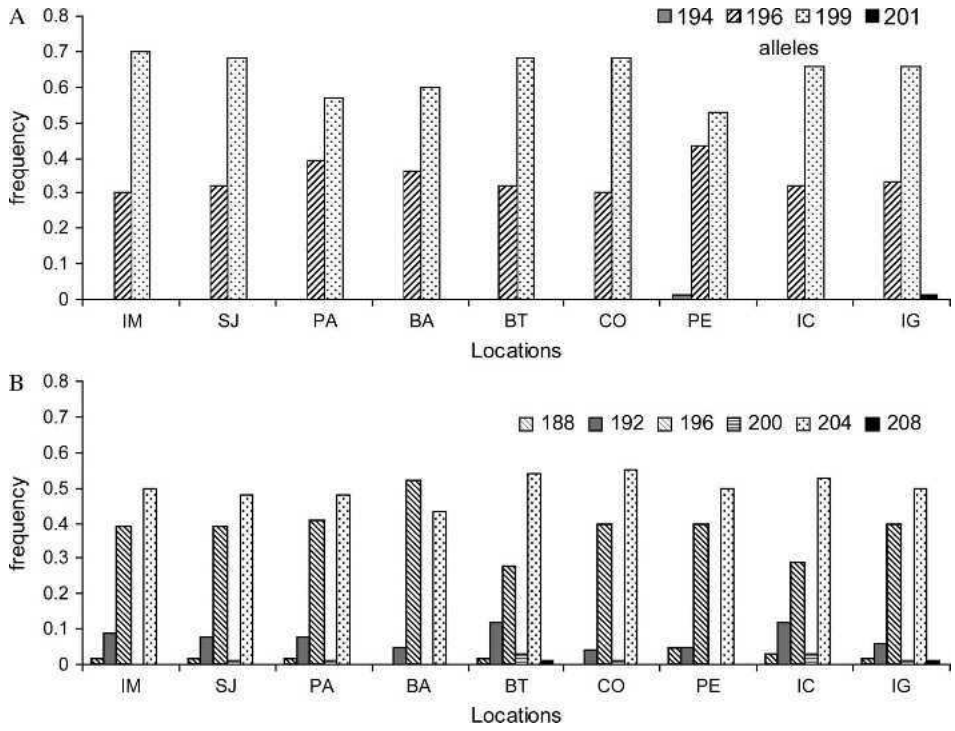

Figure 4. Allele frequencies of two loci *Hful260* (A), and *Hful603* (B) of *Haliotis fulgens* at each location from the west coast of Baja California Peninsula.

the number of individuals sampled from each population was based on the suggestions of Ruzzante (1998) who showed that samples of 50 or more per population are appropriate for population studies based on microsatellites.

Based on the AMOVA and the pairwise F_{ST} analyses it appears that, with the exception of Isla Guadalupe, the green abalone distributed along the west coast Baja California Peninsula conform to a single panmictic population, with no apparent restrictions to gene flow. This supports the observations of Zúñiga et al. (2000) who, based on allozymes, found no differences among locations in the central region of Baja California from north of Punta Eugenia to Bahia Tortugas. The differentiation between Isla Guadalupe and the rest of the locations suggests that the main reason to limited gene flow would be geographic distance, as this island is located 334 km away from the nearest location (Isla Cedros).

Abalone planktonic larval stage usually lasts between 3–5 days before its settlement (Leighton 1974), and therefore recruitment is in abalone beds near their parental stock (Prince et al. 1988, Guzmán de Próo et al. 2000). These conditions are believed to limit larval dispersal and gene flow. However, the area is heavily influenced by ocean and tidal currents (Guzmán del Próo et al. 2000), and it does not seem that these elements are sufficient to maintain a genetic isolation between the populations of the Peninsula. This is not the case of Isla Guadalupe for which the ocean currents might not be sufficient to assure larval dispersal over a few hundred kilometers.

The California current system that transports subarctic water along the western coast of the Baja California Peninsula towards the equator, reaches its maximum intensity during the spring and is remarkably reduced during summer/autumn, whereas the coastal countercurrent (CCC) intensifies of

TABLE 2.

Analysis of molecular variance of population differences of *Haliotis fulgens* with four loci at nine sites of Baja California.

Source of Variation	df	Sum of Squares	Variance Components	Percentage of Variation
Among populations	8	4.230	0.00031	0.06
Within populations	859	428.714	0.49909	99.94
Total	867	432.945	0.49939	
Fixation Index F_{ST}: 0.00062*				

* Significance test (1023 permutations) $P = 0.00196$.

TABLE 3.

Pairwise F_{ST} estimates among sites. IM, Isla Magdalena; SJ, San Juanico; PA, Punta Abreojos; BA, Bahía Asunción; BT, Bahía Tortugas; CO, Corralito; PE, Punta Eugenia; IC, Isla de Cedros; IG, Isla Guadalupe.

	SJ	PA	BA	BT	CO	PE	IC	IG
IM	0.4608	0.5458	0.0838	0.3191	0.6591	0.0055	0.0130	0.0003*
SJ		0.8552	0.0527	0.2778	0.4647	0.0019	0.1666	0.00083*
PA			0.2058	0.6222	0.5005	0.1636	0.2091	0.0003*
BA				0.0055	0.2258	0.0958	0.0011*	0.0011*
BT					0.5916	0.0003*	0.2252	0.0003*
CO						0.0222	0.0572	0.0028
PE							0.0003*	0.0003*
IC								0.0003*

* Significant value $P < 0.00138$ after Bonferroni adjustment.

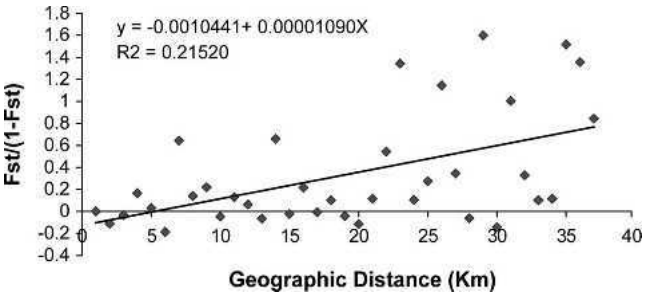

Figure 5. Isolation-by-distance: regression between paired F_{ST} values and the logarithm of the geographic distance of *Haliotis fulgens* between locations the west coast of Baja California Peninsula.

autumn/winter and carries water of tropical origin towards the north (Lluch-Belda 2000), because the abalone spawning season takes place mostly in autumn and early winter (Leon & Muciño 1996), there is greater probability that this coastal countercurrent is the responsible for larval transport. If this is true, Isla Guadalupe is the less influenced location, therefore explaining a reduced larval (gene) flow.

Because genetic differentiation patterns in other abalone species, such as *H. rubra*, *H. laevigata, H. roei*, (Brown 1991, Brown & Murray 1992, Hancock 2000, Huang et al. 2000, Conod et al. 2002), *H. rufescens* (Gaffney et al. 1996, Burton & Tegner 2000, Kirby et al. 1998), *H. cracherodii* (Hamm & Burton 2000), and *H. corrugata* (Del Rio Portilla & Gonzalez Avilés, 2001) are rather contrasting, it appears that the biological characteristics of each species, in combination with environmental factors, determine the specific gene flow patterns and no generalizations can be made.

Green abalone from Isla Guadalupe has some times been considered either as a subspecies or as a subpopulation of *H. fulgens* (Geiger & Poppe 2000). Our data would support the hypothesis of this location as a subpopulation.

Based on our results it can be suggested that abalone population is one large single panmictic population along the west coast of Baja California, with gene flow barriers acting on Isla Guadalupe population. In spite of the abalone resource having been severely exploited, the genetic composition and structure has not been compromised, and it appears that the effective population size of *H. fulgens* remains sufficient to maintain the high genetic diversity observed. Therefore, we suggest that there are still genetic resources available that can lead to the recovery of wild populations so long as adequate management strategies are designed and complemented by both government and fishermen.

There are still some questions to be answered concerning the direction of the larval migration and the connectivity between abalone beds that have to be analyzed to get a better understanding of the resource.

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