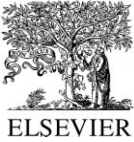

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Genetic structure of populations of the red sea urchin, *Strongylocentrotus franciscanus*

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Abstract

Population subdivision was evaluated in the red sea urchin, *Strongylocentrotus franciscanus*, using DNA sequence data from 134 adult individuals collected in 1995 and 1996. On average 22 individuals were sequenced from six geographic locations between Alaska and Baja California ($N = 134$), nearly the full extent of the species range. DNA sequence data was obtained from direct sequencing of a 273 base pair region of the bindin gene, which encodes a sperm fertilization protein. Results indicate that bindin is sufficiently polymorphic to serve as a genetic marker. We identified 14 unique alleles present in the entire range sampled with a maximum of eight alleles at a specific site. Mean pairwise comparison of the 14 unique alleles indicates moderate sequence diversity (p-distance = 1.06). Although there is conflicting evidence to suggest that Alaska populations may deviate from the Hardy–Weinberg expectations, analysis of bindin genotype frequencies indicate that it is not possible to reject the null hypothesis of random mating throughout the species range. The results of a chi-square test with pooling conform to Hardy–Weinberg expectations for all populations ($P > 0.05$) except for the Alaska population ($P = 0.037$). Inbreeding coefficients are consistent with this result and suggest that for the bindin locus, there is high gene flow. These results are compared with previously published results of genetic substructuring in sea urchins to examine relationships among population structure, dispersal potential and biogeography. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Bindin; DNA sequencing; Pacific Coast; Population genetics; Urchin

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1. Introduction

Throughout its species range off the Pacific Coast of North America, the red sea urchin, *Strongylocentrotus franciscanus*, is important both ecologically and economically. Kelp forest communities are characterized by strong species interactions between primary producers (such as *Macrocystis sp.* and *Pteryogophora sp.*), herbivores such as urchins (*Strongylocentrotus sp.*) and abalone (*Haliotis sp.*) and secondary consumers such as sea otters (*Enhydra lutris*) and spiny lobsters (Estes et al., 1982). Because red sea urchins are a valuable export product, there has been rapid growth in the commercial fishery since its inception in California in 1971 (Leet et al., 1992). An understanding of the genetic structure of this widespread and ecologically important species will aid ecologists and population geneticists and may force re-evaluation of current management practices, which assume genetic homogeneity throughout the species range (Duffy, personal communication).

The range of red sea urchins, *S. franciscanus*, extends from Northern Japan to Alaska and Baja California, Mexico (Morris et al., 1980). Throughout this range, red sea urchin spawning is concentrated in the spring with suspected release of gametes year-round (Strathmann, 1978; Schroeter et al., 1992). The planktotrophic red sea urchin larvae have a long pelagic stage that lasts from 61 to 131 days (Strathmann, 1978). Therefore, a red sea urchin larva traveling in the California current (average velocity approximately 0.10 m/s, Lynn and Simpson, 1990) could traverse a total distance exceeding 1000 km before settlement. These oceanographic factors suggest the potential for high dispersal, large gene flow and genetic homogeneity (Scheltema, 1971).

Despite the potential for long distance dispersal leading to high gene flow, previous studies of genetic structure in other marine organisms with pelagic larvae suggest the simple assumption that species with long lived planktonic larvae will be genetically homogeneous is often unfounded. Burton (1983) reviewed several cases where electrophoretic data indicated local genetic heterogeneity despite long planktonic life history stages. Some of these organisms included the sea urchin *Arbacia punctulata* (Marcus, 1977), the lobster *Homarus americanus* (Tracey et al., 1975) and the mussel *Mytilus edulis* (Koehn, 1975).

Potential biotic and abiotic factors can drastically reduce larval movement away from natal populations. Oceanographic features such as eddies, gyres and countercurrents may retain larvae for their entire larval period. The presence of boundary currents and fronts can act as physical barriers to dispersal (Ward, 1989). Differential selection against specific genotypes once larvae settle on the bottom can differentiate populations with multiple sources of parent populations (Hartl and Clark, 1989). Behaviors such as vertical migration, swimming, and benthic residence can also keep larvae closer than expected to parent populations (Raimondi and Keough, 1990). These biological and oceanographic characteristics could result in red sea urchin genetic differentiation despite their long planktonic stage.

For red sea urchins, previous work identifies geographic variation in at least one life history characteristic. Weekly monitoring of temporal and spatial patterns of red sea urchin larval settlement revealed a seasonal pattern with peak settlement occurring in spring (Schroeter et al., 1992). However, settlement is highly unpredictable depending

on location and year. For example, larvae settle at the Orange County site in southern California approximately every other month, yet at Point Cabrillo in northern California, there has been only one substantial recruitment event in 3 years (Schroeter et al., 1992). It is possible that these geographic variations in settlement imply that there are barriers to larval movement that restrict genetic exchange between populations with different settlement patterns.

To investigate the population structure of red sea urchins, this work uses a seemingly unusual population marker, the gamete interaction molecule bindin (Vacquier et al., 1995). Like other traits associated with fitness, gamete interaction molecules are assumed to be under strong selection (Li and Graur, 1991). In studies of population genetics, an appropriate genetic marker is sufficiently polymorphic to detect genetic differences and the polymorphisms are the result of neutral evolution rather than selection (Hartl and Clark, 1989). Work in several laboratories has identified high levels of interspecific sequence variation in gamete interaction molecules such as sea urchin bindin and abalone lysin (Metz and Palumbi, 1996; Vacquier et al., 1997). Additionally, recent examination of red urchin bindin sequence variation suggests that the portion of the bindin molecule used for this study is evolving neutrally (Debenham et al., in press and see Section 4). Thus, the bindin locus serves as a good marker for population genetics of *S. franciscanus*.

Based on the stochastic nature of dispersal, settlement, selection and recruitment, it is not possible to predict a priori whether Pacific coast red sea urchin populations are genetically differentiated or homogeneous. Analysis of DNA sequence data here shows that although the region of the bindin gene examined has sufficient polymorphism to detect genetic structure, there is no evidence for geographic substructuring in red sea urchin populations between Alaska and Baja California, Mexico. Additionally, although Alaska populations may deviate from Hardy–Weinberg expectations, the remaining five populations clearly conform to Hardy–Weinberg equilibrium expectations.

2. Materials and methods

2.1. Sample collection

Between August 1995 and February 1996, we collected 298 adult *S. franciscanus*. Approximately 50 animals were collected from each of six sites: Ketchikan, AK; Port Townsend, WA; Port Orford, OR; Ft. Bragg, CA; Santa Barbara, CA and Ensenada, Baja California, Mexico (Fig. 1). All animals were collected from a depth range of 10–15 m using SCUBA and then shipped overnight to the laboratory. To ensure that all individuals were adults, the test diameter was at least 80 mm (Morris et al., 1980).

2.2. DNA extraction

DNA extractions were performed as described in Milligan (1992). Briefly, 25–100 μg of gonadal tissue was homogenized in 700 μl of CTAB buffer (100 mM Tris–HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2% hexadecyltrimethylammoniumbromide) pre-

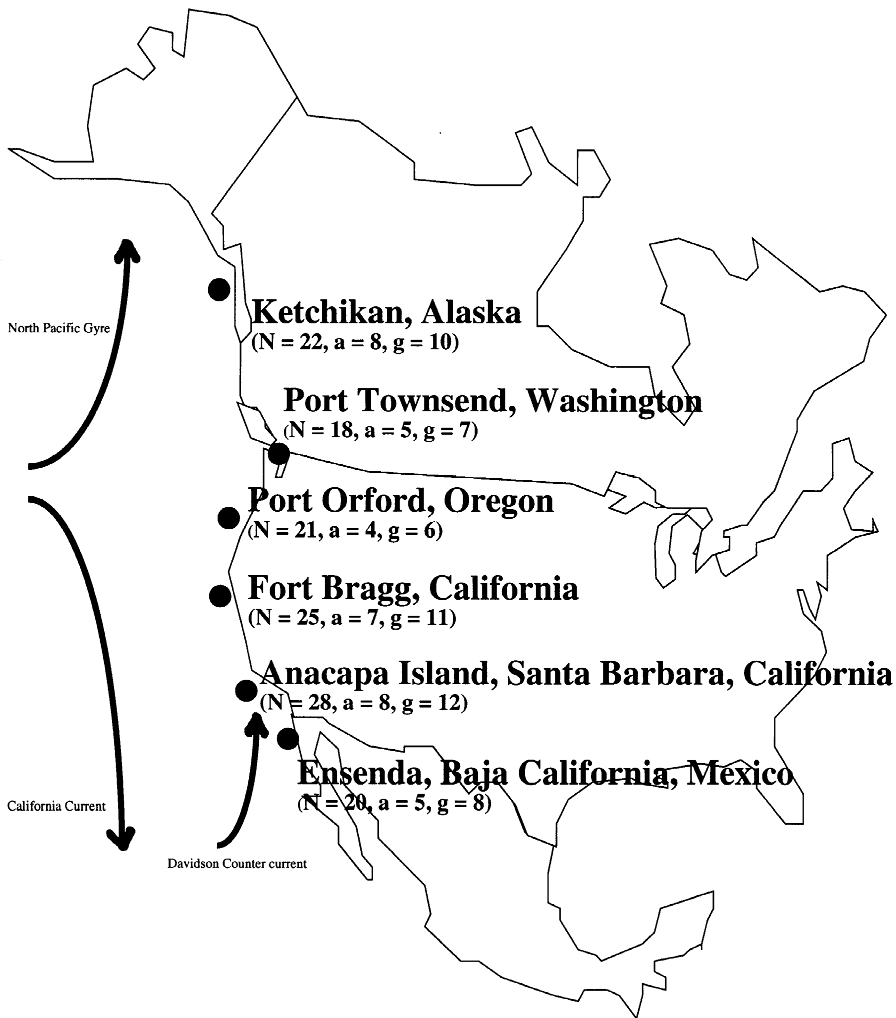

Fig. 1. *Strongylocentrotus franciscanus*. Six sampling locations of populations located along the Pacific Coast of North America. Numbers under sampling location indicate number of individuals sequenced (N), number of alleles (a) and number of genotypes (g) identified at each sampling location. For entire species range $N = 134$, $a = 14$ and $g = 21$.

warmed to 60°C and supplemented with 1% polyvinylpyrrolidone (PVP-360, w/v); 0.2% 2-mercaptoethanol (v/v, added just before use). All chemicals were from Sigma Chemical Co., St. Louis, MO, USA, unless specified otherwise. At the end of a 30–60-min incubation at 60°C, the samples were extracted with phenol–chloroform–isoamyl alcohol multiple times until the interface was clear. Ice-cold isopropanol (2/3 vol) was added to the aqueous phase and the precipitated DNA was washed with 500 μl

of 70% ethanol, 10 mM ammonium acetate, dried and resuspended in 20–30 μl of Tris–EDTA pH 8.0 containing 1 μl of RNAse (10 mg/ml).

2.3. Double-stranded PCR amplification

To obtain DNA sequences and determine allele and genotype frequencies, we directly sequenced PCR products (for both the 5′ and 3′ direction) that were created by asymmetric single-stranded amplification. The primers FNbindin5′(5′-AGTCGACGTT-CGACAGACGAC-3′) and FNbindin3′(5′-TTACATGGTCCATTATAGTATGCC-3′) amplify a 431 base pair region at the 5′ end of the bindin cDNA and the first intron. Amplification followed standard procedures (Saiki et al., 1988) in that we used a reaction volume of 25 μl and a final $MgCl_2$ concentration of 2 mM. The thermocycler (Perkin Elmer Cetus, Norwalk, CT, USA) profile for all double stranded reactions was: one cycle of 95°C for 5 min, followed by 30 cycles of 94°C for 1 min, then 60°C for 1 min and 72°C for 2 min. Each PCR reaction was resolved on a 2% Nu Sieve agarose (FMC, Inc., Rockland, ME, USA) and the PCR product gel isolates were stored at −20°C.

2.4. Single-stranded PCR amplificationand direct sequencing

Each gel isolate was heated to 65°C for approximately 5–10 min and used as template for the single-stranded PCR amplification. The amplification conditions were identical to those above for amplification of the double-stranded products except that the 5′ primer (FNbindin 5′) was used at a final concentration of 0.5 μM as opposed to 10 μM used in the double-stranded reaction. The amplification conditions to amplify the 3′ single-stranded product required a lower annealing temperature (58°C) and a lower $MgCl_2$ concentration (1.2 mM was the final concentration). The concentration of the 3′ limiting primer was 2.5 uM. The single-stranded PCR products were washed in Centricon filter units (30 000 MWCo; Amicon, Inc.) and resuspended in 7 μl of H_2O for Sanger dideoxy sequencing (Sequenase ver. 2.0, U.S. Biochemical, Cleveland, OH, USA) with internal sequencing primers Ktseq5′(5′-GGAGCGCGTAAGAAGCGTTAT-3′) and Ktseq3′(5′-ATACACACGATGGTCAAG-3′).

2.5. Cloning of bindin DNA from heterozygous individuals

In the direct sequencing process used here, a heterozygote was detected by two bands at one nucleotide position in the sequencing. For example, a double band at position 26 indicates that one allele has a ‘G’ and the second allele has an ‘A’ at that position. In order to confirm the exact sequence of all representative alleles and to rule out PCR incorporation errors, PCR products were cloned and sequenced for representative genotypes. We cloned between two and seven individuals for each genotype. In addition, two homozygous genotypes were cloned to confirm rare alleles. In the case where the same genotype occurred in more than one geographic location, at least one individual was cloned and sequenced from each site. Approximately 3.6 colony picks were sequenced in both the 5′ and 3′ direction for each individual.

Hare and Palumbi (1999) identified that 2 out of 30 PCRs products from diploids amplified one allele. If this were the case in this work, approximately 8 or 9 of the individuals scored as heteroygotes could have been falsely scored as homozygotes. It is important to identify this possible source of error that could impact detected allele frequencies.

Double-stranded PCR products were amplified with primers KBRS5′(5′-CGCGGAT-CCAGTCGACGTTCGACAGACGAC-3′) and KBRS3′(5′-GCCAAGCTTTTACATG-GTCCATTATAGTATGCC-3′), incorporating restriction sites to facilitate directional cloning. PCR products were resolved on 2% agarose gel, and the excised fragment was purified using Quiaquick spin columns (Quiagen, Valencia, CA, USA) and then digested with BamHI and HindIII. The fragment was ligated into pBMKS (Stratagene) and transformed into *E. coli* DH5α. Plasmid DNA was purified with the alkaline lysis method (Sambrook et al., 1989), and the inserts were sequenced on both strands. The DNA from at least four transformants was sequenced for each PCR product. PCR products from several individuals with the same genotype but from different geographic locations were cloned and sequenced to verify sequence consistency among alleles found at different geographic locations.

2.6. Sequence and statistical analyses

Sequences were aligned with Seq-Ap ver 1.9a multiple sequence alignment program for the Macintosh (Gilbert, 1994). Molecular Evolutionary Genetic Analysis ver. 1.01 (MEGA, Kumar et al., 1993) was used to calculate nucleotide sequence divergence. Individual genotypes were coded as paired alphabetical characters and analyzed with BIOSYS (Swofford, 1989) to obtain estimates of the following: allele frequencies, conformance to Hardy–Weinberg equilibrium, Wright's (1978) *F*-statistics and Nei's (1972) minimum genetic distance in pairwise comparisons. The phylogenetic relationship of the 14 unique alleles was determined by a parsimony analysis (PAUP, Swofford and Begle, 1993).

Conformance to Hardy–Weinberg proportions was estimated using a chi-square test with pooling of rare and common categories. This method was chosen because of a relatively high number of rare singleton alleles. Qualitatively similar conclusions are obtained using other analytical tests such as a contingency chi-square analysis with Levene's (1949) correction for small sample size, a significance test with exact probabilities, and a Monte Carlo simulation of a chi-square contingency test as per Roff and Bentzen (1989) performed using 1000 runs (data not shown). Average heterozygosity was calculated using BIOSYS, Swofford, 1989.

3. Results

Out of 134 *S. franciscanus* individuals from six geographic locations along the Pacific Coast of North America (Fig. 1), 14 bindin alleles and a total of 21 genotypes were identified, suggesting a high degree of polymorphism at this locus (Table 1). The

Table 1
Polymorphic nucleotide positions in each unique allele for 273 base pair region of the bindin gene for *Stronglyocentrotus franciscanus*

				1	1	1	1	1	1	1	1	1	1
Nucleotide[a]	9	9	9	0	0	0	0	0	0	1	1	1	1
Position[a]	4	7	8	1	1	2	4	4	5	1	4	5	8
	9	8	0	1	2	8	0	6	6	9	2	3	6
Published[a]	G	G	A	G	G	G	G	G	A	T	G	T	C
Allele													
A	·[b]	·	·	·	·	·	·	·	·	·	·	·	·
B	·	·	G	·	·	·	·	A	·	·	·	C	·
C	·	·	G	·	·	·	·	A	·	·	·	·	·
D	·	·	G	·	·	·	·	·	G	·	·	·	·
E	·	·	·	·	·	·	A	·	·	·	·	·	·
F	·	·	·	A	·	·	·	·	·	·	·	·	·
G	·	·	G	·	·	·	·	·	·	·	·	·	·
H	·	·	G	·	·	·	·	A	·	·	C	C	·
I	·	·	·	·	·	·	·	·	·	A	·	·	·
J	·	·	G	·	·	·	·	A	·	·	·	·	T
K	·	·	G	·	·	A	·	·	·	·	·	·	·
L	·	·	G	A	C	·	·	·	G	·	·	·	·
M	A	·	·	·	·	·	·	·	·	·	·	·	·
N	·	C	·	·	·	·	·	·	·	·	·	·	·

[a] As reported in Minor et al. (1991).
[b] Indicates identity with published sequence.

number of alleles per sampling site ranged from four to eight. The number of genotypes at each geographic location ranged from 6 to 12, with a mean of nine genotypes per site. There was no obvious trend in the number of alleles or the number of genotypes at a geographic location.

Within the 273 base pair region examined in 134 individual red urchins, there were a total of 13 variable nucleotide positions in all 14 alleles (Table 1). The number of nucleotide differences between any two sequences is low, ranging between one (0.35%) and six (2.1%) nucleotide substitutions in the 273 base pair region. On average, there are 2.9 nucleotide positions that vary between any two unique alleles. The average nucleotide-sequence diversity is 1.06% (Tajima and Nei, 1984, excluding correction for multiple hits). Efforts to evaluate the phylogenetic relationship of the 14 unique alleles showed no supportable allelic geneologies (data not shown).

Table 2 presents the frequency data for each allele and each geographic location. Four alleles are predominant. Allele A is the most common allele (51%). Alleles B, C and D are the next most common alleles with frequencies of 16, 20 and 8%, respectively. All other alleles are rare, representing $< 1\%$ of the total population. Allele G is present in three and allele F is present in two individuals out of the total 134 individuals sequenced. All remaining alleles (E, H, I, J, K, L, M and N) are present in only a single individual. A bootstrap resampling analysis of alleles from each geographic location was conducted to estimate the fraction of total alleles sampled in the study. The results of

Table 2
Allele frequency data of 14 unique alleles (A–N) from six geographic locations[a]

Allele	AK ($N=22$)	WA ($N=18$)	OR ($N=21$)	NC ($N=25$)	SB ($N=28$)	BJ ($N=20$)	Total ($N=134$)	Total no. of allele copies in all pops.
A	0.545	0.528	0.548	0.480	0.500	0.475	0.511	137
B	0.205	0.167	0.167	0.120	0.143	0.150	0.160	43
C	0.114	0.194	0.214	0.240	0.143	0.275	0.201	54
D	0.045	0.083	0.071	0.100	0.125	0.075	0.078	21
E	0.000	0.000	0.000	0.020	0.000	0.000	0.004	1
F	0.000	0.000	0.000	0.020	0.018	0.000	0.007	2
G	0.023	0.028	0.000	0.000	0.036	0.000	0.011	3
H	0.023	0.000	0.000	0.000	0.000	0.000	0.004	1
I	0.000	0.000	0.000	0.000	0.000	0.025	0.004	1
J	0.000	0.000	0.000	0.020	0.000	0.000	0.004	1
K	0.000	0.000	0.000	0.000	0.018	0.000	0.004	1
L	0.023	0.000	0.000	0.000	0.000	0.000	0.004	1
M	0.023	0.000	0.000	0.000	0.000	0.000	0.004	1
N	0.000	0.000	0.000	0.000	0.018	0.000	0.004	1

[a] AK = Alaska, WA = Washington, OR = Oregon, NC = Northern California, SB = Santa Barbara, BJ = Baja California, Mexico.

this analysis (data not shown) indicated that the total number of alleles at each location was reaching a plateau and that further sampling would not likely increase the number of alleles significantly. Thus, the sample sizes presented here are sufficient for estimating the number of alleles present in natural populations.

Analysis of genotype frequencies in Alaska using a chi-square statistic with pooled data (see Section 4) indicated a significant deviation from Hardy–Weinberg expectations ($P = 0.037$); all other populations conform to Hardy–Weinberg expectations (Table 3). To evaluate population subdivision over all geographic scales, F-statistics and hierarchical F-statistics were calculated for all possible combinations of geographic location. All

Table 3
Heterozygosity and tests for conformance to Hardy–Weinberg equilibrium in the region of the bindin gene analyzed for *Strongylocentrotus franciscanus*[a]

	AK	WA	OR	NC	SB	BJ
Heterozygosity						
Direct count (% of pop. heterozygous)	0.818	0.722	0.667	0.680	0.714	0.600
Estimate based on HW expectation	0.644	0.648	0.621	0.686	0.691	0.670
Unbiased estimate[b]	0.659	0.667	0.636	0.700	0.704	0.687
D[c]	0.242	0.083	0.047	− 0.029	0.015	− 0.127
Hardy–Weinberg statistics (P-values)[d]	0.037	0.402	0.88	0.768	0.509	0.147

[a] See text; data calculations with BIOSYS, Swofford, 1989.
[b] Levene, 1949; Nei, 1978.
[c] Coefficient for heterozygosity.
[d] Chi-square with pooling.

combinations of regional grouping were statistically insignificant (all data not shown, however, all values for $F_{st} \leq 0.008$). For example, analysis of Alaska as one group versus a second grouping of Washington, Oregon, Northern California, Santa Barbara and Baja California, Mexico indicated insignificant deviations from zero.

4. Discussion

It is unusual to use a gamete interaction molecule, such as bindin, as a population marker. An appropriate genetic marker is moderately polymorphic and these polymorphisms are the result of neutral evolution and not directed selection (Hartl and Clark, 1989). Most traits associated with reproductive success are assumed to have low intraspecific genetic variation as a result of purifying selection (Li and Graur, 1991). As stated previously, detailed analysis of intraspecific variation in the portion of the bindin locus used here suggests that this portion of the bindin molecule is evolving as a result of neutral evolution (Debenham et al., in press). To summarize the results of Debenham et al. (in press), it was not possible to reject the null hypothesis that sequence variation observed in *S. franciscanus* bindin is a result of neutral evolution. Statistical evaluation of expected proportions of replacement and silent nucleotide substitutions, observed versus expected proportions of radical replacement substitutions, Tajima and Nei's (1984) *T*-test, and conformance to the McDonald and Kreitman (1991) test of neutral evolution, all fail to reject the neutral origin of observed polymorphisms for bindin. All of these data presented in Debenham et al. (in press) suggest that the polymorphism observed in bindin is most likely the result of neutral random mutations and that selection is not confounding the pattern of genetic heterogeneity.

As suggested by this and previous work on bindin (Vacquier et al., 1995; Metz and Palumbi, 1996), the bindin locus does appear to be an acceptable genetic marker with sufficient polymorphism to detect genetic structure. All six populations of *S. franciscanus* are highly polymorphic with at least four and up to eight alleles at a single geographic site. With the amount of polymorphism present in bindin, genetic isolation could result in changes in gene frequencies over evolutionary time periods. Although bindin sequence variation (1.06%) is moderate, it is approximately equal to variation in mtDNA (COI) examined in *S. purpuratus*, *S. droebachiensis* and *Echinometra sp.* (Palumbi and Wilson, 1990; Palumbi and Metz, 1991; Edmands et al., 1996; Palumbi et al., 1997).

All measures of genetic variation in bindin make it impossible to reject the null hypothesis that the red sea urchin, *S. franciscanus*, is panmictic throughout the entire range examined from Baja California to Alaska. There are no statistically significant differences in genotype frequencies among sites and no evidence of a genetic break among any of the sampling sites. Gene flow appears sufficient to prevent detectable genetic divergence within *S. franciscanus*, at least at the bindin locus, along the Pacific Coast of North America. Given the constancy of bindin frequencies throughout the range examined, there is no evidence that genetic isolation by distance exists for *S. franciscanus*.

One of the major biogeographic features along the Pacific coast is Point Conception, a prominent headland (Fig. 1). As a major boundary of the Oregonian and Californian biogeographic provinces (Valentine, 1973), Point Conception divides two regions with disparate sets of co-occurring species and marks the limit of distribution for many species. It is hypothesized that biogeographic boundaries can also be boundaries for gene flow and result in population subdivision (see Briggs, 1958, 1974; Avise, 1994; Palumbi, 1995). The California Current, flowing predominantly southward from Alaska to Baja California, turns seaward at Point Conception, potentially demarcating northern and southern water masses (Hickey, 1979). For a species existing on a linear coastline with a unidirectional current, genetic isolation created by Point Conception would predict a greater amount of genetic variation in the south compared to the north. Burton (1998) reviewed the available literature regarding interspecific phylogeography across Point Conception and failed to find any correspondence between genetic differentiation and Point Conception.

Based on the results of a hierarchical analysis of F-statistics, no regional genetic patterns are apparent in *S. franciscanus* nuclear DNA. Similarly, southern populations of *S. purpuratus* are apparently not genetically isolated from populations north of Point Conception (Palumbi and Wilson, 1990; Palumbi and Kessing, 1991; Edmands et al., 1996). Although flow of the California Current is predominantly southward, reversals in flow direction are quite common (Pirie et al., 1975). As a result of an El Nino Southern Oscillation (ENSO) event, warm tropical waters can travel past Point Conception as far north as Vancouver Island (Crowe and Schwartzlose, 1972). In addition, every winter, the southern California and Davidson Counter currents send water from southern California northward (Hickey, 1979). Homogeneity throughout the range examined here suggests that *S. franciscanus* larvae are able to move south with the California Current as well as north with either a periodic ENSO event or annually with the Davidson Counter current.

Unlike the five other *S. franciscanus* sampling sites, the Alaska site does not conform to Hardy–Weinberg expectations. One explanation for this observation is that mating is non-random in the Alaska population. It is also possible that selection results in a deviation from Hardy–Weinberg equilibrium.

The purple sea urchin, *S. purpuratus,* is a congener of the red sea urchin that has an overlapping range and shares several life history characteristics. The results here are consistent with previous work evaluating population structure in *S. purpuratus* and suggest genetic homogeneity throughout its range. Britten et al. (1978) reported no difference in the thermal renaturation of DNA from two urchins separated by 2000 km compared to the reassociation of DNA of each urchin compared to itself, suggesting a lack of genetic subdivision. Palumbi and Wilson (1990) used restriction fragment length polymorphism (RFLP) of mitochondrial DNA (mtDNA) and saw no genetic differentiation in 28 *S. purpuratus* individuals from populations separated by 1500 km along the Pacific Coast of the USA. Additionally, sequence data of cytochrome oxidase I (COI; a mitochondrial gene) in 30 individuals collected from three sites between Washington and Santa Barbara, CA (approximately 2500 km) showed approximately 1% sequence variation yet no genetic heterogeneity among populations and no population subdivision (Palumbi and Kessing, 1991).

Whereas the above work examined large geographic ranges, Edmands et al. (1996) examined genetic variation in much larger sample sizes from the southern part of the distribution of *S. purpuratus*. Their sequence data of a mtDNA gene revealed a significant heterogeneity among locations with a contingency chi-square analysis (Edmands et al., 1996; $N = 147$, $X^2 = 115.05$ df $= 90$, $P < 0.05$). Analyzing the same data with an Analysis of Molecular Variance, AMOVA (Excoffier et al., 1992), Edmands et al. (1996) did not find significant differentiation among locations ($F_{st} = 0.017$, $P > 0.05$). However, based on a regional analysis, there was a statistically significant genetic break approximately 300 km south of Pt. Conception in central California ($F_{st} = 0.064$, $P < 0.05$).

Moberg and Burton (in press) examined the population structure of adult and juvenile size classes for *S. franciscanus*. Like those presented here, their results indicate that there is no regional pattern of differentiation in the red sea urchin. However, on smaller geographic scales, neighboring populations were often more differentiated than distant populations. Interestingly, recruit samples differed significantly from adult samples and showed extensive between-year variation. These results indicated less mixing of the larval pool than would be predicted for organisms with a life hitory stage that includes long residence times in the water column. The authors suggest that pre- and post-settlement selection and high interfamily variance in reproductive success might contribute to the observed genetic differences between adults and new recruits.

Analysis of DNA sequences in a segment of the bindin gene in red sea urchin populations along the Pacific Coast of North America (except for a possible exception in Alaska populations) suggests that there is no apparent regional subdivision of this species. These results imply that current assumptions (that the red sea urchin is one randomly mating population) made in relation to the management of red sea urchin populations may be sufficient. However, geographic variation in settlement and adult dynamics along the species range suggests that different sections along the coast have very different ecological dynamics, which could likely warrant regional management plans. Therefore, although the work here suggests that over long evolutionary time scales, there is a large amount of genetic exchange, Moberg and Burton's (in press) result of local heterogeneity supports the possibility that over ecological and intergenerational time scales, genetic exchange may be limited. Thus local populations are more isolated than larval dynamics may suggest, which would also warrant regional management plans.

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