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S. Edmands · P. E. Moberg · R. S. Burton

Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*

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Abstract Despite high potential for dispersal, the purple sea urchin *Strongylocentrotus purpuratus* was found to have significant genetic subdivision among locations. Ten geographic locations along the coast of California and Baja California were sampled between 1994 and 1995. Samples from some locations included both adult and recruit urchins. Allozyme analyses revealed a genetic mosaic, where differentiation over short geographic distances could exceed differentiation over much larger distances. Significant allozyme differentiation was found among subpopulations of adults (standardized variance, $F_{ST} = 0.033$), among subpopulations of recruits ($F_{ST} = 0.037$), and between adults and recruits from the same location. DNA-sequence data for the mitochondrial cytochrome oxidase I gene also showed significant heterogeneity among locations, with a mild break in haplotype frequencies observed $\simeq 300$ km south of Point Conception, California. Repeated sampling over time is necessary to determine whether these patterns of differentiation are stable and to begin to understand what forces produce them.

Introduction

Gene flow from the dispersal of long-lived planktotrophic larvae is generally predicted to homogenize marine populations over broad geographic distances (Scheltema 1971, 1978). While much empirical evidence supports this prediction (see Palumbi 1992), exceptions abound for a variety of reasons (see Burton 1983; Palumbi 1994). Marine species may not realize their greatest dispersal potential because behavioral mechanisms may favor local recruitment or planktonic transport may pose hazards, as has been suggested to explain the extensive population subdivision found in the intertidal copepod *Tigriopus californicus* (Burton and Feldman 1981). Effective dispersal may also be limited by physical processes including large-scale current patterns (Incze et al. 1990) and smaller-scale eddies and upwelling zones (Ebert and Russell 1988). Further, genetic differentiation may occur *in spite* of extensive dispersal due both to differential post-settlement mortality, as suggested by the steep genetic clines in the mussel *Mytilus edulis* (Koehn et al. 1980), and to temporal variation in the genetic composition of larvae (Kordos and Burton 1993) or recruits (Johnson and Black 1982, 1984; Watts et al. 1990).

The sea urchin *Strongylocentrotus purpuratus* presents a good model to test for population subdivision in spite of its high dispersal capacity because of its abundance and the accumulated knowledge of its development, ecology and genetics. Factors expected to promote genetic homogeneity in this species include external fertilization, extremely high fecundity (individuals spawn millions of eggs at a time), a lengthy feeding planktonic larval period of 4 to 21 wk (Strathmann 1978), and a continuous coastal distribution from Vancouver Island, British Columbia, to Isla Cedros, Baja California (Morris et al. 1980). Conversely, local differentiation might be promoted by the highly variable recruitment patterns in this species (Ebert and Russell 1988), while regional differentiation might be promoted by the biogeographic boundary at Point Conception (Fig. 1), which marks the range limit of numerous species (Valentine 1973; Jablonski et al. 1985). This boundary is driven by the California current, which carries colder water southward along the west coast of North America to the Point Conception region, where it then moves offshore allowing warmer water to accumulate along the southern coast. For species spanning the boundary, this

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S. Edmands (✉) · P. E. Moberg · R. S. Burton
Marine Biology Research Division, Scripps Institution of Oceanography, University of California. San Diego, La Jolla, California 92093-0202, USA

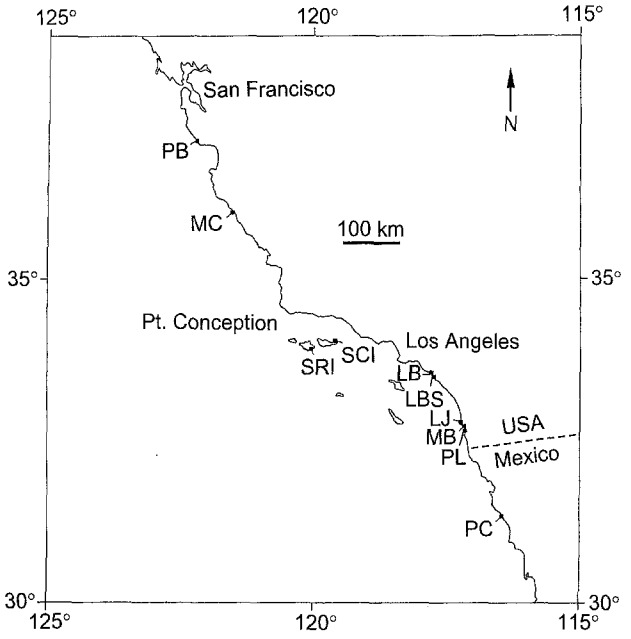

Fig. 1 Location of collection sites in California, USA, and Baja California, Mexico (*PB* Panther Beach, May 1995; *MC* Mill Creek, May 1995; *SCI* Scorpion Anchorage, Santa Cruz Island, July 1994; *SRI* Ford Point, Santa Rosa Island, November 1994; *LB* Laguna Beach, January 1995; *LBS* Laguna Beach South, March 1995; *LJ* La Jolla, September 1994; *MB* Mission Bay, March 1994; *PL* Point Loma, October 1994; *PC* Punta Cabras, October 1994)

current pattern might be expected to cause genetic discontinuity.

Previous work on *Strongylocentrotus purpuratus* (Palumbi and Wilson 1990; Palumbi 1995) found no mitochondrial DNA (mtDNA) differentiation among samples from Washington State, central California and southern California. Neither mtDNA restriction-fragment analysis of 38 individuals (Palumbi and Wilson 1990) nor a 910 base pair (bp) sequence of the mitochondrial cytochrome oxidase I gene for 31 individuals (Palumbi 1995) revealed any evidence of geographic structure over this 1500 km region. While these samples were convincingly homogeneous, the data are limited to mitochondrial DNA (which acts essentially as a single locus) in adult individuals from three locations. Similarly, an earlier study (Britten et al. 1978) of nuclear DNA variation estimated by thermal stability did not find populations from southern California to British Columbia to be "grossly distinct", yet the possibility of a subtler level of population structure remains.

Given that no mtDNA variation has been detected in *Strongylocentrotus purpuratus* over a large geographic scale, we asked whether genetic structure occurs over a finer spatial scale and whether nuclear and mitochondrial markers reveal the same patterns. We assessed allozyme and mitochondrial DNA variation in samples from ten locations, including both adult and recruit *S. purpuratus* at some locations. The value of assessing both allozymes and DNA has been illustrated by recent studies in which these two types of markers achieved strikingly different results. While allozyme studies (Buroker 1983) of the Atlantic oyster *Crassostrea virginica* provided little evidence of population subdivision along the east coast of North America, both mtDNA (Reeb and Avise 1990) and nuclear DNA data (Karl and Avise 1992) revealed a sharp genetic break between Atlantic and Gulf coast populations. Similarly, extreme DNA-sequence divergence between northern and southern *Tigriopus californicus* clades was not reflected in extensive allozyme data (Burton and Lee 1994). However, allozyme studies are comparatively time- and cost-effective, and thus allow sampling on a spatial and temporal scale not practical for DNA studies. The virtue of extensive population sampling was made particularly clear by a series of studies on limpets and urchins (Johnson and Black 1982, 1984; Watts et al. 1990), in which shifting patterns of genetic heterogeneity could be understood only through rigorous sampling over space and time. These studies also illustrate the importance of including different age classes to determine if observed patterns are caused by the geographic source of recruits or by pre- or post-recruitment selection. While most population genetic studies have implicitly assumed that different age classes collected from the same location represent a single deme, this assumption is coming under increasing scrutiny (e.g. Johnson and Black 1984; Macleod et al. 1985; Lewis and Thorpe 1994).

Materials and methods

Sample collection and storage

Strongylocentrotus purpuratus were sampled from ten intertidal or shallow subtidal locations in California, USA and Baja California, Mexico between September 1994 and May 1995 (Fig. 1). When both recruits ($\leq$ 20 mm test diam) and adults ($>$ 20 mm test diam) were present, they were sampled from overlapping regions (15 to 60 individuals of each size class per location). Urchins were dissected soon after collection or held temporarily in flowing seawater aquaria. Test diameter was measured using vernier calipers, and tissues (gonad, digestive gland, tube feet, muscle of Aristotle's lantern) were separated before being frozen at -70 °C.

Enzyme electrophoresis

Samples were analyzed by both horizontal starch-gel electrophoresis and polyacrylamide-gel electrophoresis. Tissue was homogenized in 1 to 2 vol of homogenization buffer (0.1 ml mercaptoethanol, 10 g sucrose, 0.1 g bromophenol blue and 25 mg $NADP^+$ per 100 ml distilled water) and centrifuged for 2 to 3 min. Starch-gel electrophoresis followed standard procedures (Murphy et al. 1990) and used 10.5% Sigma starch gels, Tris maleate buffer (Selander et al. 1971), and 1:1 dilutions of tissue to homogenization buffer absorbed onto Whatman # 1 filter paper. Polyacrylamide-gel electrophoresis followed Kordos and Burton (1993), and utilized 4 µl of tissue homogenate (diluted 1:1 or 1:2) loaded onto 0.8 mm thick, vertical slab gels of 6% total acrylamide in a continuous Tris-boric acid-EDTA buffer system. Starch gels were run under ice at 90 V for 7 h in

a 10 °C refrigerator. Polyacrylamide gels were run at 120 V for 4.5 h at 10 °C. Stains for IDH, MPI, GPI and PGM were prepared as agar overlays following recipes in Murphy et al. (1990), with 0.05 g pyruvic acid added to the MPI stain to suppress lactic dehydrogenase activity. GOT was assayed using a florescent stain and filter-paper overlay as described in Burton and Feldman (1981).

Preliminary analyses of at least 15 individuals from each of two populations showed the following enzymes to be monomorphic: catalase (CAT; EC 1.11.1.6), esterase-D (ESTD; EC 3.1.-.-), malate dehydrogenase (MDH; EC 1.1.1.37), $NADP^+$malic enzyme (sMEP; EC 1.1.1.40), and phosphogluconate dehydrogenase (PGDH; EC 1.1.1.44). Five enzymes (encoded by six loci) were found to be polymorphic: glutamic-oxaloacetic transaminase (GOT; EC 2.6.1.1; 2 loci), isocitrate dehydrogenase (IDH; EC 1.1.1.42), mannose-6-phosphate isomerase (MPI; EC 5.3.1.8), glucose-6-phosphate isomerase (PGI; EC 5.3.1.9) and phosphoglucomutase (PGM; EC 5.4.2.2). All five polymorphic enzymes were assayed on starch gels, and GOT was also assayed on polyacrylamide gels, which generally provided better resolution of *Got*-1. Initial allozyme surveys used all four tissue types, but gonad tissue provided the greatest resolution for these five enzymes and was therefore used for all subsequent assays.

The electrophoretic data were analyzed using BIOSYS-1 (Version 1.7; Swofford and Selander 1981, 1989) to calculate allele frequencies, observed and expected heterozygosities, and the extent of population subdivision (F_{ST}; Nei 1977). Statistical significance of the variation in allelic counts among samples (with rare alleles pooled) was tested with heterogeneity chi-square. Significance of the F_{ST} values was also determined by chi-square analysis, using equations in Waples (1987).

DNA analyses

DNA was extracted from gonad tissue using Chelex 100 (Walsh et al. 1991). A 519 bp segment of the cytochrome oxidase I (COI) gene was amplified by the polymerase chain reaction (PCR) using the following primers based on the complete *Strongylocentrotus purpuratus* mtDNA sequence (Jacobs et al. 1988): primer COIC, (5'-TCGTCTGATCCGTCTTTGTCAC-3', Positions 6335 to 6356) and primer COIJ (5'-CAATACCTGTGAGTCCTCCTA-3', Positions 6833 to 6853). Amplification followed standard procedures (Saiki et al. 1988), using a reaction volume of 50 µl and a magnesium chloride concentration of 2 m*M*. The following thermocycle profile was used: 94 °C, 60 s; 50 °C, 60 s; 72 °C, 90 s for 35 cycles, followed by 5 min at 72 °C. PCR products were purified using Microcon 100 spin filters (Amicon), and sequenced using Taq-DyeDeoxy Terminator Cycle sequencing (Applied Biosystems). All samples were sequenced using primer COIC, and ambiguous sites were resolved by also sequencing the opposite strand using primer COIJ. Products of sequencing reactions were purified on Centri-Sep columns (Princeton Separations) and vacuum-dried. Dried samples were resuspended and electrophoresed on an Applied Biosystems 373A DNA sequencer.

Sequences were aligned using ClustalV (Higgins et al. 1992) or PileUp (Genetics Computer Group 1994). A 305 bp segment (corresponding to Positions 6390 to 6694; Jacobs et al. 1988) was analyzed. Nucleotide diversity, the number of nucleotide substitutions per site between paired sequences, was determined by the method of Jukes and Cantor (1969). Comparisons were also made using haplotypes composed of only nucleotide sites that were unambiguous and polymorphic at the 5% level. Haplotype numbers were compared among demes by contingency-table analysis, with significance tested by a Monte Carlo randomization method to correct for small sample size (Roff and Bentzen 1989). Haplotype numbers were also used to calculate variance components and *F*-statistic analogs using the AMOVA (analysis of molecular variance) program (Excoffier et al. 1992), which incorporates information on DNA haplotype divergence.

Results

For allozymes, sample sizes were large enough for adults and recruits of *Strongylocentrotus purpuratus* to be analyzed separately. Table 1 shows allele frequencies for each of the six polymorphic loci at each location, with sample sizes and observed and expected heterozygosities averaged over all loci. Both chi-square and F_{ST} analyses revealed significant differentiation among populations of both size classes (Table 2). The overall level of population differentiation among adults (mean $F_{ST} = 0.033$) was similar to that among recruits (mean $F_{ST} = 0.037$). For the five locations where both adults and recruits were sampled, significant allelic frequency differences were found for *Got-2* at two locations (SRI and LBS; Table 3 and for *Idh* at three locations (MC, SRI and LBS; Table 3). The most striking contrast was for the $Got\text{-}2^{103}$ allele at SRI, which was in very low frequency in adults (frequency of $Got\text{-}2^{103} = 0.020$, $N = 51$) but in high frequency in recruits (frequency = 0.676, $N = 34$). These differences were confirmed by rerunning the samples side by side on the same gel. Little geographic patterning was apparent in the allozyme data. Geographic patterns were tested by linear regressions of the frequencies of each allozyme vs location number (ordered from north to south). These regressions were significant in only 4 out of 61 tests ($Got\text{-}2^{100}$ in adults, Mpi^{100} in recruits, Pgi^{98} in recruits and Pgm^{98} in adults). Averaging over all populations and all loci, both adults and recruits showed a mean deficit of heterozygotes with respect to Hardy-Weinberg expectations (adults: $D = -0.131$, SE = 0.032; recruits: $D = -0.049$, SE = 0.029) [D = (observed heterozygosity – expected heterozygosity)/expected heterozygosity]. Chi-square analyses revealed significantly more heterozygote deficits than heterozygote excesses in adults (37:11, $p < 0.01$), while the difference was not significant in recruits (27:14, $p > 0.05$).

A 305 bp segment of the mitochondrial COI gene was sequenced in 147 individuals. For this region, nucleotide-sequence diversity was 0.0138 ± 0.0001 (mean $\pm$ SE, calculated according to Jukes and Cantor 1969). Condensing these data to include only nucleotide sites which are phylogenetically informative and unambiguous leaves 23 nucleotide sites and 42 different composite haplotypes (Fig. 2). The level of nucleotide diversity for these haplotypes shows no obvious trends from north to south (PB, 0.1143; MC, 0.1306; SCI, 0.1471; SRI, 0.1316; LB, 0.0904; LBS, 0.1446; LJ, 0.1088; MB, 0.0712; PL, 0.0980; PC, 0.1838; all locations combined, 0.1280; fullsite names in legand to Fig. 1). Because the majority of the 23-nucleotide haplotypes were singletons, the data were further condensed to include only those nucleotide sites which were polymorphic at the 5% level, leaving 6 nucleotide positions and 11 different haplotypes. Between 9 and 17 adults and/or recruits were sequenced from each of the ten

Table 1 *Strongylocentrotus purpuratus.* Allele frequencies, sample sizes (N mean number of individuals scored per locus) and mean heterozygosities (H_o observed heterozygosity; H_e expected heterozygosity) for adults (a) and/or recruits (r) from ten geographic locations listed from north to south (*ND* no data; – zero; *location abbreviations* as in legend to Fig. 1)

Locus, allele	Location (adult or recruit)														
	PB		MC		SCI		SRI		LB	LBS		LJ	MB	PL	PC
	(a)	(r)	(a)	(r)	(a)	(r)	(a)	(r)	(a)	(a)	(r)	(r)	(a)	(r)	(a)
Got-1															
107	–	–	–	–	–	–	–	–	–	0.012	–	–	–	0.017	0.013
103	0.214	0.177	0.151	0.242	0.031	0.154	0.280	0.208	0.286	0.488	0.392	0.231	0.093	0.267	0.316
100	0.667	0.630	0.663	0.617	0.813	0.692	0.574	0.625	0.469	0.325	0.373	0.462	0.759	0.483	0.553
97	0.119	0.177	0.186	0.133	0.156	0.154	0.146	0.167	0.245	0.175	0.235	0.038	0.148	0.150	0.118
93	–	–	–	0.008	–	–	–	–	–	–	–	0.269	–	0.083	–
90	–	0.016	–	–	–	–	–	–	–	–	–	–	–	–	–
Got-2															
106	–	–	–	0.008	–	–	–	0.074	–	–	–	–	–	–	–
103	0.102	0.025	0.039	0.016	0.060	–	0.020	0.676	0.082	0.014	–	–	0.031	–	0.021
100	0.827	0.841	0.873	0.869	0.900	0.964	0.931	0.250	0.887	0.945	0.750	0.962	0.906	0.846	0.936
97	0.071	0.134	0.088	0.107	0.040	0.036	0.049	–	0.031	0.041	0.250	0.038	0.063	0.154	0.043
Idh															
102	0.010	–	0.018	0.009	0.016	–	–	–	–	0.014	–	0.036	–	0.017	–
100	0.612	0.598	0.519	0.636	0.640	0.531	0.727	0.300	0.424	0.614	0.342	0.535	0.421	0.690	0.521
98	0.327	0.317	0.309	0.301	0.281	0.469	0.212	0.650	0.489	0.329	0.625	0.429	0.526	0.276	0.427
94	0.031	0.061	0.127	0.036	0.047	–	0.061	0.050	0.076	0.029	0.033	–	–	0.017	0.052
88	0.020	0.024	0.027	0.018	0.016	–	–	–	0.011	0.014	–	–	0.053	–	–
Mpi															
109	0.032	–	0.043	–	–	–	–	ND	0.011	–	–	–	–	–	0.017
105	0.096	0.171	0.163	0.107	0.083	0.067	0.129	ND	0.136	0.171	0.098	0.107	0.020	0.038	0.138
100	0.479	0.474	0.370	0.500	0.317	0.500	0.597	ND	0.433	0.475	0.383	0.357	0.500	0.328	0.483
95	0.234	0.224	0.293	0.232	0.467	0.400	0.242	ND	0.295	0.220	0.284	0.393	0.420	0.538	0.293
92	–	–	–	0.009	–	–	–	ND	0.011	0.049	–	–	–	–	–
90	0.106	0.092	0.098	0.107	0.133	–	–	ND	0.091	0.085	0.147	0.143	0.060	0.077	0.052
86	0.053	0.039	0.033	0.045	–	0.033	0.032	ND	0.023	–	0.088	–	–	0.019	0.017
Pgi															
113	–	–	–	–	–	–	–	–	0.010	–	–	–	–	–	0.012
109	0.029	0.022	0.045	0.008	–	0.088	0.009	0.025	0.010	0.010	0.017	0.031	–	–	0.012
105	0.346	0.293	0.250	0.335	0.283	0.265	0.232	0.275	0.245	0.231	0.267	0.313	0.318	0.286	0.280
100	0.596	0.674	0.687	0.641	0.700	0.618	0.722	0.675	0.715	0.749	0.683	0.625	0.667	0.657	0.684
98	0.029	0.011	0.018	0.016	0.017	0.029	0.037	0.025	0.020	0.010	0.033	0.031	0.015	0.057	0.012
Pgm															
108	–	–	–	0.008	–	–	–	–	–	–	–	–	–	–	–
105	–	0.012	0.020	0.017	0.017	0.094	0.020	–	0.039	0.010	0.033	–	0.040	0.033	0.010
102	0.067	0.083	0.100	0.034	0.033	0.063	0.020	0.042	0.127	0.094	0.041	0.167	0.100	0.033	0.060
100	0.471	0.440	0.500	0.594	0.683	0.531	0.610	0.791	0.589	0.604	0.541	0.700	0.680	0.684	0.610
98	0.375	0.429	0.340	0.297	0.234	0.312	0.290	0.167	0.216	0.240	0.311	0.133	0.160	0.233	0.230
95	0.087	0.036	0.040	0.042	0.033	–	0.060	–	0.029	0.052	0.074	–	0.020	0.017	0.090
92	–	–	–	0.008	–	–	–	–	–	–	–	–	–	–	–
(N)	(48.5)	(39.8)	(50.2)	(59.2)	(27.2)	(15.2)	(43.3)	(17.6)	(48.3)	(42.2)	(54.8)	(14.2)	(26.8)	(27.2)	(42.2)
H_o	0.496	0.489	0.447	0.409	0.375	0.463	0.447	0.371	0.389	0.417	0.512	0.484	0.425	0.507	0.381
H_e	0.531	0.525	0.534	0.502	0.436	0.478	0.448	0.399	0.528	0.486	0.558	0.505	0.448	0.497	0.493

geographic locations. Both size classes were sequenced from only two locations (SRI and LBS). Contingency chi-square analyses (following Roff and Bentzen 1989) revealed no significant differences between the 6-nucleotide haplotype distributions of adults and recruits from these two locations; therefore the two size classes were pooled together. Comparison of haplotype numbers at each location (Table 4) by contingency chi-square revealed significant heterogeneity among locations ($\chi^2 = 115.05$ $df = 90$, $p < 0.05$), indicating that the *distribution* of haplotypes differs. The same data were also compared by an AMOVA (Excoffier et al. 1992) incorporating information on the number of nucleotide differences between haplotypes. This comparison did not show significant differentiation among locations ($F_{ST} = 0.017$, $p > 0.05$), indicating

Table 2 *Strongylocentrotus purpuratus.* Chi-square values (χ^2) and standardized variance (F_{ST}) for tests of heterogeneity in allele frequencies (six loci) among locations. Recruits from Site SRI were excluded from this analysis because they were not scored for *Mpi* (*SE* standard error; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$)

Comparison	Locus						Mean ± SE
	Got-1	*Got-2*	*Idh*	*Mpi*	*Pgi*	*Pgm*	
Adults (8 locations)							
χ^2	59.95***	11.02	38.38***	39.54**	12.07	21.52	
F_{ST}	0.074***	0.014	0.040***	0.026***	0.008	0.020***	0.033 ±0.010
Recruits (6 locations)							
χ^2	24.03**	12.66*	33.47***	33.01**	1.018	15.37	
F_{ST}	0.051***	0.049***	0.052***	0.032***	0.005	0.035***	0.037 ±0.007

Table 3 *Strongylocentrotus purpuratus.* Chi-square values from tests of heterogeneity in allele frequencies (six loci) between adults and recruits from same location ($* p < 0.05$; $** p < 0.01$; $*** p < 0.001$; *nd* no data; *location abbreviations* as in legend to Fig. 1)

Location	Locus					
	Got-1	*Got-2*	*Idh*	*Mpi*	*Pgi*	*Pgm*
PB	1.636	0.072	0.389	2.779	1.557	0.599
MC	2.796	0.007	6.884*	3.925	3.877	5.068
SCI	1.132	1.045	1.067	3.511	0.666	2.288
SRI	0.502	91.515***	9.477**	nd	0.126	2.790
LBS	1.705	11.562***	13.322***	5.966	1.213	1.396

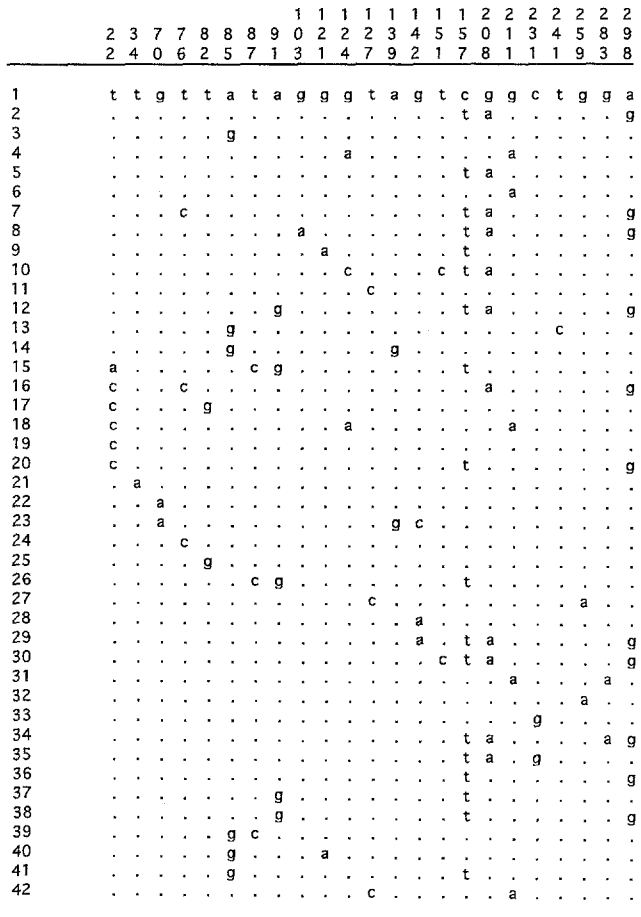

Fig. 2 *Strongylocentrotus purpuratus.* Nucleotide variation in 42 mitochondrial DNA haplotypes (*Numbers on top abscissa* nucleotide positions; *dots* positions identical to Haplotype 1) Position 1 corresponds to Position 6390 in sequence published by Jacobs et al. (1988)

that nucleotide variation for all locations combined was not significantly greater than the mean nucleotide variation within locations.

Both allozyme and mitochondrial DNA data were examined for a break at Point Conception. Of the ten locations sampled, two are north of Point Conception (PB and MC), two are in what might be considered a transition zone (SCI and SRI) and the remaining six are south of Point Conception. Table 5 shows the proportion of the total variation attributable to differences among regions (F_{RT}) for four different regional groupings. Whether the two transitional locations are grouped with the southern region, grouped with the northern region, or considered as a separate "central" group, both allozyme and mitochondrial DNA indicate no significant regional differentiation. However, the DNA data do indicate a significant break well south of Point Conception, between Laguna Beach and La Jolla, California. This break was not observed in the data for all allozyme loci combined (Table 5). Of the four cases where an allozyme cline was detected, only one (Mpi^{100} in recruits) showed a significant break consistent with the mtDNA break (between LBS and LJ) when population allele frequencies were compared by analysis of variance.

Discussion

The principal finding of this study is that both allozyme and mitochondrial DNA data show significant differentiation among subpopulations of *Strongylocentrotus purpuratus*. The allozyme pattern is reminiscent of the genetic mosaics found in limpets and sea urchins

Table 4 *Strongylocentrotus purpuratus*. Mitochondrial DNA haplotype numbers (Nucleotide Positions 85, 124, 157, 208, 211, 298) at ten geographic locations listed from north to south (– zero; *location abbreviations* as in legend to Fig. 1)

Haplotype	Location										Totals
	PB	MC	SCI	SRI	LB	LBS	LJ	MB	PL	PC	
agcgga	6	6	2	7	8	10	4	7	6	4	60
agtagg	3	7	3	7	2	14	2	1	1	–	40
ggcgga	2	2	4	–	1	4	2	2	–	1	18
aacgaa	–	–	1	2	–	1	–	1	1	1	7
agtgga	1	–	–	3	–	–	1	–	1	–	6
agtaga	–	1	2	1	–	–	–	–	1	–	5
agcgaa	1	1	–	–	–	1	–	2	–	–	5
agtggg	–	–	–	1	–	–	–	–	–	2	3
actaga	–	–	–	–	1	–	–	–	–	–	1
agcagg	–	–	–	–	–	–	–	–	–	1	1
ggtgga	–	–	–	–	–	1	–	–	–	–	1
Totals	13	17	12	21	12	31	9	13	10	9	147

Table 5 *Strongylocentrotus purpuratus*. Components of variance attributable to differences among regions (F_{RT}) for four different regional groupings. Significance was calculated according to Waples (1987) for allozyme data and according to Excoffier et al. (1992) for mitochondrial DNA (mtDNA) data. (* $p < 0.05$)

Regional grouping	F_{RT}	
	allozymes	mtDNA
(1) (PB, MC) vs (SCI, SRI, LB, LBS, LJ, MB, PL, PC)	– 0.003	– 0.024
(2) (PB, MC, SCI, SRI) vs (LB, LBS, LJ, MB, PL, PC)	– 0.003	0.001
(3) (PB, MC) vs (SCI, SRI) vs (LB, LBS, LJ, MB, PL, PC)	0.012	– 0.013
(4) (PB, MC, SCI, SRI, LB, LBS) vs (LJ, MB, PL, PC)	– 0.002	0.064*

studied in Australia (Johnson and Black 1982, 1984; Watts et al. 1990), where genetic differences over short geographic distances in some cases greatly exceeded differences over much larger distances. The mtDNA pattern also shows substantial differentiation over short distances (e.g. between LB and LBS), with a mild regional break well south of Point Conception. Our finding of significant mtDNA differentiation contrasts with previous studies on *S. purpuratus* (Palumbi and Wilson 1990; Palumbi 1995), which did not detect significant mtDNA population subdivision. This difference is apparently due both to the greater number of populations sampled in the present study and to the geographic ranges studied, with our samples focusing more on the southern end of the species' range.

The small but significant level of population subdivision detected by allozymes ($F_{ST} = 0.033$ for adults, $F_{ST} = 0.037$ for recruits) is comparable to that found in other marine invertebrates with long-lived pelagic larvae (Nishida and Lucas 1988; Hurst and Skibinski 1995). The extent of population subdivision detected with mtDNA was somewhat milder than that detected with allozymes, as significant mtDNA differences were revealed by chi-square analysis but not by analysis of variance. This may be due to a real difference in the level of nuclear versus mitochondrial DNA diversity. Estimates of single-copy nuclear DNA diversity in *Strongylocentrotus purpuratus* are $\simeq 8$ times higher than mtDNA diversity (reviewed in Palumbi and Wilson 1990). The mechanism underlying this difference is unclear.

An alternative explanation for the stronger differentiation in allozymes vs mtDNA concerns the lack of statistical power inherent in the smaller DNA data set relative to the allozyme data set (for the six allozyme loci the average number of genomes sampled was > 1100, while for the single mtDNA locus the total number of genomes sampled was 147). Although we chose to test the significance of the mtDNA F_{ST} by a Monte Carlo method (Excoffier et al. 1992) because of the small sample size, the chi-square method of determining significance (Waples 1987) illustrates the linear dependence on sample size [$\chi^2 = 2N\, F_{ST}(k-1)$, where N is the total number of diploid individuals and k is the number of alleles]. In future studies we plan to increase the mtDNA sample size by doing restriction-fragment analyses rather than sequencing the entire fragment. Of the three most polymorphic nucleotide sites (Positions 157, 208 and 298), restriction enzymes are available which recognize two of them (Enzyme *Sty*I at Position 208 and Enzyme *Tha*I at Position 298).

Comparing the level of genetic subdivision among different age classes is one means of discerning the mechanisms driving population differentiation. For example, if population differentiation is driven by post-settlement selection under localized conditions, then subpopulations of adults should be more heterogeneous than subpopulations of recruits (e.g. Koehn et al. 1980). However, if differentiation is due to the geographic source of larvae or to pre-recruitment selection, then recruits should be at least as heterogeneous as adults (e.g. Johnson and Black 1982). Our results showed similar levels of allozyme differentiation among subpopulations of adults and recruits, indicating that pre-recruitment effects may be important.

Mechanisms driving population differentiation can also be inferred by comparing genotype frequencies in different age classes. Heterozygote deficits are common in a range of marine taxa (e.g. Bucklin and Hedgecock 1982; Zouros and Foltz 1984; Watts et al. 1990). In marine bivalves in particular, these deficits are often reduced in older organisms (e.g. Koehn et al. 1976; Johnson and Black 1982; Zouros and Foltz 1984), presumably by post-recruitment selection for heterozygotes. Instead, our results show *greater* heterozygote deficits in older organisms. This resembles the pattern found by Watts et al. (1990) for the sea urchin *Echinometra mathei*, where greater heterozygote deficits in adults were hypothesized to result from the accumulation of genetically differentiated cohorts. This sort of temporal Wahlund effect would be expected to be most pronounced in relatively long-lived organisms such as urchins. It should be noted, however, that in the present study the data sets for adults and recruits are not wholly comparable, and that the "recruits" included individuals up to 20 mm in diameter, which may already have undergone considerable post-settlement selection. The optimal test of the mechanisms driving population structure would require equal sample sizes of adults and newly-settled recruits collected simultaneously from multiple locations, with replication over time.

Perhaps the most dramatic result of this study is the extreme allelic-frequency differences at the *Got-2* locus for adults and recruits collected from one tidepool on Santa Rosa Island at the same time. Specifically, the recruits were found to have an anomalously high frequency of the 103 allele. The difference does not appear to be a developmental change, because the pattern was not found in other pairs of adults and recruits. The difference is clearly not attributable to a species misidentification, since the remaining four allozyme loci and the mtDNA-sequence data showed both adults and recruits to be typical *Strongylocentrotus purpuratus*. The remaining possible explanations are that the recruits came from an unusual geographic source or that the difference may be caused have arisen by selection on the *Got-2* locus or on a closely linked locus. These possibilities cannot be distinguished with the present data, but previous studies indicate that the same enzyme may be under selection in other marine organisms (Grahame et al. 1992; Kordos and Burton 1993; Johannesson et al. 1995).

Genetic structure in *Strongylocentrotus purpuratus* might be expected to show a break at Point Conception, due either to the current pattern or to the abrupt change in near-shore temperature regimes. Because the primary water flow, the California Current, runs from north to south, southern populations might also be expected to have higher genetic diversity than northern populations, as mutations arising in the south would be less likely to be move northward than vice versa. Neither of these two genetic predictions is supported by the present data. Neither the allozyme nor the mtDNA data suggest a difference between the regions north and south of Point Conception, and no obvious geographical trends were found in the level of observed or expected allozyme heterozygosity or of mtDNA haplotype diversity. The explanation for the mild genetic break $\simeq$ 300 km south of Point Conception is not clear, but it is intriguing that a recent study of *S. purpuratus* settlement patterns showed a break at approximately the same location (Ebert et al. 1994). While this discontinuity was not detected in the overall allozyme data, the mild clines observed for individual alleles warrant further study. Whatever the cause of the regional genetic differentiation south of Point Conception, it is far subtler than that shown in populations spanning biogeographic boundaries on the southeast coast of North America (reviewed in Avise 1992). Although previous studies of population genetic structure along the west coast have not focused on Point Conception, those studies spanning the boundary have generally not found an obvious break (Levinton and Suchanek 1978; Ford and Mitton 1983; Palumbi and Wilson 1990; Hellberg 1994). One reason for the poor fit between genetic structure and predictions based on current patterns may be the variability in the California current. Changes in larval transport during ENSO (El Niño Southern Oscillation) events, for example, have been suggested to result in "reverse dispersal" from south to north (Palumbi 1995).

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References

Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63: 62–76

Britten RJ, Cetta A, Davidson EH (1978) The single-copy DNA sequence polymorphism of the sea urchin *Strongylocentrotus purpuratus*. Cell 15: 1175–1186

Bucklin A, Hedgecock D (1982) Biochemical genetic evidence for a third species of *Metridium* (Coelenterata: Actiniaria). Mar Biol 66: 1–7

Buroker NE (1983) Population genetics of the American oyster *Crassostrea virginica* along the Atlantic coast and the Gulf of Mexico. Mar Biol 75: 99–112

Burton RS (1983) Protein polymorphisms and genetic differentiation of marine invertebrate populations. Mar Biol Lett 4: 193–206

Burton RS, Feldman MW (1981) Population genetics of *Tigriopus californicus*. II. Differentiation among neighboring populations. Evolution 35: 1192–1205

Burton RS, Lee B-N (1994) Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. Proc natn Acad Sci USA 91: 5197–5201

Ebert TA, Russell MP (1988) Latitudinal variation in size structure of the west coast purple urchin: a correlation with headlands. Limnol Oceanogr 33: 286–294

Ebert TA, Schroeter SC, Dixon JD, Kalvass P (1994) Settlement patterns of red and purple sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*) in California, USA. Mar Ecol Prog Ser 111: 41–52

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among haplotypes: applications to human mitochondrial DNA restriction data. Genetics, Austin, Tex 131: 479–491

Ford MJ, Mitton JB (1993) Population structure of the pink barnacle, *Tetraclita squamosa rubescens*, along the California coast. Molec mar Biol Biotechnol 2: 147–153

Genetics Computer Group (1994) Program manual for the GCG package. Version 8. Genetics Computer Group, 575 Scenic Drive, Madison, Wisconsin 53711

Grahame J, Mill PJ, Double M, Hull SL (1992) Patterns of variation in *Aat-1* allele frequencies in rough periwinkles (*Littorina*) suggest similar selection regimes rather than conspecificity. J mar biol Ass UK 72: 499–502

Hellberg ME (1994) Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. Evolution 48: 1829–1854

Higgins DG, Bleasby AJ, Fuchs R (1992) Clustal V: improved software for multiple sequence alignment. CABIOS 8: 189–191

Hurst CD, Skibinski DOF (1995) Comparison of allozyme and mitochondrial DNA spatial differentiation in the limpet *Patella vulgata*. Mar Biol 122: 257–263

Incze LS, Ortner PB, Schumacher JD (1990) Microzooplankton, vertical mixing and advection in a larval fish patch. J Plankton Res 12: 365–379

Jablonski D, Flessa K, Valentine JW (1985) Biogeography and paleobiology. Paleobiology 11: 75–90

Jacobs HT, Elliot DJ, Math VB, Farquharson A (1988) Nucleotide sequence and gene organization of sea urchin mitochondrial DNA. J molec Biol 202: 185–217

Johannesson K, Johannesson J, Lundgren U (1995) Strong natural selection causes microscale allozyme variation in a marine snail. Proc natn Acad Sci USA 92: 2602–2606

Johnson MS, Black R (1982) Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. Mar Biol 70: 157–164

Johnson MS, Black R (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38: 1371–1383

Jukes TH, Cantor CR (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism. Academic Press, New York, pp 21–132

Karl SA, Avise JC (1992) Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science, NY 256: 100–102

Koehn RK, Milkman R, Mitton JB (1976) Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel, *Mytilus edulis*. Evolution 30: 2–32

Koehn RK, Newell RIE, Immerman F (1980) Maintenance of an aminopeptidase allele frequency cline by natural selection. Proc natn Acad Sci USA 77: 5385–5389

Kordos LM, Burton RS (1993) Genetic differentiation of Texas Gulf coast populations of the blue crab *Callinectes sapidus*. Mar Biol 117: 227–233

Levinton JS, Suchanek TH (1978) Geographic variation, niche breadth and genetic differentiation at different geographic scales in the mussels *Mytilus californianus* and *M. edulis*. Mar Biol 49: 363–375

Lewis RI, Thorpe JP (1994) Temporal stability of gene frequencies within genetically heterogeneous populations of the queen scallop *Aequipectin* (*Chlamys*) *opercularis*. Mar Biol 121: 117–126

Macleod JAA, Thorpe JP, Duggan NA (1985) A biochemical genetic study of population structure in queen scallop (*Chlamys opercularis*) stocks in the Northern Irish Sea. Mar Biol 87: 77–82

Morris RH, Abbott DP, Haderlie EC (1980) Intertidal invertebrates of California. Stanford University Press. Stanford, California

Murphy RW, Sites JW Jr, Buth DG, Haufler CH (1990) Proteins. I. isozyme electrophoresis. In: Hillis DM, Moritz C (eds) Molecular systematics. Sinauer Associates, Sunderland, Massachusetts, pp 45–126

Nei M (1977) *F*-statistics and analysis of gene diversity in subdivided populations. Ann hum Genet 41: 225–233

Nishida M, Lucas JS (1988) Genetic differences between geographic populations of the crown-of-thorns starfish throughout the Pacific region. Mar Biol 98: 359–368

Palumbi SR (1992) Marine speciation on a small planet. Trends Ecol Evolut 7: 114–118

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. A Rev Ecol Syst 25: 547–572

Palumbi SR (1995) Using genetics as an indirect estimator of larval dispersal. In: McEdward LR (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton, pp 369–387

Palumbi SR, Wilson AC (1990) Mitochondrial DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. Evolution 44: 403–415

Reeb CA, Avise JC (1990) A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. Genetics, Austin, Tex 124: 397–406

Roff DA, Bentzen P (1989) The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. Molec Biol Evolut 6: 539–545

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science, NY 239: 487–491

Scheltema RS (1971) Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. Biol Bull mar biol Lab, Woods Hole 140: 284–322

Scheltema RS (1978) On the relationship between dispersal of pelagic veliger larvae and the evolution of marine prosobranch gastropods. In: Battaglia B, Beardmore JA (eds) Marine organisms: genetics, ecology and evolution. Plenum Press, New York, pp 303–322

Selander RK, Smith MH, Yang SY, Johnson WE, Gentry JB (1971) IV. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Stud Genet, Austin, Tex 6: 49–90 (Univ Texas Publ No. 7103)

Strathmann RR (1978) The length of pelagic period in echinoderms with feeding larvae from the northeastern Pacific. J exp mar Biol Ecol 34: 23–27

Swofford DL, Selander RB (1981) BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J Hered 72: 281–283

Swofford DL, Selander RK (1989) BIOSYS-1: a computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey, Champaign, Illinois

Valentine JW (1973) Evolutionary paleoecology of the marine biosphere. Prentice-Hall, Englewood Cliffs, New Jersey

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for the simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10: 506–513

Waples RS (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41: 385–400

Watts RJ, Johnson MS, Black R (1990) Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. Mar Biol 105: 145–151

Zouros E, Foltz DW (1984) Possible explanations of heterozygote deficiency in bivalve molluscs. Malacalogia 25: 583–591