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# PATTERNS OF REPRODUCTION, GENETIC DIVERSITY, AND GENETIC DIFFERENTIATION IN CALIFORNIA POPULATIONS OF THE GENICULATE CORALLINE ALGA *LITHOTHRIX ASPERGILLUM* (RHODOPHYTA)[1]

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ABSTRACT

*The reproductive composition and genetic diversity of populations of the red seaweed* Lithothrix aspergillum *Gray (O. Corallinales) were studied at three southern California sites (Shaw's Cove and Treasure Island, Laguna Beach; Indian Rock, Santa Catalina Island) and at a fourth site (Bodega Bay) located in northern California. Sexually reproducing populations were confined to southern California. Diploid individuals were numerically dominant over haploid (gametophytic) individuals at all sites. Intertidal and subtidal subpopulations from Shaw's Cove differed in their reproductive profiles. Most intertidal specimens found on emersed surfaces were densely branched, turf-forming, and bore tetrasporangial (68.6%), carposporangial (11.4%), or spermatangial (5.7%) conceptacles, reflecting a sexual life history; none produced asexual bispores. In contrast, 74.3% of the larger, loosely branched subtidal specimens bore bisporangial conceptacles indicative of asexual reproduction. Nearly 70% of the Indian Rock thalli showed no evidence of conceptacle formation. Only asexual, diploid bispore-producing thalli were obtained from the Bodega Bay site. Genetic diversity (mean number of alleles per locus, percent of polymorphic loci, and average expected heterozygosity) of diploid* L. aspergillum *populations varied with life-history characteristics and geographic location. A total of 30 alleles was inferred from zymograms of 16 loci examined by starch-gel electrophoresis; of these loci, 11 were polymorphic. The genetic diversity of sexual, diploid populations of* L. aspergillum *(alleles per locus [A/L] = 1.4–1.5; percent polymorphic loci [%P] = 37.5–50.0) was relatively high compared with other red seaweeds. Lowest diversity (A/L = 1.0; %P = 0.0) occurred in the exclusively asexual Bodega Bay population which consisted of genetic clones. All sexual* L. aspergillum *populations deviated significantly from Hardy–Weinberg expectations due to lower than expected heterozygosity. Genetic differentiation (Wright's $F_{statistic}$ [$F_{ST}$]; Nei's Genetic Distance [D]) among sexually reproducing southern California populations was low ($F_{ST}$ = 0.030) on a local scale (ca. 5 km), suggesting high levels of gene flow, but high genetic differention ($F_{ST}$ = 0.390 and 0.406) occurred among southern California populations separated by ca. 70 km. Very high genetic differentiation ($F_{ST}$ = 0.583–0.683) was obtained between northern and southern California populations separated by 700–760 km. Our genetic and reproductive data suggest that the* L. aspergillum *population from Bodega Bay is sustained by perennation, vegetative propagation, or asexual reproduction by bispores and may represent an isolated remnant or a population established by a founder event.*

*Key index words:* Corallinales; *coralline algae; genetic variation; isozyme electrophoresis;* Lithothrix aspergillum; *reproduction; Rhodophyta; seaweeds*

Knowledge of the genetic structure of seaweed populations is limited compared with other groups of organisms (Innes 1984, Cheney 1985, van der Meer 1986). The few available studies performed on seaweeds have relied on gel electrophoresis to analyze enzyme polymorphisms within and among populations (Malinowski 1974, Cheney and Babbel 1978, Miura et al. 1979, Innes and Yarish 1984, Fujio et al. 1985, 1987, Innes 1987, 1988, Sosa and Garcia-Reina 1992, 1993, Intasuwan et al. 1993, Lindstrom 1993, Neefus et al. 1993, Lu and Williams 1994, Sosa et al. 1996, Williams and Di Fiori 1996). This previous research has established the usefulness of protein electrophoresis for studies of the population genetics of marine macroalgae.

Genetic diversity appears to be low in seaweed populations where asexual reproduction is common (Malinowski 1974, Cheney and Babbel 1978, Innes and Yarish 1984, Fujio et al. 1985, 1987, Innes 1987, 1988, Sosa and Garcia-Reina 1992, 1993, Intasuwan et al. 1993, Sosa et al. 1996). Genetic differentiation among such populations, however, varies and can be almost nonexistent (e.g. Malinowski 1974, Sosa et al. 1996) or quite high, even over short geographic distances (e.g. Miura et al. 1979, Fujio et al. 1985, 1987, Innes 1987, 1988). In contrast, high levels of genetic diversity, together with low levels of genetic differentiation, have been reported over small geographic distances for exclusively sexual populations of *Halidrys dioica,* an obligate outcrossing seaweed with broad dispersal abilities (Lu and Williams 1994). Hence, although data are too limited yet for well-founded generalizations, evidence suggests that the genetic structure of seaweed populations appears to be related to reproductive and life history characteristics and to dispersal ability. Here, we report results of the first study to describe genetic patterns corresponding with geographic variations in reproductive mode and life-history type over the distributional range of a single species, the monotypic red alga *Lithothrix aspergillum* Gray.

*Lithothrix aspergillum,* a geniculate, calcified seaweed, is confined to the eastern North Pacific and ranges from Isla Magdalena, Baja California, Mexico

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(24°55′ N, 112°10′ W), to Cook Inlet, Alaska (ca. 59°30′ N, 151°40′ W) (Scagel et al. 1989). However, this northern limit has been questioned by Tyrell and Johansen (1995), who indicate a more certain range termination at Melville Island, British Columbia (54°22′ N, 130°45′ W). *Lithothrix aspergillum* is believed to exhibit a triphasic life history of the *Polysiphonia* type (Murray and Dixon 1992) only in southern California where gametangial, carposporangial, and tetrasporangial phases have been reported (Ganesan and Desikachary 1970, Gittins 1975, Tyrell and Johansen 1995). Throughout most of its range, *L. aspergillum* produces bisporangial thalli that release uninucleate bispores (Gittins 1975, Tyrell and Johansen 1995). In the Corallinales, bisporangia have not been reported on gametangial thalli, and uninucleate bispores are believed to be mitotic homologues of tetraspores that function in the asexual reproduction of the tetrasporangial phase (Johansen 1981, Guiry 1990, Murray and Dixon 1992). Examination of specimens collected outside of southern California by Gittins (1975) and Tyrell and Johansen (1995) revealed only bisporangial or sterile, vegetative thalli. Studies have also demonstrated that *L. aspergillum* can regenerate upright axes rapidly from basal remnants and regrow from detached fragments (Gittins 1975, Tyrell and Johansen 1995). These observations suggest that *L. aspergillum* maintains populations by perennation, vegetative propagation, or the asexual production of uninucleate bispores where sexual individuals have yet to be reported. In addition, Tyrell and Johansen (1995) provide evidence to suggest that sexually reproducing southern California populations of *L. aspergillum* show spatial segregation of reproductive types on a local scale.

The purpose of this study was to test the hypothesis that the genetic diversity of *L. aspergillum* populations varies in relation to life-history characteristics over local, regional, and latitudinal geographic scales. We predicted that reduced genetic diversity occurs within *L. aspergillum* populations reproducing mostly or exclusively by asexual means and that sexually reproducing populations are characterized by greater diversity. To test this hypothesis, we sampled *L. aspergillum* from a northern California population believed to be exclusively asexual and compared its reproductive and genetic characteristics with southern California *L. aspergillum* populations that exhibit both asexual and sexual reproduction. A second purpose of our research was to determine whether patterns of genetic diversity and genetic differentiation among southern California *L. aspergillum* populations can be predicted from their reproductive profiles, which, as suggested by Tyrell and Johansen (1995), differ on local and regional scales. To answer these questions, we sampled *L. aspergillum* from three distinct sites in southern California to learn if a relationship exists between genetic and geographic distance.

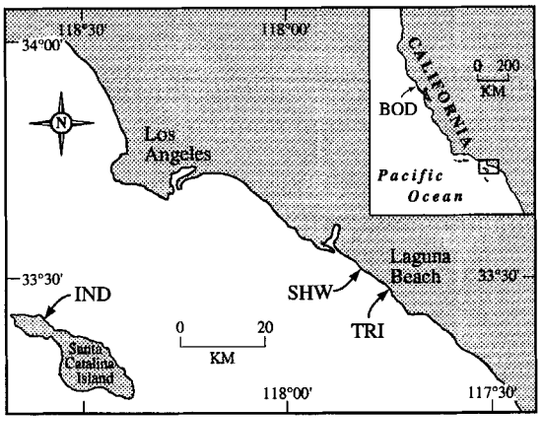


FIG. 1. Map showing the four California sites from which populations of *Lithothrix aspergillum* were sampled.

## MATERIALS AND METHODS

*Lithothrix aspergillum* samples were collected from three southern California populations, and a fourth population was collected 700–760 km to the north near Bodega Bay (38°15′ N, 122°57′ W) (Fig. 1). Two of the southern California populations were located in Laguna Beach within 5.5 km of each other at Shaw's Cove (33°32.8′ N, 117°48.2′ W) and Treasure Island (33°30.8′ N, 117°45.5′ W). The third southern California population was obtained from a site ca. 70 km southwest of Laguna Beach at Indian Rock, Santa Catalina Island (33°28.1′ N, 118°31.5′ W). Intertidal (0.5–1.0 m above MLLW) collections were made at the Shaw's Cove (April 1994), Treasure Island (March 1995), and Bodega Bay (December 1994) sites, whereas samples were obtained from lower intertidal and shallow subtidal habitats (0.5–2 m below MLLW) at Indian Rock (September 1994) and from the shallow subtidal (2–3 m below MLLW) at Shaw's Cove (April 1994) using snorkeling gear. Preliminary analyses of the reproductive status of intertidal and subtidal subpopulations from Shaw's Cove were performed during January and August of 1993. These preliminary results, together with our reported data for April of 1994 and subsequent observations, indicate that seasonal shifts in reproductive composition in this perennial seaweed are minimal and that the data reported herein are representative of the populations occupying each of our study sites.

At each site, individual thalli (ca. 2 g wet weight) were collected haphazardly by hand, placed separately in 20-mL vials or small plastic bags with ca. 5 mL of seawater, and transported to the laboratory on ice where they were held at 10° C for a maximum of 3 (enzyme extractions) or 4 (reproductive determinations) days. Samples were obtained from representative habitat and over similar intertidal areas at each site and were not collected from contiguous clumps to ensure that each represented a distinct individual. At all southern California sites, samples collected from emersed, intertidal surfaces consisted of small, densely branched, turf-forming thalli, whereas larger, less branched specimens were obtained from the subtidal zone, intertidal pools, and the lower intertidal zone. The latter arborescent form also characterized the Bodega Bay *L. aspergillum* population, which was collected from low intertidal surfaces.

*Determination of reproductive status.* For each *L. aspergillum* population, ca. 1 g (wet weight) of each specimen was examined with a stereomicroscope for the presence of conceptacles. The contents of conceptacles (tetraspores, bispores, carpospores, and spermatangia) can be used to establish whether fertile thalli are gametangial (presumably haploid) or tetrasporangial (presumably diploid); bisporangia in the coralline red algae are believed to occur only on sporangial and presumably diploid thalli (Guiry 1990). Except for the nature of their conceptacles, fertile *L. aspergillum* thalli are morphologically indistinguishable from sterile thalli. A total of 60–75 specimens was analyzed for each population except for the Shaw's Cove site, where 75 individuals were

examined for both intertidal and subtidal collections. Where present, conceptacles were excised from the genicular surface using a razor blade, and the exposed spore contents were examined to determine reproductive status. Male gametangial thalli were easily recognized based on the small size and high intergenicular density of their conceptacles (Tyrell and Johansen 1995). Thalli lacking conceptacles were recorded as sterile. With the exception of male thalli, the reproductive status of individuals with conceptacles but without spores could not be determined.

*Genetic diversity.* The genetic composition of each sampled *L. aspergillum* population or subpopulation was determined from isozyme phenotypes using starch-gel electrophoresis. Seaweeds can be extremely difficult subjects for molecular study (Olsen 1990, Neefus et al. 1993). Hence, several extraction buffers, gel buffers, and enzyme stains were screened prior to identifying a protocol that optimized visualization and interpretation of zymograms of enzyme systems.

Specimens of *L. aspergillum* (n = 60–75) were carefully cleaned of epiphytes, blotted dry, and ground with a chilled mortar and pestle for ca. 3 min in an extraction buffer specified by Lindstrom and South (1989). The extraction buffer (4 mM disodium EDTA, 20 mM sodium metabisulfite, 50 mM 3-[N-Morphalino]propanesulfonic acid (MOPS), 200 mM ascorbic acid, 5% w/v PVP40, and 5 mM β-mercaptoethanol; pH 7.5) was prepared in two parts; two-thirds of the volume was mixed without β-mercaptoethanol and used for vigorous, primary grinding (1 mL buffer per 1 g of sample), whereas β-mercaptoethanol was added to the other one-third volume (0.5 mL buffer per 1 g of sample) and used to complete the grinding process. The extraction slurry was then transferred to a 1.5-mL microcentrifuge tube and centrifuged at ca. 13,000 rpm for 30 s. Samples were kept cool at all times in ice and gel-filled storage racks when not refrigerated.

Starch gels were prepared using 36 g starch, 9 g sucrose, and 300 mL gel buffer. A tris-ethylenediaminetetra acetate borate (TEB) gel buffer (0.18 M Tris, 0.004 M $Na_2EDTA$, and 0.10 M boric acid) was used for electrophoresis of all enzyme systems. A 1:3 TEB:deionized water solution was used for the gel buffer, whereas full-strength TEB served as the electrode buffer. The TEB gel buffer (225 mL) was brought to a boil in a microwave oven and then carefully poured into a 2-L Florence flask containing 75 mL of gel buffer to which starch and sucrose had been added. The resulting solution was vigorously shaken to ensure thorough mixing, returned to the microwave, and brought to a gentle boil. The gel solution was then immediately removed from the microwave oven, shaken, degassed using a vacuum hose, and poured into Plexiglas gel forms (141 mm L × 182 mm W × 9 mm D). Poured gels were allowed to cool and then sealed with thin plastic wrap to prevent dehydration and stored at 10° C until use.

Two 75 mm × 30 mm wicks made of Whatman #1 filter paper were dipped into the supernatant from each extracted sample and loaded together into a horizontal slit in the gel located ca. 3 cm from the anode. Extracts from 25 algal samples (including reference thalli) were loaded into each gel, along with a single wick containing a Bromo-Phenol Blue tracking dye. Gels were covered with plastic wrap and run horizontally at 10° C for 4 h at 30 mA and 200 V. After ca. 15 min, the wicks were removed, the sample slit closed, and all bubbles gently squeezed out to ensure a uniform current across the gel.

Following electrophoresis, gels were sliced horizontally into thin sections. Each section was then placed into a glass baking dish (17.5 cm × 27.5 cm) and stained according to protocols of Weeden and Wendel (1987) for one of the following enzymes: alanine aminotransferase (ALT, E.C. 2.6.1.2), aldolase (ALD, E.C. 4.1.2.13), fructose-bisphosphatase (FBP, E.C. 3.1.3.11), glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), hexokinase (HEX, E.C. 2.7.1.1), isocitrate dehydrogenase (IDH NADP form, E.C. 1.1.1.42), malate dehydrogenase (MDH, E.C. 1.1.1.37), phosphoglucomutase (PGM, E.C. 5.4.2.2), ribulose-bisphosphate carboxylase (RBC, E.C. 4.1.1.39), and triose-phosphate isomerase (TPI, E.C. 5.3.1.1). Staining trays were placed in the dark at ca. 24° C until bands appeared. TPI, GPI, and RBC were scored within 3 h when resolution was best, while all other enzyme systems took as long as 24 h to develop fully. Many additional enzyme systems were tested (Table 1) but not used because of poor resolution or because no activity could be detected using the described methodology.

TABLE 1. *Allele frequencies inferred from zymograms of 16 consistently scorable enzyme*[a] *loci for diploid California populations and subpopulations of* Lithothrix aspergillum. *Loci and their alleles are designated numerically and alphabetically based on the degree of anodal migration.*[b] *Sample sizes are given in parentheses.*

| Locus | Allele | Laguna Shaw's Cove (intertidal) (n = 56) | Laguna Shaw's Cove (subtidal) (n = 69) | Laguna Treasure Is. (intertidal) (n = 55) | S. Catalina Is. Indian Rock (subtidal) (n = 53) | Bodega Bay (intertidal) (n = 75) |
|---|---|---|---|---|---|---|
| ALD-1 | a | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| ALT-1 | a | 0.921 | 0.929 | 0.919 | 1.000 | 1.000 |
| | b | 0.079 | 0.071 | 0.081 | 0 | 0 |
| FBP-1 | a | 0.543 | 0.507 | 0.655 | 0 | 1.000 |
| | b | 0.457 | 0.493 | 0.645 | 1.000 | 0 |
| GPI-1 | a | 1.000 | 1.000 | 1.000 | 0.917 | 1.000 |
| | b | 0 | 0 | 0 | 0.083 | 0 |
| GPI-2 | a | 0.964 | 0.971 | 0.939 | 0.333 | 0 |
| | b | 0.036 | 0.029 | 0.029 | 0.667 | 1.000 |
| GPI-3 | a | 0.471 | 0.493 | 0.852 | 0 | 0 |
| | b | 0.529 | 0.507 | 0.148 | 0 | 0 |
| | c | 0 | 0 | 0 | 0 | 1.000 |
| | d | 0 | 0 | 0 | 1.000 | 0 |
| HEX-1 | a | 0.293 | 0.307 | 0.405 | 0.267 | 1.000 |
| | b | 0.707 | 0.693 | 0.595 | 0.733 | 0 |
| HEX-2 | a | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| IDH-1 | a | 0.286 | 0.221 | 0.209 | 0.783 | 1.000 |
| | b | 0.714 | 0.779 | 0.791 | 0.217 | 0 |
| IDH-2 | a | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| MDH-1 | a | 0 | 0 | 0 | 0 | 1.000 |
| | b | 0.279 | 0.200 | 0.230 | 1.000 | 0 |
| | c | 0.721 | 0.800 | 0.770 | 0 | 0 |
| PGM-1 | a | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| RBC-1 | a | 0.507 | 0.579 | 0.743 | 1.000 | 1.000 |
| | b | 0.493 | 0.421 | 0.257 | 0 | 0 |
| RBC-2 | a | 1.000 | 1.000 | 1.000 | 0.217 | 1.000 |
| | b | 0 | 0 | 0 | 0.783 | 0 |
| TPI-1 | a | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| TPI-2 | a | 1.000 | 1.000 | 1.000 | 0.842 | 1.000 |
| | b | 0 | 0 | 0 | 0.158 | 0 |

[a] Additional enzyme systems tested but not consistently resolved or for which activity was not detected included; alcohol dehydrogenase (E.C. 1.1.1.1), alkaline phosphatase (E.C. 3.1.3.1), aspartate aminotransferase (E.C. 2.6.1.1), catalase (E.C. 1.11.1.6), esterase (E.C. 3.1.1.–), formate dehydrogenase (E.C. 1.2.1.2), fumarase (E.C. 4.2.1.2), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), glutamate dehydrogenase (E.C. 1.4.1.2), glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12), lactate dehydrogenase (E.C. 1.1.1.27), malic enzyme (E.C. 1.1.1.40), NAD(P)H dehydrogenase (E.C. 1.6.99), peroxidase (E.C. 1.11.1.7), shikimate dehydrogenase (E.C. 1.1.1.25), sorbitol dehydrogenase (E.C. 1.1.1.14), and superoxide dismutase (E.C. 1.15.1.1).

[b] Loci abbreviations: ALD, aldolase; ALT, alanine aminotransferase; FBP, fructose-bisphosphatase; GPI, glucose-6-phosphate isomerase; HEX, hexokinase; IDH, isocitrate dehydrogenase; MDH, malate dehydrogenase; PGM, phospho-glucomutase; RBC, ribulose-bisphosphate carboxylase; TPI, triose-phosphate isomerase.

The phycobiliprotein pigment found in *L. aspergillum* produced a monomorphic band for all specimens and served as a consistent reference for calculating the relative electrophoretic mobility of each allelic form. A sample extracted from a *L. aspergillum* specimen collected from the midintertidal subpopulation at Shaw's Cove was run in all gels for reference purposes. The genotype of each locus was inferred directly based on the number of bands present for each individual, with single banding patterns inter-

TABLE 2. *Analysis of the reproductive status of California populations of* Lithothrix aspergillum *obtained from the four study sites. Data shown are number of individuals (and percentage composition for the site) by reproductive category. Thalli-bearing carposporangial and spermatangial conceptacles are considered to be gametophytes, whereas thalli with tetrasporangial and bisporangial conceptacles are treated as sporophytes. The Shaw's Cove data are presented for both intertidal and subtidal subpopulations. Site and collection details are provided in the text (n = sample size).*

| Reproductive status | Laguna Shaw's Cove (intertidal) (n = 70) | | Laguna Shaw's Cove (subtidal) (n = 70) | | Laguna Treasure Is. (intertidal) (n = 75) | | S. Catalina Is. Indian Rock (subtidal) (n = 60) | | Bodega Bay (intertidal) (n = 75) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Thalli with conceptacles: | | | | | | | | | | |
| Gametophytic thalli | | | | | | | | | | |
| Carposporangial | 8 | (11.4%) | 0 | (0.0%) | 8 | (10.6%) | 3 | (5.0%) | 0 | (0.0%) |
| Spermatangial | 4 | (5.7%) | 1 | (1.4%) | 8 | (10.6%) | 2 | (0.3%) | 0 | (0.0%) |
| Sporophytic thalli: | | | | | | | | | | |
| Tetrasporangial | 48 | (68.6%) | 17 | (24.3%) | 37 | (49.3%) | 5 | (8.3%) | 0 | (0.0%) |
| Bisporangial | 0 | (0.0%) | 52 | (74.3%) | 0 | (0.0%) | 0 | (0.0%) | 75 | (100.0%) |
| Unknown (empty conceptacles): | 0 | (0.0%) | 0 | (0.0%) | 7 | (9.3%) | 9 | (15.0%) | 0 | (0.0%) |
| Thalli lacking conceptacles: | 10 | (14.2%) | 0 | (0.0%) | 15 | (20.0%) | 41 | (68.3%) | 0 | (0.0%) |

preted as homozygotes and multiple bands as heterozygotes (Ayala 1982).

Genetic diversity of each sampled *L. aspergillum* population and subpopulation was described using the following measures (Nei 1987): allele frequencies, mean number of alleles per locus (A/L), proportion of polymorphic loci (P = the number of polymorphic loci divided by the total number of loci observed within a population), observed heterozygosity ($H_o$ = the proportion of individuals sampled that are heterozygous), and expected heterozygosity ($H_e$ = the number of expected heterozygotes based on Hardy–Weinberg equilibrium). The chi-square statistical test was used to evaluate whether sampled populations or subpopulations deviated from Hardy–Weinberg equilibrium. Wright's F statistics, a hierarchical approach to apportioning genetic diversity (Wright 1978), and Nei's genetic distance (D) were used to measure the levels of genetic differentiation among populations. Wright's F statistics ($F_{IS}$, $F_{IT}$, and $F_{ST}$) were calculated for all possible site comparisons; only Wright's $F_{ST}$ values are reported here. Significance of single locus $F_{ST}$ values were determined by chi-square analysis using equations in Waples (1987). Variances were obtained for mean $F_{ST}$ values by jackknifing over loci, omitting one locus at a time, as recommended by Weir and Cockerham (1984). Genetic distance, which ranges upwards from 0, the case where the same alleles in the same frequency are found in both populations, was calculated from Nei's (1972) genetic identity (I) using the formula ($D = -\ln I$). A dendrogram based on D and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering algorithm was used to further examine genetic differentiation among sampled populations. Calculations of genetic diversity and genetic differentiation were made using the computer program BIOSYS-1 (Swofford and Selander 1989).

Separate A/L, P, $H_o$, and $H_e$ values for diploid tetrasporophyte and haploid gametophytic thalli, which without conceptacles are morphologically indistinguishable from one another, were not calculated because of the variable and small number of identifiable haploid individuals obtained at each site (Table 2). Consequently, measures of genetic diversity are presented only for diploid *L. aspergillum* populations and are based on the sample sizes indicated in Table 3. Individuals lacking conceptacles could not be assigned haploid or diploid status based only on morphological criteria. In these cases, zymograms were used to distinguish diploid individuals, which were included so that our genetic data represent the contributions of both sterile and fertile diploid individuals.

TABLE 3. *Measures of genetic diversity for each sampled diploid population and subpopulation of* Lithothrix aspergillum*: average number of alleles per locus (A/L), percent polymorphic loci (%P), heterozygosity observed ($H_o$), and heterozygosity expected ($H_e$). Only thalli determined to be diploid by conceptacle type or, if sterile, by zymogram data were included in the analyses.*

| Measure | Laguna Shaw's Cove (intertidal) (n = 56) | Laguna Shaw's Cove (subtidal) (n = 69) | Laguna Treasure Is. (intertidal) (n = 55) | S. Catalina Is. Indian Rock (subtidal) (n = 53) | Bodega Bay (intertidal) (n = 75) |
|---|---|---|---|---|---|
| A/L | 1.5 | 1.5 | 1.5 | 1.4 | 1.0 |
| %P | 43.8 | 43.8 | 50.0 | 37.5 | 0 |
| $H_o$ | 0.077 | 0.044 | 0.087 | 0.085 | 0 |
| $H_e$ | 0.185[a] | 0.175[a] | 0.170[a] | 0.126[a] | 0 |

[a] Populations deviating significantly ($P < 0.001$) from Hardy–Weinberg equilibrium; no test was performed for the Bodega Bay population because of the absence of polymorphic loci.

## RESULTS

*Reproductive status of* L. aspergillum *populations.* The reproductive status of *L. aspergillum* populations varied among the four sites and also between subtidal and intertidal subpopulations at Shaw's Cove (Table 2). Conceptacle-bearing thalli at Shaw's Cove were very abundant in both intertidal (86%) and subtidal (100%) subpopulations, which differed morphologically. Evidence for sexual reproduction in the turf-forming, intertidal subpopulation was strong, since 68.6% of all individuals had tetrasporangial conceptacles, 11.4% were found to be fertile gametophytes bearing carposporangial conceptacles, and no asexual bisporangial thalli were observed. This profile differed markedly from the subtidal subpopulation, which was strongly dominated by bispore-producing sporophytes; 74.3% of subtidal *L. aspergillum* thalli bore bispores, and only 24.3% contained tetrasporangial conceptacles. The turf-forming, intertidal population from nearby Treasure Island also included no bisporangial thalli and had an otherwise similar reproductive profile to the intertidal *L. aspergillum* subpopulation at Shaw's Cove (Table 2). However, very different reproductive profiles were detected for the Indian Rock and Bodega Bay *L. aspergillum* populations, which were composed of larger, less densely branched thalli similar in form to those obtained subtidally at Shaw's Cove (Table 2). No bisporangial thalli were observed in our Indian Rock population, where the vast majority of individuals sampled (68.3%) lacked conceptacles. This suggests that this population is

TABLE 4. *Genetic differentiation between diploid* Lithothrix aspergillum *subpopulations and populations based on Wright's $F_{ST}$ statistic for each polymorphic locus. Values presented are $F_{ST}$ results between subpopulations and among populations occurring at indicated sites over different distance scales; for all comparisons with populations at other sites, Shaw's Cove samples are pooled and treated as a single population. Results of chi-square tests of $F_{ST}$ values for individual loci are indicated where significant. Site abbreviations: Shaw's Cove (SHW), Treasure Island (TRI), Indian Rock (IND), and Bodega Bay (BOD).*

| Enzyme locus | SHW (intertidal) SHW (subtidal) (0.03 km) | SHW TRI (5.5 km) | SHW IND (68 km) | TRI IND (72 km) | SHW BOD (752 km) | TRI BOD (758 km) | IND BOD (707 km) |
|---|---|---|---|---|---|---|---|
| FBP-1[a] | 0.001 | 0.013* | 0.348*** | 0.457*** | 0.319*** | 0.229*** | 1.000*** |
| MDH-1 | 0.004 | 0.005 | 0.629*** | 0.549*** | 0.701*** | 0.658*** | 1.000*** |
| IDH-1 | 0.012 | 0.013* | 0.276*** | 0.385*** | 0.577*** | 0.705*** | 0.116*** |
| HEX-1 | 0.003 | 0.007 | 0.001 | 0.014 | 0.553*** | 0.467*** | 0.594*** |
| GPI-1 | — | — | — | 0.050** | — | — | 0.050*** |
| GPI-2 | 0.002 | 0.009 | 0.429*** | 0.359*** | 0.931*** | 0.849*** | 0.205*** |
| GPI-3 | <0.001 | 0.122*** | 0.600*** | 0.749*** | 0.600*** | 0.749*** | 1.000*** |
| RBC-1 | 0.008 | 0.027** | 0.305*** | 0.183*** | 0.305*** | 0.183*** | — |
| RBC-2 | — | — | 0.606*** | 0.606*** | — | — | 0.606*** |
| TPI-2 | — | — | 0.098*** | 0.098*** | — | — | 0.098*** |
| ALT-1 | <0.001 | 0.003 | 0.040*** | 0.058*** | 0.040*** | 0.058*** | — |
| Mean | 0.004 | 0.030 | 0.390 | 0.406 | 0.583 | 0.587 | 0.683 |
| 95% CI | 0.001 | 0.004 | 0.016 | 0.018 | 0.017 | 0.018 | 0.027 |

[a] Loci abbreviations: FBP, fructose-bisphosphatase; MDH, malate dehydrogenase; IDH, isocitrate dehydrogenase; HEX, hexokinase; GPI, glucose-6-phosphate isomerase; RBC, ribulose-bisphosphate carboxylase; TPI, triose-phosphate isomerase; ALT, alanine aminotransferase.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

maintained by recruitment of spores received from contiguous areas or is sustained by perennation from basal crusts or by asexual vegetative propagation (Table 2). In marked contrast, 100% of the individuals examined from the Bodega Bay site formed asexual, bisporangial conceptacles.

*Genetic diversity within diploid* L. aspergillum *populations.* A total of 30 alleles was inferred from zymograms of 16 consistently scorable loci, and all interpreted loci conformed to monomeric or dimeric enzyme structures. A total of 11 loci was polymorphic, exhibiting a minimum of two alleles in at least one of the sampled *L. aspergillum* populations (Table 1). Five loci (ALD-1, HEX-2, IDH-2, PGM-1, and TPI-1) were monomorphic and appeared to be represented by identical alleles in all populations sampled. The most polymorphic of the examined loci was GPI-3, where four distinct allelic morphs were observed. The remaining 10 loci consistently exhibited only two allelic forms with the exception of MDH-1 for which three allelic morphs were recorded.

The diploid *L. aspergillum* subpopulations and populations from Shaw's Cove and Treasure Island in Laguna Beach exhibited highly similar genetic profiles based on four measures of genetic diversity (Table 3). The intertidal and subtidal subpopulations from Shaw's Cove had identical A/L ratios (A/L = 1.5) and showed an equivalent percentage of polymorphic loci ($P$ = 43.8%), despite differences in reproductive status. Both diploid subpopulations showed significant deviations from Hardy–Weinberg equilibrium (Table 3). The A/L ratio for the *L. aspergillum* diploid population from Treasure Island was also 1.5, and the observed heterozygosity was similar to the Shaw's Cove intertidal subpopulation ($H_o$ = 0.087 vs. $H_o$ = 0.077); however, the percentage of polymorphic loci (50.0%) was slightly greater for the Treasure Island population, which also deviated significantly from Hardy–Weinberg expectations (Table 3).

Relative to the Laguna Beach populations, diploid *L. aspergillum* populations from Indian Rock and Bodega Bay showed reduced genetic diversity (Table 3). Although possessing similar A/L ratios to the Laguna Beach populations, the Indian Rock diploid population, which consisted mostly of individuals lacking reproductive conceptacles, had a lower percentage of polymorphic loci ($P$ = 37.5%), lower expected heterozygosity ($H_e$ = 0.126), and also showed significant deviation from Hardy–Weinberg equilibrium. Further reductions in diversity were observed for the genetically uniform Bodega Bay population, which averaged the fewest number of alleles per locus (A/L = 1.0) and also had zero polymorphic loci ($P$ = 0.0%) and expected ($H_e$) and observed ($H_o$) heterozygosity values of 0.000 (Table 3). All of the asexual Bodega Bay individuals sampled were diploid clones based on our zymogram data.

*Genetic differentiation among* Lithothrix *populations.* Based on $F_{ST}$ and D, genetic differentiation among *L. aspergillum* populations was found to increase with geographic separation (Table 4, Fig. 2). Despite strong differences in morphological and reproductive profiles, very little genetic differentiation (mean $F_{ST}$ = 0.004; no $F_{ST}$ values significantly >0 for all eight shared loci) and low genetic distance (D = 0.001) were observed between the Shaw's Cove intertidal and subtidal diploid subpopulations, indicating high gene flow and that individuals from this site should be viewed as belonging to a single population. Genetic differentiation (mean $F_{ST}$ = 0.030; $F_{ST}$ values significantly >0 for only 2 of 8 shared loci) and genetic distance (D = 0.013) between in-

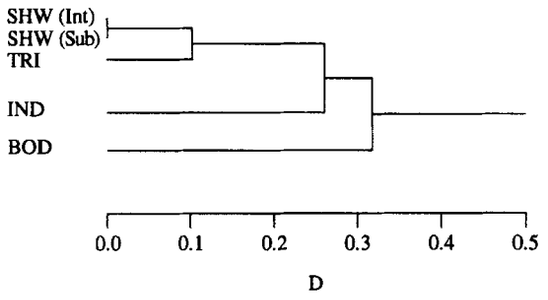


FIG. 2. Dendrogram formed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm showing Nei's genetic distance (D = −ln I) between subpopulations and populations of *L. aspergillum*.

tertidal and subtidal Shaw's Cove samples and the Treasure Island *L. aspergillum* diploid population were also very low. The Indian Rock population, located approximately 70 km across a deep ocean channel, was clearly differentiated from the Shaw's Cove and Treasure Island populations, providing evidence of regional differences in genetic composition (Table 4, Fig. 2). Mean $F_{ST}$ values (mean $F_{ST}$ = 0.390–0.406) calculated with the inclusion of the Indian Rock population were more than 10 times greater than the values obtained for the two Laguna Beach populations alone; $F_{ST}$ values for individual loci were significantly >0 for 9 of 10 and 10 of 11 loci shared with the Shaw's Cove and Treasure Island populations. As expected, all three southern California *L. aspergillum* diploid populations were strongly differentiated (mean $F_{ST}$ = 0.583–0.683; $F_{ST}$ values significantly >0 for all shared loci) from the Bodega Bay population (Table 4). Thus, based on its genetic makeup, the Bodega Bay population, located >700 km from Laguna Beach, was the most distinctive of all *L. aspergillum* populations examined (Fig. 2).

## DISCUSSION

Our results support previous findings concerning geographic variation in the reproductive composition of *Lithothrix aspergillum* populations (Tyrell and Johansen 1995) and provide evidence that the genetic diversity of a seaweed species varies predictably with life-history characteristics over its geographic range. Further, similar to *Halidrys dioica* (Lu and Williams 1994) and *Pelvetia compressa* (as *P. fastigiata*. Williams and Di Fiori 1996), sexually reproducing *L. aspergillum* populations appear to be genetically similar over short geographic distances. This suggests at least moderate levels of gene flow among local *L. aspergillum* populations, despite the hypothetically limited dispersal potential of this calcified red seaweed that lacks gas-filled flotation structures and produces only nonmotile, sinking spores.

*Reproductive status of* L. aspergillum *populations.* Our observations substantiate the view (Ganesan and Desikachary 1970, Gittins 1975, Tyrell and Johansen 1995) that the reproductive status of *L. aspergillum* populations varies over local, regional, and latitudinal scales and that gametangial and tetrasporangial thalli occur only in southern California. No evidence for sexual reproduction was detected in our Bodega Bay population, which, based on conceptacle type and zymogram patterns, consisted exclusively of asexual and diploid bisporangial thalli. These results are consistent with those of Tyrell and Johansen (1995), who, based on examination of herbarium specimens collected by E. Y. Dawson and others, concluded that only sterile or bisporangial thalli occurred outside of southern California. Further evidence in support of this geographic pattern has been provided by DeWreede and Vandermeulen (1988), who failed to find conceptacles on thalli during an intensive 3-year study performed near the northern distributional limit for *L. aspergillum* in the Strait of Georgia.

The relative abundances of haploid gametangial and diploid tetrasporangial life-history phases often vary geographically in red algal populations (Dixon 1965, 1973). In Europe, for example, Dixon (1965, 1973) observed that tetrasporangial thalli often occur farther north than gametangial thalli and that, at the extreme northern limits, many red algae are totally sterile, implying dependence on vegetative propagation or perennation. As pointed out by Druehl (1981), the wider geographic distribution often exhibited by tetrasporangial thalli suggests that the diploid life-history phase tolerates a broader range of environmental conditions than the haploid gametangial phase in red seaweeds. Thus, *L. aspergillum* appears to be an eastern North Pacific species with a geographic reproductive pattern that conforms to Dixon's model for several European red algae, where a typical triphasic life history exists in one part of the geographic range and a nonsexual life history in another. The occurrence of both sexual and apomeiotic nonsexual life histories, with the latter dependent on asexual bispores, has also been reported for other coralline red algae besides *L. aspergillum*, including the nongeniculate species *Titanoderma corallinae* (Crouan et Crouan) Woelkerling (as *Lithophyllum corallinae*) and *T. pustulatum* (Crouan et Crouan) Chamberlain (as *Dermatolithon litorale*) (Suneson 1950, 1982). Additional research is required to learn the degree to which this "field" life-history pattern occurs in other eastern North Pacific red algal species with isomorphic gametangial and tetrasporangial phases. As emphasized by Druehl (1981), the recognition of phase and ploidy level in red algae with isomorphic free-living phases is often quite difficult and usually requires not only direct observations of reproductive structures but also information on chromosome number, DNA content, or allelic pattern.

Consistent with earlier observations by Gittins (1975) and Tyrell and Johansen (1995), sexual reproduction was much greater in intertidal, turf-

forming Laguna Beach subpopulations, whereas asexual reproduction predominated in the subtidal, where thalli were larger and less densely branched. Together with genetic evidence of low levels of differentiation between subtidal and intertidal subpopulations at Shaw's Cove, this finding strongly suggests habitat-based effects on spore viability or survivorship and morphological and reproductive development between the life-history phases of *L. aspergillum.* The preponderance of sexually reproducing *L. aspergillum* thalli in intertidal habitats (Gooch and Schopf 1970, Black and Johnson 1981, Innes 1988) known for their high spatial heterogeneity and steep environmental gradients is perhaps not surprising. Intertidal environments support a level of microhabitat diversity exceeding that to which a single genotype can uniformly adapt (Koehn and Gaffney 1984, Zouros et al. 1980, Sarver and Foltz 1993). Hence, in red algae with triphasic life histories, such as *L. aspergillum,* the meiotic production of tetraspores and the recombination and amplification of genotypes during fertilization and carpospore production should hypothetically result in large pools of genetically variable spores and increase the number of microhabitats available for successful colonization (Loveless and Hamrick 1984, Grace 1993). In contrast to intertidal environments, subtidal habitats are characterized by greater environmental homogeneity. Under these circumstances, asexual reproduction can theoretically conserve a successful genotype without the expenditure of resources requisite for the sexual production of new individuals, an event that could result in large numbers of individuals with less well-adapted genotypes (Russell 1986, DeWreede and Klinger 1988, Grace 1993).

The absence of reproductive conceptacles on nearly 70% of the thalli collected from our Indian Rock population is enigmatic. We believe the absence cannot be ascribed to the season of collection, since evidence of conceptacle production can be detected on intergenicular segments long after the spore contents have been shed. Together with our zymogram data (Table 1), the absence of conceptacles or evidence of prior conceptacle production on the majority of these *L. aspergillum* thalli suggests important roles for perennation or vegetative propagation, processes long known (Dixon 1965, 1973) to be vital to the maintenance of many red algal populations. However, more detailed spatial and temporal studies of reproductive mechanisms in this population must be performed to test this hypothesis. If sterile thalli occur in the same proportions as thalli assignable to a reproductive type, as is the case for *Gracilaria* sp. (Bird 1976), then gametangial and tetrasporangial individuals should occur at a 1:1 ratio at our Indian Rock site. However, a 1:1 ratio was not apparent, since conceptacle analyses and heterozygous zymogram banding patterns indicated that more than 70% of the sampled population consists of diploid individuals. Thus, our Indian Rock population appears similar in ploidy composition to *L. aspergillum* populations from Laguna Beach and Bodega Bay, where diploid tetrasporangial or bisporangial thalli dominate over haploid gametangial individuals. Such numerical dominance of the diploid phase is not unusual for red algae and, among other species, has been reported for *Gelidium* (Akatsuka 1986, Sosa and Garcia-Reina 1992, 1993), *Gracilaria* spp. (Hoyle 1978, Trono and Azanza-Corrales 1981, Whyte et al. 1981, Hay and Norris 1984), *Hypnea cervicornis* J. Ag. (Mshigeni 1976), *Mazzaella lilacina* (Post. & Rupr.) Leister (as *Iridaea cordata,* Hansen and Doyle 1976, Dyck et al. 1985), and *Polysiphonia denudata* (Dillwyn) Grev. ex Harv. in W. J. Hooker (Kapraun 1978).

*Genetic diversity within* L. aspergillum *populations.* This study is the first to demonstrate in seaweeds that over the geographic range of a single species, genetic diversity of populations varies in a predictable way with the field life history. As expected from previous research on seed plants and other organisms (Loveless and Hamrick 1984), sexually reproducing *L. aspergillum* populations exhibited higher genetic diversity compared with a population that appears to reproduce exclusively by asexual means.

Our measurements of genetic diversity for diploid *L. aspergillum* populations are on the higher end of the range of values reported for other marine algae (Table 5), but are low compared with most species of seed plants and many invertebrates (Hamrick et al. 1979). With the exception of some wild haploid populations of *Porphyra yezoensis* from Japan (Miura et al. 1979, Fujio et al. 1985, 1987), A/L, %P, and $H_e$ for sexually reproducing subpopulations and populations of *L. aspergillum* from Laguna Beach are higher than values for other red algal species and only slightly less than those reported for sexual populations of the brown alga *Halidrys dioica* (Lu and Williams 1994), a dioecious obligate outcrosser with floating reproductive fronds that facilitate long-range dispersal.

We hypothesized lower genetic diversity within the *L. aspergillum* subpopulation occupying subtidal habitats at Shaw's Cove because of the predominance of asexual bisporangial individuals (Table 3). Fewer heterozygotes were observed within this subtidal subpopulation than within any other southern California population, which suggests a high level of clonal propagation (Malinowski 1974, Cheney and Babbel 1978, Innes 1984). However, our measures of genetic diversity and differentiation indicate high gene flow between intertidal and subtidal subpopulations at Shaw's Cove and provide little evidence for the development of differences due to genetic drift or the differential selection pressures that hypothetically characterize intertidal and subtidal habitats.

As hypothesized, genetic diversity of the exclusively asexual diploid *L. aspergillum* population from Bo-

TABLE 5. *Range in genetic diversity found within seaweed populations and subpopulations from various locales.*

| Species (location) | Life history phases | No. of populations or sub-populations | Sample size range | No. of loci detected | No. of alleles per locus (A/L) | % Polymorphic loci | Heterozygosity expected | Source |
|---|---|---|---|---|---|---|---|---|
| Chlorophyta | | | | | | | | |
| *Codium fragile* (Sur.) Har. subsp. *tomentosoides* (Van Goor) Silva (Long Island, New York, U.S.A.) | diploid | 10 | — | 14 | — | 31.0 | 0.150 | Malinowski 1974 |
| Phaeophyceae | | | | | | | | |
| *Halidrys dioica* Gardn. (Southern California, U.S.A.) | diploid | 5 | 55–72 | 5 | 1.60–2.00 | 40.0–60.0 | 0.169–0.235 | Lu and Williams 1994 |
| *Pelvetia compressa* (J. Ag.) DeToni (as *P. fastigiata* (J. Ag.) DeToni) (Southern California, U.S.A.) | diploid | 5 | 30–60 | 7 | 1.00–2.00 | 0.0–28.6 | 0.000–0.088 | Williams and DiFiori, 1996 |
| Rhodophyta | | | | | | | | |
| *Eucheuma isiforme* (C. Ag.) J. Ag. var. *denudatum* Cheney (as *E. nudum*) (Florida, U.S.A.) | diploid, haploid | 3 | 50–68 | 11–12 | 1.27–1.33 | 27.3–33.3 | — | Cheney and Babbel 1978 |
| *Eucheuma isiforme* var. *isiforme* (C. Ag.) J. Ag. (Florida, U.S.A.) | diploid, haploid | 3 | 24–56 | 11 | 1.36 | 36.4 | — | Cheney and Babbel 1978 |
| *Gelidium arbuscula* Bory (Canary Islands) | diploid | 3 | 31–49 | 22 | 1.18–1.32 | 13.6–27.3 | 0.065–0.074 | Sosa and Garcia-Reina 1992 |
| | haploid | 3 | 11–22 | 22 | 1.04–1.09 | 4.5–9.1 | 0.015–0.021 | Sosa and Garcia-Reina 1992 |
| *Gelidium canariensis* Grun. (Canary Islands) | diploid | 3 | 23–50 | 22 | 1.14–1.23 | 13.6–22.7 | 0.039–0.063 | Sosa and Garcia-Reina 1993 |
| | haploid | 3 | 11–18 | 22 | 1.05–1.14 | 4.5–13.6 | 0.017–0.053 | Sosa and Garcia-Reina 1993 |
| *Gracilaria cervicornis* (Turner) J. Ag. (Canary Islands) | haploid | 3 | 2–63 | 16 | 1.06 | 5.9 | 0.025–0.030 | Sosa et al. 1996 |
| *Gracilaria chilensis* Bird, McLach. & Oliv. (New Zealand) | diploid | 17 | ca. 20 | 14 | — | 0.0–14.3 | 0.000–0.056 | Intasuwan et al. 1993 |
| *Lithothrix aspergillum* Gray (Laguna Beach, California, U.S.A.) | diploid | 3 | 55–69 | 16 | 1.50 | 43.8–50.0 | 0.158–0.185 | This study |
| *Lithothrix aspergillum* Gray (Indian Rock, Santa Catalina Island, California, U.S.A.) | diploid | 1 | 53 | 16 | 1.40 | 37.5 | 0.122 | This study |
| *Lithothrix aspergillum* Gray (Bodega Bay, California, U.S.A.) | diploid | 1 | 75 | 16 | 1.00 | 0.0 | 0.000 | This study |
| *Meristiella gelidium* (J. Ag.) Cheney & Gabrielson (as *Eucheuma gelidium* and *E. acanthocladum*) (Florida, U.S.A.) | diploid, haploid | 2 | 12–29 | 7–8 | 1.25–1.29 | 25.0–28.6 | — | Cheney and Babbel 1978 |
| *Porphyra yezoensis* Ueda (Japan) | haploid | 3 | 23–228 | 12 | 1.58–1.75 | 33.3 | 0.100–0.141 | Fujio et al. 1985 |
| *Porphyra yezoensis* Ueda (Japan) | haploid | 11 | 23–111 | 12 | 1.83–2.75 | 41.7–91.7 | 0.123–0.344 | Fujio et al. 1987 |
| *Porphyra yezoensis* Ueda (Japan) | haploid | 11 | 12–171 | 8 | 1.00–1.90 | 0.0–75.0 | — | Miura et al. 1979 |
| *Porphyra yezoensis* Ueda (Cultivated) (Japan) | haploid | 9 | 20–219 | 8 | 1.00 | 0.00 | — | Miura et al. 1979 |

dega Bay was much lower than the diversity of sexually reproducing diploid populations from southern California. Asexual reproduction, including vegetative propagation, has previously been hypothesized to contribute to the low levels of genetic diversity reported for natural populations of *Codium fragile* spp. *tomentosoides* (Malinowski 1974), *Enteromorpha linza* (L.) J. Ag. (Innes and Yarish 1984, Innes 1987, 1988), *Eucheuma* and *Meristiella* species (Cheney and Babbel 1978), *Gelidium arbuscula* and *G. canariensis* (Sosa and Garcia-Reina 1992, 1993), *Gracilaria cervicornis* (Sosa et al. 1996), and *Gracilaria chilensis* (Intasuwan et al. 1993), whereas extensive selfing together with asexual propagation was thought to cause the low diversity observed in Japanese populations of *Porphyra yezoensis* (Miura et al. 1979, Fujio et al. 1985, 1987).

With the exception of our unusual, genetically uniform population from Bodega Bay, significant deviations of allele frequencies from Hardy–Weinberg expectations and low observed heterozygosity were found for each of our *L. aspergillum* populations, as appears to be the case for all previous studies of seaweeds where these parameters have been evaluated (Cheney and Babbel 1978, Innes and Yarish 1984, Fujio et al. 1987, Sosa and Garcia-Reina 1992, 1993, Lu and Williams 1994, Williams and Di Fiori 1996). Clearly, it appears that in most seaweed populations the assumptions required for Hardy-Weinberg equilibrium are rarely met probably due to nonrandom mating, selection pressures, and the common occurrence (Russell 1986, Santelices 1990) of asexual reproduction.

*Genetic differentiation among* L. aspergillum *populations.* Seaweeds exhibit a variety of life histories and dispersal abilities which are thought to be important determinants of genetic differentiation (Innes 1984). For example, sexual reproduction and high dispersibility can result in high gene flow and promote low levels of differentiation among populations separated by even small geographic distances. In contrast, asexual reproduction can magnify the effects of selective factors and result in differentiation even on a microgeographic scale (Black and Johnson 1981, Innes 1987, 1988). Based on interpretations of $F_{ST}$ as an index of genetic differentiation (Hartl 1981), we observed (Table 4) little differentiation ($F_{ST}$ = 0.004 and 0.030) over spatial scales of 30 m and 5.5 km among local Laguna Beach subpopulations and populations of *L. aspergillum* but found very great differentiation when we analyzed populations that were separated by distances of ca. 70 km (mean $F_{ST}$ = 0.390–0.406) and 700–760 km (mean $F_{ST}$ = 0.583–0.683). These results are similar to those for sexually reproducing populations of *Halidrys dioica,* where little differentiation (mean $F_{ST}$ = 0.018) occurred among four San Diegan subpopulations separated by less than 4 km but great differentiation (mean $F_{ST}$ = 0.194) was obtained with the inclusion of a site 90 km to the north (Lu and Williams 1994). Working with *Pelvetia compressa,* a monecious seaweed that reproduces only by sexual means and which, like *L. aspergillum,* is characterized by limited dispersal, Williams and Di Fiori (1996) also found little genetic differentiation (mean $F_{ST}$ = 0.005) among populations separated by 3 to 640 m but very great differentiation (mean $F_{ST}$ = 0.806) over distances of 80 to 130 km. Like *P. compressa,* the genetic differentiation between southern California and Bodega Bay *L. aspergillum* populations (mean $F_{ST}$ = 0.583–0.683), which are separated by more than 700 km, is very great (Table 4, Fig. 2) and falls close to the amount of divergence generally reported to characterize differentiation of subspecies (Ayala 1982).

Interestingly, no relationship between genetic and geographic distance was detected in studies of Canary Island populations of *Gelidium arbuscula* (Sosa and Garcia-Reina 1992) and *G. canariensis* (Sosa and Garcia-Reina 1993), separated by as much as 100 km. In contrast, high levels of genetic differentiation have been reported even over small geographic distances for predominantly asexual populations of *Enteromorpha linza* from Long Island Sound (Innes and Yarish 1984, Innes 1987, 1988). These high levels have also been observed among Japanese populations of the red seaweed *Porphyra yezoensis,* which not only propagate asexually but are also characterized by a high occurrence of selfing (Miura et al. 1979, Fujio et al. 1985, 1987). *Chondrus crispus* populations from New Hampshire and the Canadian maritimes (Cheney and Mathieson 1979) have also exhibited high levels of genetic differentiation. At a scale (<6 m) previously uninvestigated in seaweeds, Williams and Di Fiori (1996) observed high genetic structure within a *P. compressa* population occupying a single intertidal reef using allele mapping and second-order analysis.

The trend of increasing genetic differentiation among *L. aspergillum* populations with progressive geographic distance suggests limited long-distance dispersal. Seaweed dispersal can occur by means of spores, gametes, and vegetative fragments, but the relative importance of these mechanisms is unknown for most species (Santelices 1990). Spores of *L. aspergillum* are large (43–51 μm) (Gittins 1975) and, based on studies by Okuda and Neushul (1981), probably sink relatively quickly. In addition, coralline algal spores are thought to require little time to adhere to the substratum (Johansen 1981). Vegetative fragmentation is also an important mode of dispersal for many seaweeds (Dixon 1965, 1973), and the ability to reattach by fragments has been reported (Gittins 1975) for *L. aspergillum.* However, the transport of fragments over long distances is very unlikely because thallus calcification results in a higher specific gravity than seawater and encourages sinking. These factors suggest that dispersal in *L. aspergillum* is limited and, together with differences in reproductive patterns among populations, may

explain the high genetic differentiation observed over distances of ca. 70 km.

The asexual Bodega Bay population exhibited the lowest genetic diversity of all populations sampled, and our zymogram data indicate that it consists of a single clone similar to Long Island Sound and southeastern Massachusetts populations of the green alga *Codium fragile* spp. *tomentosoides* (Malinowski 1974, as reported by Innes 1984) and *Gracilaria cervicornis* populations from the Canary Islands (Sosa et al. 1996). For *C. fragile* spp. *tomentosoides* and *G. cervicornis,* low genetic diversity and little differentiation among populations are thought to be due to founder events followed by repeated vegetative propagation (Malinowski 1974, Sosa et al. 1996). Canary Island populations of the red seaweeds, *Gelidium arbuscula* and *G. canariensis,* are also thought to be the results of founder events, but in these cases, some genetic differentiation has occurred presumably due to original differences among founders, high levels of asexual reproduction, or differential selection pressures (Sosa and Garcia-Reina 1992, 1993). Interestingly, in these *Gelidium* populations, the degree of differentiation is much greater among gametophytes than sporophytes, suggesting higher levels of interpopulation gene flow among sporophytes and possibly reflecting the predominance of asexual reproduction, which can decrease gene flow and increase genetic differences between the two life-history phases within a single population (Sosa and Garcia-Reina 1992, 1993). Although the ultimate origin of the Bodega Bay population of *L. aspergillum* can never be surely known, our reproductive and genetic data suggest that this population, and possibly other *L. aspergillum* populations in the Pacific Northwest, are either isolated remnants of prior populations or were introduced through founder events, perhaps attached to the rock ballast discarded by sailing vessels during the previous two centuries (Carlton 1985, 1992). We hypothesize that these northerly *L. aspergillum* populations persist today outside of the window of environmental conditions required for sexual reproduction and are entirely dependent on processes that fix allele frequencies, including perennation, vegetative propagation, and, as in the case of our Bodega Bay population, asexual spore production.

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