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RESEARCH ARTICLE

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Small-scale genetic structure in the sea palm *Postelsia palmaeformis* Ruprecht (Phaeophyceae)

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Abstract Documenting the scale of movement among populations is an important challenge for marine ecology. Using nine microsatellite markers, evidence of genetic structure in a marine kelp, the sea palm *Postelsia palmaeformis* Ruprecht, was examined in the vicinity of Cape Flattery, Washington state, USA (48° 24′ N, 124°44′ W). Genetic clustering analysis implemented without reference to geographic structure strongly suggested that a number of distinct genetic clusters existed among the 245 plants sampled in August in the years 1997–2001. Subsequent analysis showed that clustering was associated with geographically defined populations both among (km scale) and within (m scale) sampling sites. F_{st} analysis of geographically defined populations revealed significant genetic differentiation among populations of plants as little as 5 m apart, evidence of genetic structuring at even smaller scales, and a sharp increase in F_{st} across populations separated by up to 23 m. F_{st} values were also high and approximately unchanging ($F_{st} = 0.470$) for populations separated by greater distances (up to 11 km), consistent with a scenario of rare dispersal by detached, floating plants carried by variable currents. The results corroborate natural history observations suggesting that *P. palmaeformis* has extremely short (1–3 m) spore dispersal distances, and indicate that the dynamics of sea palm populations are more affected by local processes than recruitment from distant populations.

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Introduction

Determining the degree of movement among populations is important for understanding the dynamics of marine communities. Because many marine organisms release their larvae and/or gametes into the water column (Thorson 1950), where they potentially can be transported hundreds of kilometers by strong ocean currents, marine systems on a local scale are often thought to be influenced by distant populations, thereby disrupting local density-dependent interactions (Roughgarden et al. 1988). In intertidal ecology, this perspective is generated in part by a focus on several groups of organisms with long-lived planktonic larval stages, particularly barnacles, mussels, some echinoderms, and limpets. Not all marine organisms share these life history traits, hence marine communities may be structured by several processes at a variety of spatial scales. For example, important neogastropod predators such as whelks (e.g., *Nucella* spp.) lay benthic egg capsules with crawl-away larvae (Spight 1974), benthic algal recruitment can correlate with local population sizes (Sousa 1984), and many ascidian and coelenterate larvae have short planktonic durations (Olson 1985; Hellberg 1994; Yund and O'Neil 2000). Although determining the degree and scale of migration among populations would be extremely valuable, direct observation of microscopic larval or gamete transport is nearly impossible except under unusual circumstances (e.g., Olson 1985).

Analysis of the genetic structure of populations can provide insight into scales of movement among marine populations (reviewed by Avise 2004; Palumbi 1994, 1995; Bohonak 1999; Grosberg and Cunningham 2001). Populations weakly connected by migration are expected to exhibit genetic differentiation at neutral alleles as a result of genetic drift, usually quantified by an index describing the expected probability that different alleles are fixed in different populations (F_{st}; Wright 1951, 1965). Therefore, if life history patterns determine the degree of movement among marine populations, we

would expect stronger population genetic differentiation at smaller scales in species with relatively low dispersal rates and short planktonic durations of their reproductive propagules. For example, previous studies with allozymes have not revealed genetic differentiation in several fish species where the larval stage is thought to last weeks or months (Waples 1987; Yoshiyama and Sassaman 1987). In contrast, those species that brood or have "crawl-away" larvae do show some genetic differentiation (Hellberg 1996; Kyle and Boulding 2000). Similarly, high levels of genetic differentiation were found in populations of monoecious algae with limited dispersal of gametes and zygotes (Williams and Di Fiori 1996; Coleman and Brawley 2004), whereas low genetic differentiation was observed in an obligately outcrossing seaweed with broad dispersal ability (Lu and Williams 1994). Factors other than dispersal ability, however, may also affect patterns of genetic structure (Coleman and Brawley 2005).

Although the duration that a propagule spends in the water column may be a general predictor of population structure (e. g., Waples 1987), at least two additional factors may be important. First, larvae or spores may have behaviors or adaptations that reduce gene flow relative to plankton passively dispersing in currents (Santelices 1990; Shulman and Bermingham 1995; Swearer et al. 1999; Jones et al. 1999). Second, a number of marine organisms can be transported as fertile adults through rafting (e. g., Dayton 1973; Worcester 1994), resulting in less genetic differentiation than would be expected based on the nature of the reproductive propagules. For example, in the ecologically and economically important kelps, both adults and spores may have differing probabilities of long-distance dispersal and corresponding differences in genetic structure (Coyer et al. 1997; Kusumo and Druehl 2000). The main dispersal mode for kelp is via meiospores, which exhibit a short-lived dispersal mode generally with a maximum swimming period of 72 h (Reed et al. 1992). Spores can continue to photosynthesize and be viable for longer periods (Kain 1964; Reed et al. 1992), however, and for those species having positive buoyancy because of pneumatocysts or hollow stipes, dislodged macrophytes may transport spore-bearing tissue (e.g., Deysher and Norton 1982). Disentangling the roles of multiple modes of potential dispersal is therefore important. Here, we report an analysis of genetic structure among populations for a marine kelp, the sea palm (*Postelsia palmaeformis* Ruprecht).

Postelsia palmaeformis occurs widely in the intertidal zone of rocky wave-swept shores of the northeast Pacific Ocean from Monterey Bay, California to the northern end of Vancouver Island, British Columbia (Abbott and Hollenberg 1976), where it grows in dense populations that exhibit some of the highest productivity rates known (Leigh et al. 1987). *P. palmaeformis* is an annual that undergoes an alternation of generations, with a conspicuous diploid sporophyte stage during the summer and a microscopic haploid dioecious gametophyte stage during the winter. The sporophyte produces flagellated meiospores that are released into the sea (Paine 1988). Dayton (1973) observed that heavy sporulation occurred in *P. palmaeformis* when the plant was exposed during low tide. Therefore, *P. palmaeformis* is thought to have low dispersal capabilities, typically limited to a maximum of 1 to 3 m from the parents based on observations of interannual spatial locations of unmanipulated populations, population removal experiments, and invasion of cleared plots adjacent to natural populations (Dayton 1973; Paine 1988; R. T. Paine personal communication; JTW and CAP unpublished data). Hence, we would predict strong genetic structuring of *P. palmaeformis* populations on a small scale. However, sporogenous plants that are ripped from the rocks by waves (Paine 1979) can float via currents to a distant spot and drop their spores, raising the possibility of an important alternative mechanism of migration. Furthermore, although male and female spores look identical and the next generation of sporophytes develops from the site of female gametophyte settlement, it is possible that male spores differentially travel greater distances or that *P. palmaeformis* sperm can travel some distance throughout the water column like ascidian sperm (e.g. Grosberg 1991; Yund 1995), thereby increasing gene flow. Coyer et al. (1997) used RAPDs and M13 fingerprinting to examine the genetic structure of *P. palmaeformis* populations <1 to 250 km apart in Central California (Coyer et al. 1997). They found strong evidence for differentiation among populations 16 and 250 km apart, and some evidence for genetic differentiation among populations as little as 25 m apart when using M13 fingerprinting but not when using RAPDs, due to the different power of resolution of the methods. The limited number of populations examined (3) and low sample size per population (3–4) could not provide strong resolution of the pattern of differentiation with distance at smaller scales (<25 m).

Here we use microsatellite markers to explore small-scale population structure in *P. palmaeformis*. Aside from providing basic information on the population structure of this ecologically significant kelp, this information is of use in addressing the scale of dispersal and the probable success of restoration programs for sea palm metapopulations. This is important because the sea palm is harvested in some areas (Kalvass 1994).

Materials and methods

Tissue collection

A total of 245 samples of *P. palmaeformis* Ruprecht were collected from nine different sites in the vicinity of Cape Flattery, at the northwestern tip of the Olympic Peninsula, Washington state, USA (Fig. 1, Table 1). Five of the sites were on Tatoosh Island (48°24′N, 124°44′W) namely West Rocks, West Island, Fingernail, North Island, and Rainbow Rock. Sites on the

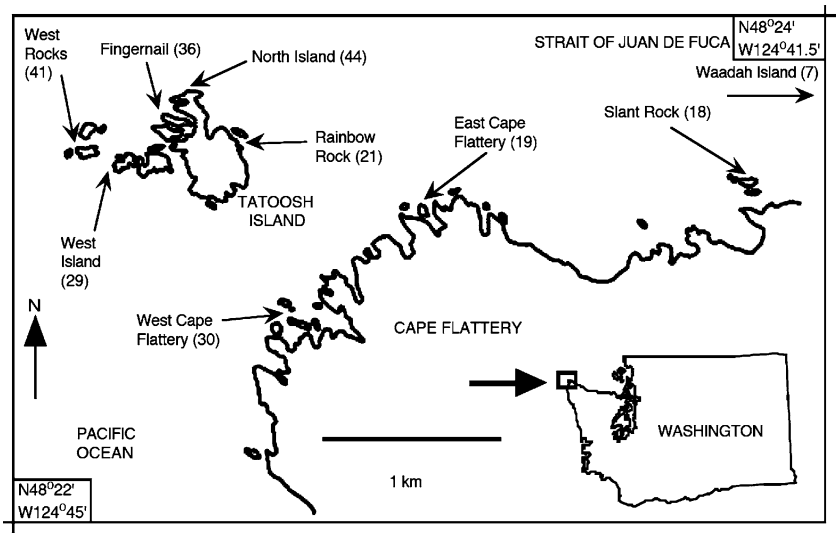

Fig. 1 The nine sites on Cape Flattery, Olympic Peninsula, Washington State, USA, where *Postelsia palmaeformis* was collected. Sample sizes in *parentheses*. *Inset* location of Cape Flattery (*box* with *arrow* pointing to it) in Washington State

Table 1 *Postelsia palmaeformis*: sample sizes of sea palm collected from each site in each year of the study

Year	Sample size								
	West Rocks	West Island	Fingernail	North Island	Rainbow Rock	West Cape Flattery	East Cape Flattery	Slant Rock	Waadah Island
1997	0	14	0	0	0	15	0	0	0
1998	0	0	0	0	0	15	0	0	0
1999	0	0	14	11	15	0	14	14	0
2000	29	0	1	7	0	0	0	0	0
2001	12	15	21	26	6	0	5	4	7

mainland were West Cape Flattery, East Cape Flattery, Slant Rock, and Waadah Island. Distances among the sites ranged from 200 to 11,000 m, a very small fraction of the range of the species. The blade tissue samples were collected from 1997 to 2001 in early August when the diploid sporophyte stage is conspicuous. Tissue collections were air-dried for storage until DNA extraction. Only clean tissue devoid of epiphytes was used. For a subset of "sites", we sampled plants at a smaller scale of spatial resolution which we term "patches". Plants were collected from different patches ranging from 5 to 33 m apart at West Rocks ($n = 41$ plants from three patches), Fingernail ($n = 21$ plants from five patches) and North Island ($n = 21$ plants from five patches) sites. Collections from the latter two sites were made explicitly to explore small-scale genetic structure, with plants collected in a single year (2001) from a relatively contiguous, linearly arrayed population, and patches defined as aggregations of plants identified at a fine scale of resolution (< 1 m). In contrast, plants from the West Rocks site were collected for use in other experiments in multiple years from three large, clearly disjunct populations defined at a coarse scale of resolution (> 5 m apart), and arrayed in an approximately triangular pattern. Hence, plants considered to be from the same patch in this site could have been collected 5 m or more away from each other.

DNA extraction

DNA extraction in seaweeds is challenging because of their high mucopolysaccharide content. Three DNA extraction methods were used: scaled-down CsCl (Fain et al. 1988), CTAB (modified from a protocol provided by Dr. R. Grosberg, University of California, Davis), or REDExtract-N-Amp Plant PCR Kits (SIGMA, Saint Louis, Missouri USA, Cat # XNA-PE). Extraction details are given in Kusumo et al. (2004).

Molecular methodology

The genetic analysis employed the nine microsatellite markers reported by Kusumo et al. (2004). Initial analyses indicated that these markers exhibited a reasonable degree of polymorphism (3–13 alleles per locus, expected heterozygosity 0.065–0.789; Kusumo et al. 2004). The amplified fragments were detected with an ABI 377 XL automatic sequencer (PE Applied Biosystems, Foster City, CA, USA) using 36 cm Long Ranger polyacrylamide gels (FMC, Rockland, ME, USA). An internal lane size standard, GGS 500-Rox, was included in each sample lane. The size standard and fluorescence signals of 6-FAM-, NED- or HEX-labeled primer fragments were analyzed using

Genescan 3.1 and Genotyper 2.5 software (PE Applied Biosystems).

Data analysis

We used two complementary approaches for exploring population genetic structure: The first, genetic cluster analysis (STRUCTURE software, Pritchard et al. 2000; Falush et al. 2003), uses the association of alleles across all loci and makes no initial assumptions about which individuals belong to particular populations. The second, an analysis of genetic structure of pre-defined populations, used *F*-statistics to analyze patterns of heterozygosity at each locus (Wright 1965). Briefly, the STRUCTURE analysis assigns individuals in the sample to a pre-defined number of categories with the goal of maximizing genetic similarity of individuals within categories, and then uses Bayesian methods under a uniform prior distribution to determine the relative probability of category assignments, and to estimate the rate of migration among categories. We explored the entire dataset for evidence of genetic structure by carrying out cluster analysis assuming a range of 1–27 clusters, making no a priori assumptions about the population membership of any individual. We repeated the analysis 11 times for each cluster set to generate error estimates for Bayesian probability values given a specified number of clusters. Given the data, we then asked whether the probability of a single cluster was lower than the probability of assuming more than one cluster, which would indicate that genetic structure existed within the dataset. We also examined the pattern of Bayesian probabilities with assumed cluster number to determine at what point the probability function reached a plateau, and used this as an indication of the number of genetic clusters present in the dataset. We then repeated the analysis, making a priori assumptions about which populations' individuals belonged to, based on the geographic location where the plant was sampled. We compared the Bayesian probability of the resulting clustering given the data to that derived assuming a single cluster, and to that assuming the same number of clusters without a priori geographic information. The former comparison evaluated the hypothesis that geographic source information provided statistically significant predictive power, whereas the latter provided insight into the extent to which genetic structure was associated with geographic structure. We repeated the analysis for the three sites (West Rocks, Fingernail, and North Island) where we had higher resolution spatial information to address similar questions at smaller spatial scales.

We also examined genetic structure among the populations that we defined a priori based on spatial patterns of the sampled individuals using relative genetic variance among versus within populations. Population genetic structure was inferred from F_{st} estimates among populations and F_{is} estimates among individuals within populations, calculated with an analysis of molecular variation (AMOVA) using Arlequin 2.0 software (Schneider et al. 2000), which is based on the methods of Weir and Cockerham (1984). Because sites were sampled at different scales of resolution, we carried out the AMOVA on different subsets of plants to look for differentiation at different scales. To analyze structure at the site scale, we identified all plants only by their source site and ran an AMOVA with a single (site) spatial scale factor, nested within year that the sample was collected. We then analyzed the West Rocks, Fingernail, and North Island populations for within-site structure at the patch scale. Overall estimates of F_{st} and estimates between pairs of sites or patches were generated with Arlequin 2.0 and tested statistically for significant deviations from zero using Monte Carlo methods (Schneider et al. 2000).

To further analyze patterns of genetic structure where we found overall statistically significant F_{st} values, we explored the relationship between pairwise F_{st} and geographic distance using break-point regression modeled as an initial linear increase in F_{st} with distance, followed by a constant F_{st} with distance. We used break-point regression because the patterns we observed were highly non-linear, and because any break between increasing and constant F_{st} with distance provides a useful point of reference in interpreting ecological processes affecting population structure. A positive relationship over a range of distances could suggest isolation by distance (stepping stone type gene flow).

Results

At the largest scale of resolution (0.2–11 km), we found clear evidence of strong genetic differentiation among populations at different sites. In genetic clustering (STRUCTURE) analysis, the Bayesian probability of the individuals being from a single genetic cluster was substantially lower than analyses assuming >1 cluster, and the Bayesian probability increased steadily with cluster number until it reached a plateau at 16–26 clusters (Fig. 2). Estimated rates of migration among clusters were very low [average admixture $= 0.030 \pm 0.001$ (SD)], suggesting little gene flow among populations at large scales. The genetic clustering that resulted from assuming that populations were structured geographically at the nine sample sites generated substantially higher Bayesian probabilities than those generated assuming a single genetic cluster (Fig. 2), indicating that spatial structure contributed to genetic structure. This conclusion was further supported by the high ability of genetic clustering information, derived either with or without reference to geographic origin, to correctly predict geographic origin from the large-scale sampling sites (94.8 or 80.9% correct assignment with or without using geographic information to derive clusters, respectively; Fig. 2). The Bayesian probability from the

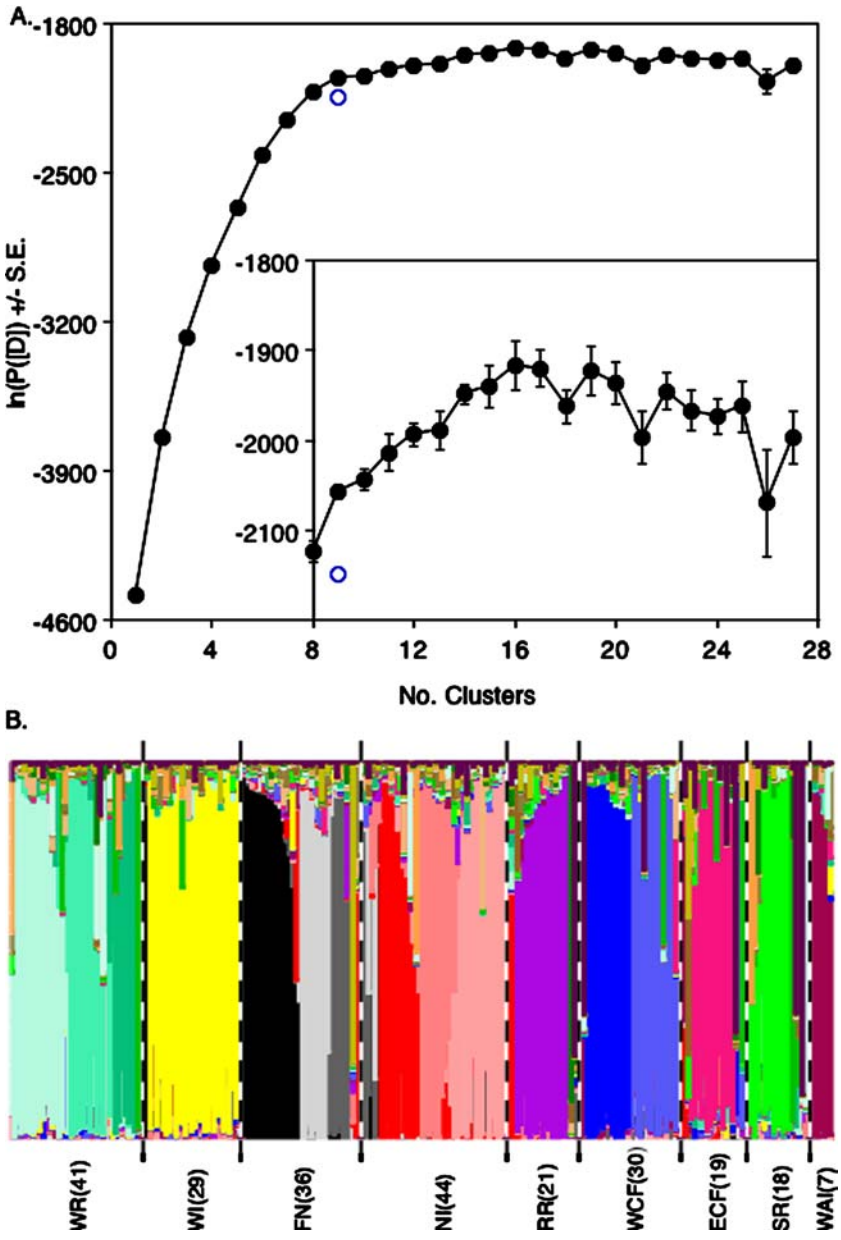

Fig. 2 *Postelsia palmaeformis*. Results of STRUCTURE analysis of genetic clustering for all plants and sites sampled. **a** Graph of average (± SE) ln(Bayesian probability) of STRUCTURE analyses ($n = 11$) without reference to geographic source for different assumed numbers of genetic clusters. *Inset* higher resolution results for >7 clusters. *Open symbol* analyses incorporating site of origin of each individual. **b** Pattern of geographic clustering among nine sample sites for best-fitting STRUCTURE result (26 clusters assumed), generated without reference to geographic source of individuals. *Columns* represent individuals, different *colors* represent proportionate contribution to an individual genotype of each genetic cluster. High correspondence of genetic clusters with geographic populations indicates strong genetic differentiation among sites

Table 2 *Postelsia palmaeformis*: genetic variance components estimated from AMOVA for between-site comparisons

Source	*df*	SS	*F*-statistic	*P*
Among years	4	199.51	0.031	0.27
Among sites within years	14	415.14	0.533	< 0.00001
Among individuals within Sites	226	329.74	0.354	< 0.00001
Within individuals	245	170.50	0.708	< 0.00001

analysis assuming site-level geographic structure was substantially lower, however, than that derived without reference to geographic structure for analyses assuming nine or more clusters, which indicates that genetic structure was not entirely explained by population subdivision at the site scale of geographic resolution (Fig. 2).

Analysis of genetic variation via *F*-statistics reached complementary conclusions. *F*-statistics indicated significant genetic differentiation among sites (Table 2, overall $F_{st} = 0.533$, $P < 0.00001$). F_{st} values among pairs of sites ranged from 0.278 to 0.658, and were all significantly differentiated from each other (all $P < 0.00001$, Table 3). Additionally, there was strong differentiation

Table 3 *Postelsia palmaeformis*: pairwise geographic (m, below diagonal) and genetic (F_{st}, above diagonal) distances between sites sampled in the study

	Sites	Pairwise F_{st}								
		West Rocks	West Island	Finger-Nail	North Island	Rainbow Rock	West Cape Flattery	East Cape Flattery	Slant Rock	Waadah Island
Geographic distance (m)	West Rocks	0	0.554	0.619	0.466	0.434	0.504	0.401	0.317	0.474
	West Island	237	0	0.592	0.515	0.539	0.569	0.469	0.528	0.630
	Fingernail	518	410	0	0.443	0.508	0.561	0.421	0.567	0.658
	North Island	712	619	209	0	0.357	0.523	0.379	0.450	0.549
	Rainbow Rock	900	705	432	381	0	0.535	0.338	0.377	0.557
	West Cape Flattery	1,479	1,237	1,266	1,324	961	0	0.318	0.328	0.414
	East Cape Flattery	1,947	1,712	1,496	1,424	1,072	1,432	0	0.278	0.377
	Slant Rock	3,747	3,768	3,305	3,147	2,863	2,646	1,838	0	0.355
	Waadah Island	11,000	10,805	10,532	10,481	10,100	8,446	7,638	5,800	0

among individuals within sites ($F_{is} = 0.354$, $P < 0.00001$), indicating that the populations were structured at smaller scales. Within sites, all populations except the less-extensively sampled Waadah Island site ($n = 7$) were out of Hardy–Weinberg equilibrium (HWE, Table 4) when analyzed across all loci, and for many individual loci, which is also consistent with population structure at smaller scales (i.e., a ''Wahlund Effect''). The identity of loci out of HWE varied among populations, but generally correlated with levels of genetic variation exhibited by a marker at a given site (Table 4). Because the marker PM8 was most consistently out of HWE, we tested its effect on the F_{st} analysis by removing it and found that it did not change our conclusions. In contrast to geographic effects, there was no significant variation in genetic structure among sample years ($F_y = .032$, $P > 0.27$), as expected for neutral microsatellite alleles.

At smaller scales of analysis (5–30 m), we also found evidence for population differentiation. For all three populations sampled systematically at smaller spatial scales, the Bayesian probability that individuals comprised a single genetic cluster was substantially lower than analyses assuming >1 cluster (Fig. 3). Bayesian probability estimates reached a plateau of 6–7 clusters for the West Rocks site, 2–6 clusters for the Fingernail site, and 3–6 clusters at the North Island site. In all three cases, the Bayesian probability of the genetic clustering derived with geographic information was substantially higher than for analyses assuming a single cluster, indicating that spatial structure contributed to genetic structure. Furthermore, at the Fingernail and North Island sites, where the patches were arrayed linearly along the shore, a gradient of genetic clustering along the array is apparent (Fig. 3b, c).

Analysis of *F*-statistics for genetic variation again yielded complementary conclusions (Table 5). There was significant differentiation among West Rocks, the Fingernail, and North Island sites ($F_{st} = 0.626$, $P < 0.00001$), as expected given the results of the larger among site analysis. There was also significant differentiation among patches within sites ($F_{st} = 0.099$, $P < 0.00001$). Of the 23 pairwise comparisons between patches within sites, 8 were significant ($P < 0.05$) following sequential Bonferroni correction, including a patch pair only 5 m apart. Finally, there was significant differentiation among individuals within patches $F_{is} = 0.625$, $P < 0.00001$), suggesting non-random gene flow at even smaller scales.

Combining all pairwise comparisons of population differentiation estimates among sites and patches, and comparing them to distances between these populations (Fig. 4, $r^2 = 0.343$, $P < 0.0002$), we found a rapid increase in population differentiation with distance over very small scales, with a break in the relationship at 23 m. Beyond this distance, there was no relationship between F_{st} and distance, but the average F_{st} was high (Fig. 4). Because the shape of this pattern might be strongly affected by long-distance comparisons, we also examined differentiation with geographic distance below

Table 4 *Postelsia palmaeformis*: observed (H_o) and expected (H_e) levels of heterozygosity for each sampling site and each microsatellite locus

Locus	Site H_o/H_e								
	West Rocks	West Island	Fingernail	North Island	Rainbow Rock	West Cape Flattery	East Cape Flattery	Slant Rock	Waadah Island
PM1	.20/.31	.14/.16	.08/.11	0/.07	.24/.34	0/0	.21/.59	0/.26*	.14/.51
PM2	.05/.20	.10/.28	.03/.33*	0/.23*	0/0	.30/.53	.05/.24	.06/.11	.43/.36
PM3	.10/.20	.07/.10	.11/.38*	.23/.29	.23/.26	.13/.36	.37/.63	.06/.10	.29/.40
PM7	.07/.20	.52/.45	.39/.39	.30/.36	.71/.65*	.47/.37	.74/.55	.72/.51	0/0
PM8	0/.46*	.03/.43*	.03/.34*	.30/.78*	.05/.63*	0/0	.05/.56*	0/.47*	0/.40
PM10	0/0	0/0	0/0	0/0	0/0	0/0	.32/.53	0/.16	0/0
PM12	0/.07	0/.10	.03/.30*	.05/.40*	0/0	0/.28*	.32/.73	.22/.41	.29/.73
PM13	.19/.53*	.07/.16	.33/.30	.25/.24	.38/.35	.33/.31	.74/.48	.67/.49	0/0
PM15	.34/.79*	.03/.10	.06/.13	.27/.56*	.29/.61	.03/.59*	0/.15	.28/.63	0/0

Asterisks indicate significant deviations from Hardy–Weinberg equilibrium (sequential Bonferroni-corrected for non-monomorphic loci, $P < 0.05$)

the site level. Breakpoint regression revealed an increasing relationship between population differentiation and geographic distance ($r^2 = 0.331$, $P = 0.004$, Fig. 5), but no statistically supported break in the relationship within this smaller scale (< 33 m).

For between-patch comparisons, sample sizes were small ($n = 3$–5) in some cases, which probably made our detection of pairwise differences conservative. F_{st} estimates were unrelated to sample size (multiple regression, $P = 0.45$) but increased strongly with distance between patches ($P < 0.005$). Probability values associated with these estimates declined significantly as F_{st} estimates and sample size increased (multiple regression, $P < 0.00001$ and $P < 0.0006$, respectively). There was no systematic variation in sample size with distance between patch pairs ($r = -0.198$, $P > 0.35$).

Discussion

Microsatellite analyses strongly corroborate the restricted spore dispersal distances (1–3 m) in *P. palmaeformis* inferred from field studies (Dayton 1973; Paine 1988; R. T. Paine, personal communication; J. T. Wootton and C. A. Pfister, unpublished data). Populations separated by as little as 5 m had genetic differentiation (F_{st}) values significantly greater than zero. Furthermore, individuals within small-scale populations were significantly differentiated, consistent with population structure at smaller spatial scales than those sampled (a "Wahlund effect"). We interpret these patterns as resulting from low dispersal. Theoretically, vegetative reproduction could also contribute to such a pattern. Vegetative reproduction of the sporophyte is not known in *P. palmaeformis* and our genetic data do not support the most plausible mechanism, budding multiple stipes with fronds from the same holdfast, because plants collected from the same tangle of holdfast were not more genetically similar compared to individuals with clearly separated holdfasts at the patch scale (H. Kusumo, C. A. Pfister, and J. T. Wootton, unpublished data). Estimates of F_{is} in our analysis were also high, which is generally considered evidence of inbreeding. Inbreeding can increase homozygosity among individuals beyond the effects of drift alone. Ecologically, inbreeding generally is associated with limited dispersal of offspring and gametes. In *P. palmaeformis*, there is no evidence for alternative mechanisms such as an active behavioral preference for mating with siblings or strong outbreeding depression. Given the small spatial scales over which *P. palmaeformis* appears to disperse, the small population sizes necessarily contained in small areas, and our ability to start experimental sea palm populations from a single individual (Paine 1988; J. T. Wootton and C. A. Pfister, unpublished data), selfing probably occurs to some extent in this species. Hence *F*-statistics, particularly F_{is}, may be increased as a result of inbreeding. Furthermore, STRUCTURE assumes no inbreeding within clusters; the implications of inbreeding for this framework are unclear, and deserve further attention. High F_{is} estimates might also be indicative of null alleles or alleles under differential selection. We have explored this possibility in depth elsewhere, and find the pattern of genetic variation to be inconsistent with these hypotheses (Kusumo et al. 2004).

The contribution of geographic separation to overall genetic structure was generally high in our analyses using STRUCTURE, but at small scales within sites, the contribution of spatial subdivision was more variable (Fig. 3). At North Island, the Bayesian probability of clusters derived with geographic information was similar to that derived without geographic information, suggesting that spatial structure could largely explain the observed genetic structure. At the West Rocks site, the clusters derived from spatial information had substantially lower Bayesian probability values than those derived without reference to spatial structure, suggesting that other factors beyond the documented structure contributed to genetic structure at this site. The

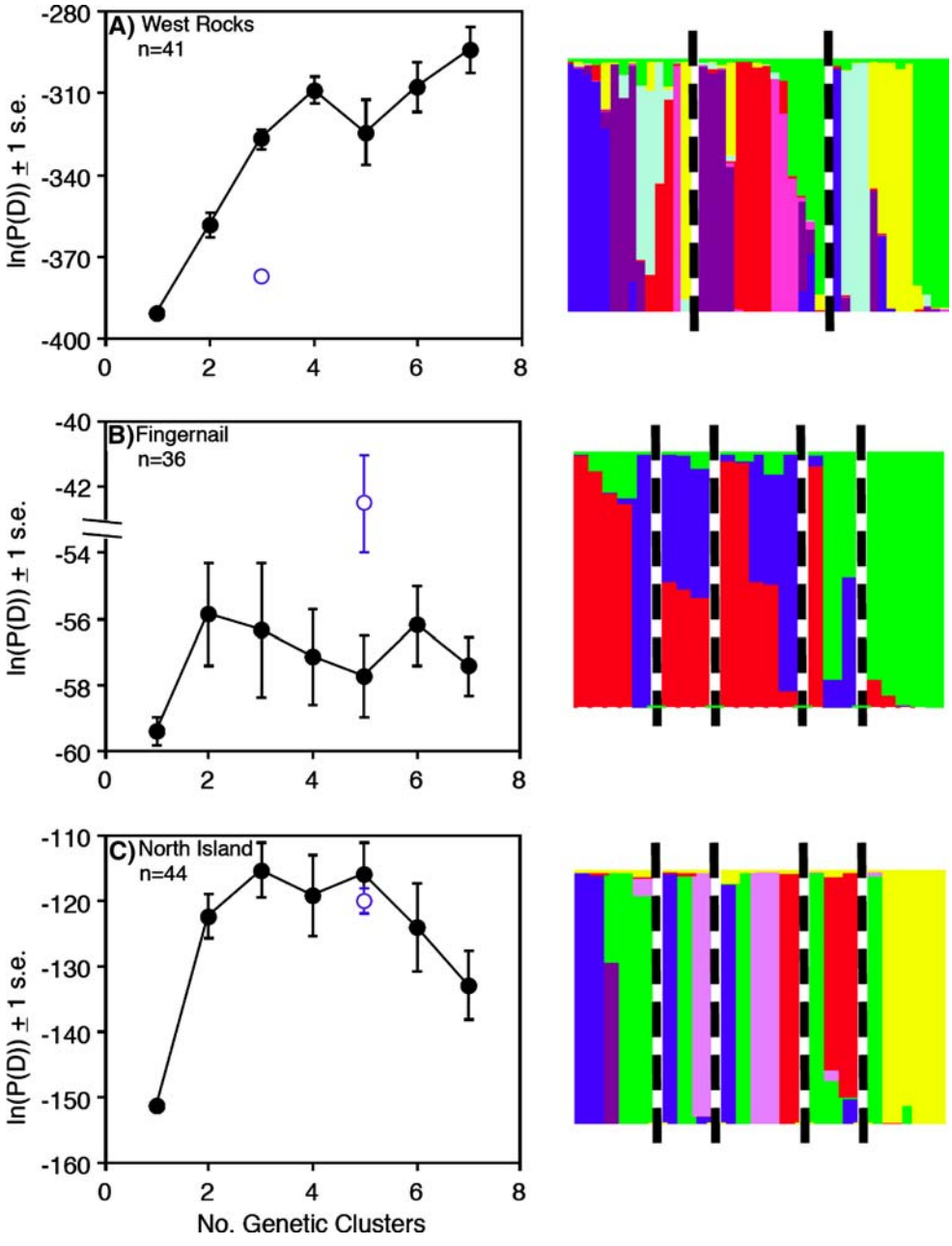

Fig. 3 *Postelsia palmaeformis*. Results of STRUCTURE analysis for fine-scale genetic clustering among subpopulations within three sites (**a** West Rocks, **b** Fingernail, **c** North Island). *Left panels* graphs of average (± SE) ln(Bayesian probability) for analyses ($n = 10$) assuming different numbers of genetic clusters. *Solid symbols* analyses made without reference to pre-defined subpopulations. *Open symbols* analyses incorporating subpopulation information. *Right panels* pattern of clustering among subpopulations for best-fitting result at each site, derived without reference to subpopulation origin. Spatial relationships of subpopulations are reflected in their ordering on the graph. *Columns* represent individuals, different *colors* represent the proportionate contribution to an individual genotype of each genetic cluster. Same *colors* represent different genetic clusters for graphs from different sites

Table 5 *Postelsia palmaeformis*: genetic variance components estimated from AMOVA for within-site, between-patch comparisons at West Rocks, the Fingernail, and North Island sites

Source	*df*	SS	*F*-statistic	*P*
Among sites	2	177.81	0.625	< 0.00001
Among patches within Sites	10	26.96	0.099	< 0.00001
Among individuals within Patches	66	102.17	0.626	< 0.00001
Within individuals	79	28.00	0.874	< 0.00001

sampling at this site differed from the other two sites in that it occurred over several years, defined populations over larger spatial extents, and was not carried out explicitly to examine small-scale genetic structure. At the Fingernail, the clusters derived with spatial information generated substantially higher Bayesian probability estimates than the average of those derived without reference to sampling location, although one replicate run without assigning spatial structure produced a similar Bayesian probability to those generated with spatial structure. The overall pattern was consistent with observed genetic structure being generated by spatial structure. Detailed exploration of the time traces of probability estimates indicated that for this data set, STRUCTURE tended to converge on parameters that minimized, rather than maximized, the Bayesian probability function when spatial structure was not incorporated a priori. The cause of this situation is uncertain, but could indicate a very sharp probability peak in

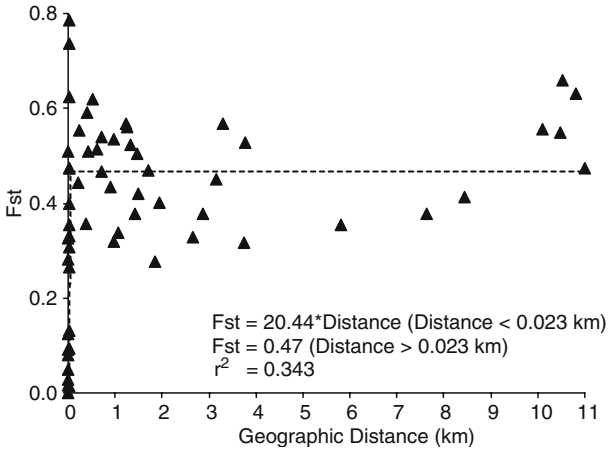

Fig. 4 *Postelsia palmaeformis*. Relationship between pairwise F_{st} estimates and geographic distance among population pairs at all scales analyzed by AMOVA. Break-point regression curve of best fit shown ($r^2 = 0.332$)

combination with a non-monotonic probability profile

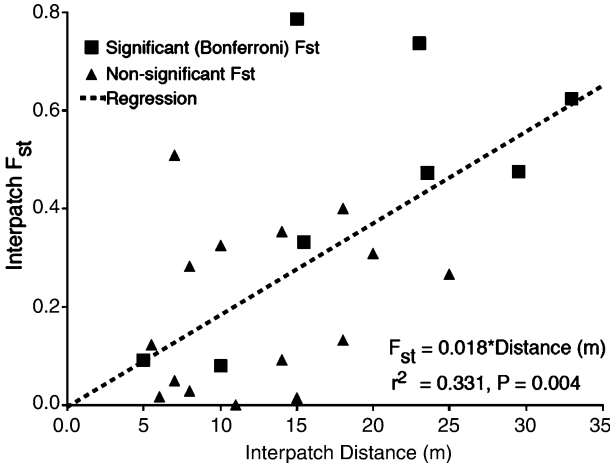

Fig. 5 *Postelsia palmaeformis*. Relationship between pairwise F_{st} estimates and geographic distance within three sites on Tatoosh Island (West Rock, Fingernail, North Island). *Square symbols* are statistically significant pairwise F_{st} estimates; *triangular symbols* are not statistically significant ($P > 0.05$) following sequential Bonferroni correction. Break-point regression curve of best fit shown ($r^2 = 0.331$)

(J. Pritchard, personal communication). In the case where the probability function was high, the estimated migration parameter among clusters was low (0.07), which is consistent with genetic structure arising from low dispersal among spatially separated populations. Furthermore, when we repeated the analysis with defined geographic structure, but assigned geographic location at random to individuals, the 95% confidence intervals did not overlap with the Bayesian probabilities estimated by assigning individuals to the correct geographic locations, indicating that geographic structure generated genetic structure.

The apparent links between dispersal modes and genetic structure found in our study correspond to patterns in intertidal kelp species (Kusumo and Druehl 2000; Martínez et al. 2003). A broader survey of diverse marine taxa with varying life histories indicate that species with actively swimming propagules that remain in the water column for a greater period of time (weeks) generally are less differentiated genetically (Levinton and Suchanek 1979; Palumbi and Wilson 1990; Duffy 1993; Williams and Benzie 1996), whereas species with short-lived and benthic propagules have demonstrated significant genetic differentiation (Duffy 1993; Hellberg 1994; McFadden 1997; Klautau et al. 1999). Recent analysis of a goby fish in the Caribbean, however, suggests that larval behavior and/or zoogeographical barriers can also result in genetic differentiation in a species with planktonic larvae (Colin 2003; Palumbi and Warner 2003; Taylor and Hellberg 2003). The F_{st} estimates exhibited by our *P. palmaeformis* populations suggest that sea palms have extremely limited dispersal. Although we acknowledge that possible differences in marker systems used among taxa make F_{st} estimates difficult to compare, we note that *P. palmaeformis* has quite high values over very small scales relative to those of most other species described in the literature, and that microsatellite markers are generally expected to exhibit low F_{st} values because of their high variability (Hedrick 1999).

The propensity for dispersal by adults may also be an important predictor of genetic structure. Although *P. palmaeformis* individuals can be ripped from the rocks, they lack flotation structures on their fronds (although their stipe is hollow) and appear to be relatively uncommon in beach drift compared with other species. For example, Coleman and Brawley (2005) reported relatively low F_{st} estimates for a brown seaweed (*Fucus spiralis* L.) that produce floating pieces of fertile thalli. However, the extent to which spore dispersal versus adult transport drives genetic structuring is unknown for any seaweed species and will be possible only with extensive individual sampling and mapping. Analyzing shifts in the pattern of isolation by distance over a range of scales, as we have done, provides some insight into this issue when combined with knowledge of general dispersal modes in different life stages of a species.

Within sites, we found a significant positive relationship between genetic differentiation and distance up to a distance of 23–33 m. Although isolation by distance is the expected result for a species with relatively limited dispersal, the evidence supporting isolation by distance in marine organisms is mixed. Studies of several marine taxa report genetic differentiation among populations but no strong relationship of F_{st} with distance (McFadden 1997; Ruckelshaus 1998; Reusch 2002). There are corresponding patterns in other marine macroalgae, including the red algae *Delisea pulchra* and *Caloglossa leprieurii* (Wright et al. 2000; Zuccarello et al. 2001), *Gracilaria gracilis* (Engel et al. 1999) and *Palmaria mollis* (Lindstrom et al. 1997). Analysis of microsatellite loci in

the kelp *Laminaria digitata* showed that isolation by distance patterns might be disrupted by habitat discontinuities where rocky substrata were unavailable (Billot et al. 2003). In contrast, there is weak or little evidence for a relationship between geographic distance and F_{st} in other macroalgae, including the kelp *Alaria marginata* (Kusumo and Druehl 2000), the fucoid *F. spiralis* (Coleman and Brawley 2005) and the green alga *Cladophoropsis membranacea* (van der Strate et al. 2003), patterns that might be ascribed to some combination of historic variability in current regimes or present day gene flow and genetic drift (*sensu* van der Strate et al. 2003). Our study of *P. palmaeformis* indicates isolation by distance over relatively short scales (within sites), but no such pattern at large scales where the F_{st} asymptotes. This pattern is consistent with the two hypothesized modes of dispersal in this species, because the scale at which the break occurs corresponds well with the scale of geographical habitat breaks between potential sea palm habitats (i.e. discrete intertidal rock benches). Within rock benches, short-distance spore and gamete dispersal would be expected to produce a slow diffusive spread of genes through the population as gametophytes mate with close neighbors, causing closer sites to be less differentiated than more distant sites. Dispersal between rock benches, however, probably requires dispersal of detached reproductive adults via drift, which is more likely to produce haphazard dispersal patterns with distance given the inconsistent directions of nearshore currents. Reusch et al. (2000) report a similar pattern for western European populations of the seagrass *Zostera marina*, with a break at around 2,000 km, but the cause of this pattern is currently unclear. As more genetic data on population structure in marine algae become available, we can assess whether isolation by distance is exceptional or typical for these taxa.

An alternative interpretation for the lack of differentiation with distance at large scales is that indices of differentiation have reached a maximal ceiling, set by the mutation rate of the marker system (Hedrick 1999). For analyses of genetic structure with multiple markers, maximum F_{st} among completely isolated populations is not 1, as it is for a single marker, but is the average homozygosity within populations (Hedrick 1999). The role of this factor in shaping our results is equivocal (Fig. 6). Our estimated values of F_{st} among pairs of sites are on average 0.22 units lower than maximum possible values, suggesting that there is sufficient variation to detect a trend in differentiation with distance. Furthermore, there is no significant relationship between maximum F_{st} and estimated F_{st} across all samples (Fig. 6; $r^2 = 0.066$, $P > 0.6$). We cannot completely rule out an effect of marker variability, however, because when we restrict our analysis to F_{st} between pairs of sites (large-scale pairs), there is a positive correlation between maximum and estimated F_{st} (Fig. 6; $r^2 = 0.457$, $P < 0.001$). Even after we accounted for this relationship statistically, however, no isolation by distance pattern emerged (multiple regression, $P > 0.1$).

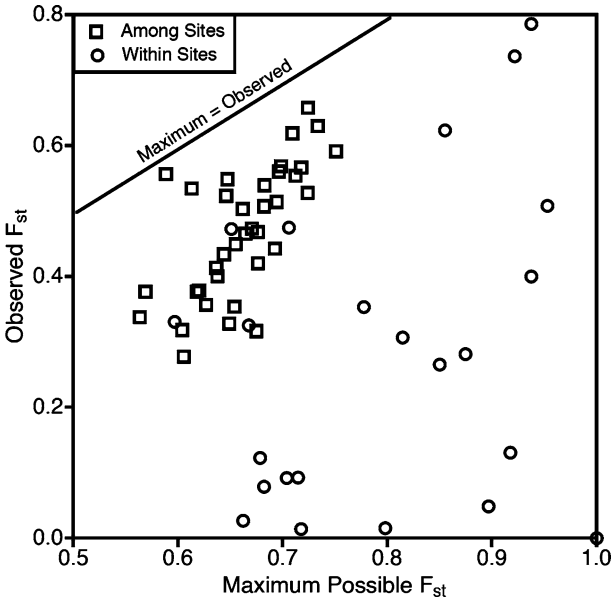

Fig. 6 *Postelsia palmaeformis*. Relationship between observed and maximum possible F_{st} estimates both between pairs of patches within sites (*open circles*) and between different pairs of sites (*open squares*). The *line* shows the expected location of points if observed F_{st} estimates equaled maximum possible F_{st} values

In an earlier study with *P. palmaeformis*, Coyer et al. (1997) found mixed evidence for population structure depending on the marker system used. Using M13 fingerprinting, they found evidence of differentiation among some population pairs as little as 25 m apart, but little structure between populations at larger scales. In contrast, RAPDs showed strong differentiation at distances 16–250 km apart, but not at smaller scales. Coyer et al. (1997) suggested that the contradictory results of the two marker systems could be explained if they had different powers of resolution at different scales. Our work agrees with their general conclusion that *P. palmaeformis* exhibits population structure and, by more intensively sampling populations at a range of smaller (< 11 km) scales using highly variable co-dominant markers, further reveals that there is strong genetic structure in this species at very small scales. Beyond simply documenting genetic structure, our level of sampling also revealed the consequences and scale of the dual dispersal modes of *P. palmaeformis*, including a shift from isolation by distance (stepping stone model) to rare random dispersal (island model).

The limited dispersal of *P. palmaeformis* strongly indicated by our findings has several implications. First, we might expect negative effects on genetic variation in local populations, which might reduce population performance. Such limited dispersal, however, might strengthen the likelihood of local adaptation if sufficient genetic variation is present. Therefore, future studies of outcrossing and inbreeding in *P. palmaeformis* would be very helpful. Second, the population dynamics of this species should be strongly influenced by local processes

including intra- and interspecific interactions, rather than through large-scale recruitment-driven fluctuations. Because other organisms share the limited dispersal capabilities of *P. palmaeformis*, this situation may apply to a number of other marine benthic species. As these species interact with long-distance dispersers such as mussels (Paine 1979), however, recruitment from remote communities may still affect *P. palmaeformis* dynamics indirectly. Hence, the development of multispecies theory accounting for regulation of populations at multiple scales may be necessary to understand the dynamics of benthic communities, and may produce some unexpected patterns. Finally, limited dispersal is of importance in the context of harvesting this species (Kalvass 1994). Reduced population size through harvesting may result in reduced local genetic diversity through enhanced genetic drift, and the loss of beneficial alleles may not be replaced because of low dispersal from other populations. Additionally, if overharvesting drives local populations extinct, the extremely limited dispersal documented here makes repopulation from other sources unlikely. Hence the management of *P. palmaeformis* harvest should explicitly account for its limited dispersal lifestyle.

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