

GENE FLOW VERSUS LOCAL ADAPTATION IN THE NORTHERN ACORN BARNACLE, *SEMIBALANUS BALANOIDES*: INSIGHTS FROM MITOCHONDRIAL DNA VARIATION

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Abstract.—In reciprocal transplant experiments, Bertness and Gaines (1993) found that *Semibalanus balanoides* juveniles that had settled in an upper Narragansett Bay estuary survived better in that estuary than did juveniles from coastal localities. The observed pattern of survivorship led to the claim that local adaptation may result from a combination of limited gene flow between and strong selection within these habitats. Here we test the hypothesis that limited gene flow has led to habitat-specific population differentiation using sequence and restriction fragment length polymorphism analyses of the mitochondrial DNA D-loop region of *S. balanoides*. Samples were analyzed from replicated coastal and estuary localities in both Narragansett Bay, Rhode Island, and Damariscotta River, Maine. The patterns of F_{ST} indicate that gene flow between coast and estuary is extensive ($Nm > 100$) and is not lower in the estuary with lower flushing rates (Narragansett Bay). Given the high estimate of genetic exchange, adaptations for unpredictable environments seem more likely than local adaptation in this species because loci that respond to selection in one generation are essentially homogenized by the next seasons' settlement. Nevertheless, these estimates of neutral gene flow can help identify the strength of selection necessary for local adaptation to accumulate in *Semibalanus*.

Key words.—Barnacle, D-loop, gene flow, mitochondrial DNA, population structure, selection, *Semibalanus balanoides*.

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In a reciprocal transplant study of the northern acorn barnacle (*Semibalanus balanoides*), Bertness and Gaines (1993) showed that the habitat of origin of transplanted barnacles affected their probability of survival. Barnacles from a thermally stressed estuary in Narragansett Bay, Rhode Island, survived significantly better in their own habitat than did barnacles from coastal Rhode Island. This pattern was not observed in a Maine estuary (Damariscotta River), where thermal stress is lower and the rate of estuary flushing is higher. Bertness and Gaines (1993) suggested that patterns of larval retention in different estuaries could contribute to local adaptation in this species.

These results present an interesting case for the analysis of gene flow and local adaptation. Marine animals with planktonic larval dispersal often exhibit little population structure (Bohonak 1999 and references therein). But clearly, the issue of local adaptation depends on the balance of effective gene flow ($N_e m$) and the difference in effective selection between habitats or populations ($N_e s$). Because habitat variation can be extreme within the effective dispersal range of many marine species, genotype-specific mortality of certain stages of the life cycle could be responsible for genetic differentiation (Johnson and Black 1984; Hedgecock 1986; Koehn and Hilbish 1987; Holm and Bourget 1994; Schmidt and Rand 1999).

The purpose of the current study is to test the hypothesis that larval retention is sufficient to contribute to local adaptation in the northern acorn barnacle. Specifically, we have used mitochondrial DNA (mtDNA) variation to estimate gene flow among the same (or very close) localities used by Bertness and Gaines (1993). These sites were situated at coastal and estuarine locations in both Maine and Rhode Island, but the two estuaries sampled in these studies differ in their rates of water flow. The flushing time of the Damariscotta River, Maine, is one to two weeks (McAlice 1977), whereas that of

Narragansett Bay, Rhode Island, is three to four weeks (Bertness and Gaines 1993). Given the two- to four-week pelagic larval phase of *S. balanoides* (Barnes 1956), these particular sites in Maine and Rhode Island were chosen based on their predicted effects on levels of larval retention and population differentiation. A larva in Narragansett Bay has a good chance of settling within the bay, whereas a larva in the Damariscotta River has a good chance of being flushed out to sea before the larval phase is over. If larval retention is an important issue in local adaptation, then genetic differentiation between estuary and coastal localities should be most pronounced in bay systems with low flushing rates (Narragansett Bay, Rhode Island). The survivorship data of Bertness and Gaines (1993) support a relationship between larval retention and local adaptation, but no independent test of the patterns of gene flow has been conducted between localities in fast-flushing versus slow-flushing estuaries.

We analyzed nucleotide variation in the D-loop of mtDNA of *S. balanoides* for several reasons. First, mtDNA is haploid and maternally inherited so its effective population size is one-quarter that of autosomal DNA (assuming an equal sex ratio) making it a more sensitive marker for population structure than nuclear markers (e.g., Birky et al. 1989). Second, compared to allozymes and functional studies such as reciprocal transplant experiments, mtDNA is more likely to be a neutral marker, making it appropriate for studies of population structure due to genetic isolation (e.g., Rand 1996; cf. Holm and Bourget 1994; Schmidt and Rand 1999). If population differentiation is not detected in mtDNA, it is not likely to exist for neutral markers with higher effective gene flow (e.g., nuclear markers). By providing an unbiased assessment of the levels of gene flow, neutral markers can help define the strength of selection that would have to exist at a nonneutral marker for local adaptation to contribute to population differentiation. Finally, neutral markers can provide a distinct view of the temporal scale of variation. Clearly,

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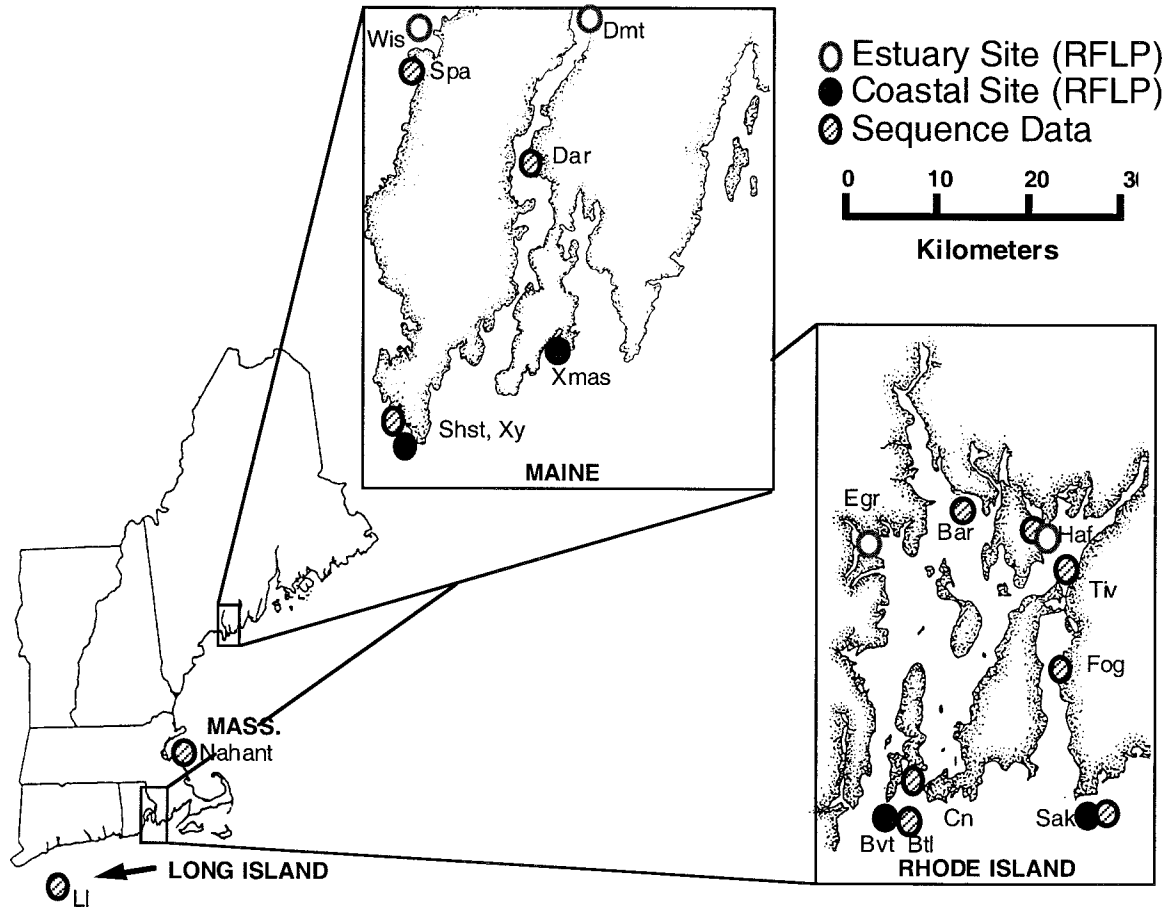


FIG. 1. Collection localities in Narragansett Bay, Rhode Island, and Damariscotta River, Maine. Estuary and coastal site (open and solid circle, respectively) refer to collecting localities for the RFLP analyses. Sequence data (hatched circle) refers to collecting localities for sequenced individuals. Abbreviations can be found in Tables 1 and 2. Note that the sites labeled Bar (Barrington), Con (Conanicut), Dar (Darling Center), and Fog (Fogland) each include several individuals listed in Table 1.

the coalescent time for a DNA polymorphism sampled from different populations spans a greater time scale than that of any experimental manipulation. Given the considerable year-to-year and site-to-site variation in recruitment in *S. balanoides* (Gaines and Bertness 1992; Minchinton and Scheibling 1993), data from neutral molecular polymorphisms may shed considerable light on the evolutionary consequences of ecological variation in this well-studied species.

MATERIALS AND METHODS

Sample Collection

Individuals were collected from the Damariscotta River, Maine, from the Sheepscot River, Maine, and from Narragansett Bay, Rhode Island. In each of these bay systems, sites were selected before collecting and identified as a coastal or an estuary site based on its proximity to the open ocean or the head of the bay, respectively. Additionally, the collecting sites were selected so that paired coastal and estuary localities could be considered (e.g., Damariscotta coast vs. Damariscotta estuary, etc.; see Fig. 1). An effort was made to match DNA samples from sites used by Bertness and Gaines (1993). In that study, transplant experiments were conducted between

Haffenreffer and Sakonnet in Rhode Island (see "Haf" and "Sak" in Fig. 1) and between The Darling Marine Center and Crow Island in the Damariscotta River, Maine (see "Dar" and near "Xmas" in Fig. 1). Additional barnacles were sampled from selected sites in Maine, Massachusetts, Rhode Island, and Long Island Sound, the last being near the southern end of this species' distribution (Barnes 1958; Wells et al. 1960). *Semibalanus balanoides* from Prince William Sound, Alaska, served as an outgroup. These additional samples, which can be assigned to coastal or estuary locations (see Table 1), were used for sequence analysis so that a phylogenetic test of mtDNA habitat association could be performed. All individuals were collected from the midintertidal zone. Additional information on collecting localities is presented in Table 1. All samples except Alaska were packed in ice for travel, then frozen and stored at -80°C . Alaska samples were stored immediately in 100% ethanol.

DNA Analyses

Individual adult barnacles were homogenized in 50 μl squish buffer (10mM Tris, 1mM EDTA, 25 mM NaCl, 1 $\mu\text{g/ml}$ proteinase K; Gloor and Engels 1991; see also

TABLE 1. Collecting localities and sample information. The abbreviations in parentheses refer to localities shown on Figure 1 and in the taxon names of the gene tree in Figure 2. The capital letters at the end of the taxon names refer to collection location (RI, ME, MA, LI) or habitat (E or C). See Materials and Methods for details.

Locality	River/Bay	Coast/Estuary	Samples size	Year
Samples analyzed in paired locality test with RFLP				
Maine				
Christmas Cove (Xmas)	Damariscotta	coast	7	1992
Damariscotta (Dmt)	Damariscotta	estuary	20	1992
Sheepscot (Shst)	Sheepscot	coast	14	1992
Wiscasset (Wis)	Sheepscot	estuary	19	1992
Rhode Island				
Beavertail (Bvt)	Narragansett Bay	coast	23	1991
Haffenraffer (Haf)	Narragansett Bay	estuary	29	1991
East Greenwich (Egr)	Narragansett Bay	estuary	23	1991
Sakonnet (Sak)	Narragansett Bay	coast	24	1991
Samples analyzed by control region sequences				
Alaska				
Prince William's Sound (AK2, AK4)			2	1993
Long Island, NY				
Stony Brook (LB5, LI4, LI5)	Long Island Sound	estuary	3	1992
Maine				
Darling Center (D1A, D2A, D4c, D8c, Dr1, DR7, DR8)	Damariscotta	estuary	7	1992
Sheepscot Pier (Spa)	Sheepscot	estuary	1	1992
Xy	Sheepscot	coast	1	1992
Massachusetts				
Nahant (NA2)		coast	1	1992
Rhode Island				
Barrington (B2e, Bar)	Narragansett	estuary	2	1992
Beavertail (Btl)	Narragansett	coast	1	1992
Conanicut (Cn1, C3c)	Narragansett	coast	2	1992
Fogland (F, FG4, FG5)	Narragansett	estuary	3	1992
Haffenraffer (Hf5)	Narragansett	estuary	2	1992
Sakonnet (Sak)	Narragansett	coast	1	1992
Tiverton (T3c)	Narragansett	estuary	1	1992

Schmidt and Rand 1999). The liquid was incubated for 15–20 min at 37°C then heated to 95°C for 2 min to denature the proteinase K.

DNA amplification was performed using primers that span the D-loop region of the mtDNA in the northern acorn barnacle. The following primer pair is internal to the isoleucine tRNA/12S RNA primer pair that amplifies the control region of many insects (cf. Simon et al. 1994) and amplifies a 550-bp fragment in *S. balanoides*: Iso2: 5'-TTA CGG GCG TAT TTT ACT TG-3' and 12Sr: 5'-TAA CCG CGA CGG CTG GCA C-3'. We constructed the Iso2 primer from a conserved region within our *S. balanoides* sequence. The 12Sr primer aligns with the *D. yakuba* 12S RNA beginning at position 14,748 (Clary and Wolstenholme 1985). An alignment of sequence data from *S. balanoides* to the 12S rRNA gene from *D. yakuba* and found an additional conserved stretch near the 5' end of the 12S gene. The reverse of this sequence was used as a more efficient right-hand primer paired with Iso2: 12S5'rev: 5'-AAT ACA ACA CGG ACC TCA AC-3'. All polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analyses were done on the 400-bp fragment amplified with Iso2 and 12S5'rev.

For each 25- μ l reaction, we used, 0.5–1.0 μ l barnacle homogenate, 1.0 μ l each primer (15 μ M), 2.5 μ l 25 mM MgCl₂, 2.0 μ l dNTP mix (2.5 mM each nucleotide), 2.5 μ l reaction buffer (Promega, Madison, WI) and 0.1 μ l of *Taq* polymerase (Promega). The samples were heated to 95°C for 2

min, then incubated for 35 cycles of 94°C (30 sec), 55°C (1 min), 72°C (2 min) on a thermal cycler.

The 400-bp PCR products from Iso2 and 12S5'rev were digested with Dde I in 20- μ l reactions using 15 μ l unpurified PCR reaction, 2.9 μ l H₂O, 2.0 μ l 10 \times reaction buffer, and 0.1 μ l Dde I provided by New England Biolabs (Beverly, MA). The resulting digests were run on 3.0% agarose gels at 75 V for 3 h. *Dde* I type A has two *Dde* I sites in the PCR fragment (producing three bands of sizes 169, 135, and 97 bp), and *Dde* I type B has three *Dde* I sites (the third 97-bp band is cut into 58-bp and 39-bp bands).

Partial sequences of the Iso2-12S5'rev PCR product were obtained as described in Rand et al. (1994). The 27 DNA sequences have been deposited in GenBank (accession nos. AF385899–AF385925).

Nucleotide Polymorphism and Neutrality Test

We compared two estimates of DNA polymorphism, π and θ (Nei 1987; Tajima 1989). π is a measure of nucleotide diversity based on pairwise comparisons of sequences and is equal to the average number of nucleotide differences between all pairs of sequences. θ is an estimate of polymorphism based on the number of variable sites, irrespective of their frequency in the sample. For a population at equilibrium for mutation and drift, π should equal θ (Tajima 1989). We calculated Tajima's *D* as one test of the neutrality of the

TABLE 2. Population structure of Dde I polymorphism.

	Dde I Haplotype			Heterozygosity	F_{IS}	F_{ST}	F_{IT}	Grand F_{ST}
	Sample size	A	B					
Rhode Island coast								
Bvt	23	5	18	0.22	0.340			
Sak	24	9	15	0.38	0.469			
RI coast total	47	14	33	0.30	0.418	0.0329		
Rhode Island estuary								
Haf	29	9	20	0.31	0.428			
Egw	23	8	15	0.35	0.454			
RI estuary total	52	17	35	0.33	0.440	-0.0018		
Rhode Island total	99	31	68	0.31	0.430		0.0023	0.0174 $Nm = 220.9$
Maine coast								
Xmas	7	2	5	0.29	0.408			
Shst	14	6	8	0.43	0.490			
ME coast total	21	8	13	0.38	0.472	0.0481		
Maine estuary								
Dmt	20	7	13	0.35	0.455			
Wis	19	9	10	0.47	0.499			
ME estuary total	39	16	23	0.41	0.484	0.0146		
Maine total	60	24	36	0.40	0.480		0.0046	0.0356 $Nm = 107.3$ -0.0057 $Nm = -88.7$
Grand total	159	55	104	0.35	0.453			

mtDNA D-loop in *S. balanoides* in an effort to determine if this molecular marker could serve as a neutral, unbiased estimator of gene flow.

Phylogenetic Analyses

The DNA sequences were aligned by eye without ambiguity. Phylogenetic analyses were performed with PAUP version 3.0s (Swofford 1991), PAUP* version 4.0b8 (Swofford 2001), MacClade 3.04 (Maddison and Maddison 1992), and MEGA version 1.01 (Kumar et al. 1993). We performed 100 bootstrap replicates in PAUP, using a heuristic search with branch swapping. For comparison to parsimony methods, a neighbor-joining tree was constructed in MEGA using 500 bootstrap replicates and Kimura two-parameter corrected distance matrix (differences between sequences were relatively small so different methods of correction produced the same results). The hypothesis of an association between habitat (coast/estuary) and mtDNA variation was assessed by performing compare-two permutation tail probability tests in PAUP* (ver. 4.0b8). The consensus tree was compared to a constrained tree where coast and estuary samples were forced into separate subtrees. Bootstrap analysis examined the difference in length between the consensus and constrained-habitat tree.

Estimates of Population Structure and Gene Flow

The sequence of the D-loop region revealed a common polymorphic site in a Dde recognition sequence (Brown 1995). This site is polymorphic in both clade 1 and clade 2 of Figure 2, and can be used to score the frequency of two distinct mtDNA haplotypes in *S. balanoides* (e.g., Schmidt and Rand 1999; Schmidt et al. 2000). This Dde I polymorphism was scored in 159 additional barnacles from paired estuary and coastal samples in replicated locations in eastern

and western Narragansett Bay and in the Sheepscott and Damariscotta Rivers, Maine (see Fig. 1, Table 2). The significance of population structure was assessed using permutation tests in AMOVA (Excoffier et al. 1992; because we are permuting a single restriction site without consideration of genetic distance, the molecular information option was turned off in WINAMOVA permutation). Gene flow, or effective migration rate, was estimated from traditional F_{ST} -values for mtDNA haplotype frequencies in various pairs of samples in Maine and Rhode Island by solving for the term $N_e m$ in the relation $F_{ST} = 1/(2N_e m + 1)$ for haploid mtDNA.

RESULTS

Nucleotide Variation and Test of Neutrality

Twenty-seven individuals were sequenced for 107 bp, and 13 variable sites were identified. Five sites were unique to Alaska and eight additional sites were polymorphic within the New England samples. All but one of the polymorphic sites were transitions. The two estimates of nucleotide polymorphism (\pm SD) were as follows: for the complete dataset of 27 sequences, $\pi = 0.087 \pm 0.011$, $\theta = 0.084 \pm 0.034$, and $D = 0.1089$. D would need to be more than an order of magnitude greater to show a significant departure from the neutral expectations (Tajima 1989). For the 25 New England sites, $\pi = 0.070 \pm 0.004$, $\theta = 0.053 \pm 0.024$, and $D = 1.040$. For 15 samples south of Cape Cod, $\pi = 0.0675 \pm 0.007$, $\theta = 0.084 \pm 0.034$, and $D = 0.56540$; and for the 10 sequences north of Cape Cod, $\pi = 0.052 \pm 0.006$, $\theta = 0.044 \pm 0.022$, and $D = 0.8728$. None of these Tajima's test is significantly different from the neutral expectation.

Phylogenetic Analysis

Two weakly supported clades within New England samples of the northern acorn barnacle are evident based on the gene

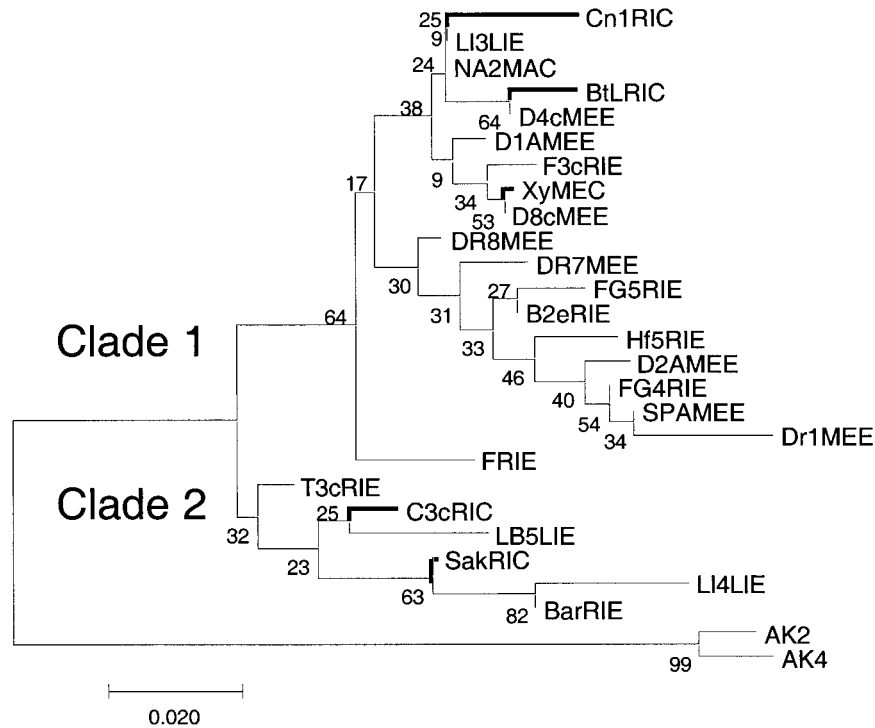


FIG. 2. Phylogenetic tree of D-loop sequences from New England samples of *Semibalanus balanoides*. The tree represents 500 bootstrap replicates constructed by MEGA. Alaska samples were chosen as an outgroup. Numbers on the tree show the percent of the 500 trees constructed that agree with this consensus tree. The last three letters of each individual identify the location (RI, Rhode Island; LI, Long Island; MA, Massachusetts; ME, Maine) and habitat of collection (C, coast site; E, estuary site). The bold branches identify individuals collected at coastal sites. There are two clades apparent, although only weakly supported (64% of the trees constructed). Clade 2 includes individuals T3cRIE, C3cRIC, LB5LIE, SakRIC, LI4LIE, and BarRIE. Clade 1 includes all other non-Alaska individuals. Statistically, each clade is an unresolved polytomy, but a phylogram is displayed to illustrate degree of sequence differentiation. The scale bar represents divergence using the Kimura two-parameter correction in MEGA (Kumar et al. 1993).

tree constructed by MEGA (clades 1 and 2, Fig. 2). There are three transitions that distinguish clade 1 from clade 2. Bootstrap analysis under parsimony also detected the same two groups with approximately the same level of support (clade 1 was recovered in 61% of the resamplings in the parsimony analysis using PAUP, 62% of the bootstrap samples in the neighbor joining analysis with Kimura two-parameter distances [Fig. 2], and 59% of the time with Tamura-Nei distances using MEGA). There is some biogeographic structure to the sequences: Individuals in the smaller clade 2 are only from south of Cape Cod, whereas clade 1 contains individuals from both north and south of Cape Cod (Brown 1995; see F_{ST} -analyses below). The two clades do not correlate with ecological habitat of the locations sampled, because coast and estuary samples are found in each clade (see Fig. 2: the last three letters of each individual refers to the state and habitat of collection, where ME, Maine; RI, Rhode Island; LI, Long Island; MA, Massachusetts; C, coast; E, estuary). If the estuary and coastal barnacles are forced into separate clades using MacClade, the three length rises from 38 (with a consistency index = 0.87) to 44+ (consistency index = 0.75) steps. Although coast-estuary tree is longer than the consensus tree, permutation tail probability tests indicate that this difference is not significant.

Qualitative evidence suggests that substantial gene flow among localities is occurring in *S. balanoides*. Three pairs of individuals showed identical sequences but were collected in

widely separated locations. Individuals D8cMEE and XyMEC were from Maine estuary and coastal sites, respectively, separated by 20 km in the Damariscotta River. Individuals SPAMEE and FG4RIE were from a Sheepscot River, Maine, estuary and a Narragansett Bay, Rhode Island, estuary separated by more than 250 km. The third pair of identical sequences (NA2MAC and LI3LIE) were from coastal Massachusetts and a Long Island estuary, separated by more than 100 km. High migration rates are implied by the fact that these haplotypes represent twigs of the gene tree.

Population Structure and Estimates of Migration Rates

The frequencies of the two mtDNA restriction haplotypes (A and B) were determined in a sample of 159 individuals. Estimates of haplotype frequencies and population subdivision (F_{ST}) are reported in Table 2. The data show that the largest proportion of the variation lies within a collecting locality and that only 0.2–0.5% of the mtDNA variation lies between the coast and estuary sampling localities in Rhode Island and Maine. These estimates of F_{ST} can be used to estimate $N_e m$, or effective gene flow, using the relation $F_{ST} = 1/(2N_e m + 1)$ for haploid mtDNA. Solving for $N_e m$ shows that gene flow is extremely high ($N_e m > 100$) between coastal and estuary sites in these samples of *S. balanoides* (see Table 2).

One goal of this study was to test the hypothesis that the flushing rate of the estuary system affects this pattern of

population structure. Because Narragansett Bay has a lower flushing rate than the Damariscotta River, this hypothesis predicts that F_{ST} should be higher between coastal and estuary sites in Narragansett Bay. This is not the case: F_{ST} is lower (and migration is higher) in the Narragansett Bay samples than in the Damariscotta River, a result opposite in direction from the prediction.

Permutation tests of population structure in AMOVA (Excoffier et al. 1992) were performed several ways. First, we performed coast-estuary tests separately for Rhode Island and for Maine (each region had two coastal sites vs. two estuary sites). All tests gave negative ϕ_{ST} -values that were not significantly different from zero (data not shown; available on request; ϕ_{ST} is analogous to F_{ST} but uses the ratio of total and within-group variance in genetic distance versus allelic state). We then pooled all the data and performed a hierarchical analysis where region (Maine vs. Rhode Island) and habitat (coast vs. estuary) were at different levels. This too produced small negative ϕ_{ST} -values that were not significantly different from zero. Negative ϕ_{ST} can result from greater variability in one sample than in the pooled sample (slightly negative F_{ST} -values are also evident in the F_{ST} -analyses in Table 2). These analyses are consistent with the F_{ST} data and indicate that the coast and estuary samples are essentially different collecting localities within a larger panmictic population.

An AMOVA comparison of all Maine samples versus all Rhode Island samples did reveal significant population structure. The variance between groups was 0.0025, variance within groups was -0.0069, and variance within populations was 0.2325; the probability of obtaining a higher value for the variance between groups was 0.0099. These results parallel weak biogeographic patterns in the sequence data: No Maine barnacles are represented in clade 2, whereas clade 1 contains barnacles from both southern and northern New England.

DISCUSSION

Analyses of nucleotide polymorphism in the D-loop region of the northern acorn barnacle (*S. balanoides*) show no statistical evidence for restricted gene flow between coastal and estuarine sampling localities in both Maine and Rhode Island. In a limited sample of DNA sequences, two clades in the gene tree do not correlate with habitat type. In a larger sample of RFLP haplotypes, effective migration rates between coast and estuary localities are estimated to be very large ($N_e m > 100$), about two orders of magnitude greater than gene flow levels that would permit population differentiation (cf. Wright 1931; Slatkin 1987). Thus, the coastal and estuarine sites we have examined are effectively different collecting localities in a larger panmictic population. Gene flow does seem to be extensive in *S. balanoides*.

The mtDNA marker we have used is resoundingly neutral by Tajima's test ($D = 0.29$) and by additional tests of both static patterns (Schmidt and Rand 1999) and direct estimates of survivorship in the wild (Schmidt et al. 2000). The neutrality of this mtDNA polymorphism provides an informative tool for discovering the movement patterns of alleles not altered by postsettlement selection. Moreover, because mtDNA is maternally inherited, its effective gene flow will

be less than that of nuclear loci (one-fourth that of autosomal loci and one-third that of X-linked loci, assuming an equal sex ratio; Birky et al. 1989). It is likely that the majority of loci responsible for local adaptation will be encoded in the nucleus. The measurements of mtDNA gene flow presented above thus provide a conservative estimate of the amount of genetic homogenization that must be overcome for true local adaptations to accumulate.

The patterns of F_{ST} for this neutral marker provide a useful contrast with the survivorship differences observed by Bertness and Gaines (1993). In that study, juvenile barnacles (approximately one to two weeks old) that had settled on cobbles were reciprocally transplanted between coastal and estuarine localities. Barnacles from a Rhode Island estuary survived significantly better in their home estuary than did coastal barnacles in one year, but this effect was not observed in the next year. In Maine, barnacles from either the coast or the estuary survived equally well in the two habitats, but as in Rhode Island, total survivorship was always much lower in the estuaries than on the coast. Maternal effects and larval nutritional state were not controlled in these transplants (cf. Jarrett and Pechenik 1997), which might contribute to the survivorship patterns observed by Bertness and Gaines (1993). This possibility aside, there is some evidence for selection in Rhode Island but not in Maine. Bertness and Gaines (1993) suggest that the higher flushing rate of the Damariscotta River in Maine could account for these regional differences. Our gene flow data are not consistent with this pattern. The Damariscotta River in Maine has a flushing rate about twice that of Narragansett Bay in Rhode Island (Gaines and Bertness 1992). Despite this greater opportunity for exchange of larvae in the Damariscotta River, estimates of gene flow are actually lower than for Narragansett Bay (see Table 2).

Although a larger sample of DNA sequences or RFLP haplotypes might uncover subtle population structure, a more likely explanation for the discrepancy between the gene flow data and the well-documented effects of flushing rates on larval retention (Gaines and Bertness 1992) is the temporal scale of resolution. Seasonal variation in rainfall and thus bay flushing can greatly alter the chances of a larva being washed out of an estuary. This would contribute to great temporal variation in recruitment on an ecological time scale. But the considerably longer coalescent time for neutral polymorphisms, coupled with the levels of gene flow documented here could result in considerable damping of allele frequency changes observed on a microevolutionary time scale (see Neigel 1997 and references therein). Thus, the extensive gene flow described here between many of the same localities that exhibited survivorship differences in the transplant experiments by Bertness and Gaines (1993) indicates that this differential mortality reflects seasonal patterns of selection rather than true local adaptations that can accumulate between populations. It appears that each season's settlement effectively randomizes genotypes between the localities we and Bertness and Gaines (1993) have studied. This suggests that adaptations to cope with heterogeneous environments would be more common than true local adaptations in this species.

How Strong Does Selection Have to Be?

Extensive gene flow for a neutral marker between coastal and estuarine localities in *S. balanoides* does not mean that

all regions of the genome are randomized between ecologically distinct habitats. Given an estimate of gene flow on the order of $N_e m = 100\text{--}200$, we can ask how strong selection must be to provide an effective barrier to gene flow for alleles with habitat-specific fitnesses. Population differentiation under selection will accumulate if effective selection ($N_e s$) is greater than effective migration ($N_e m$). At a minimum, then, alleles with habitat-specific selection coefficients on the order of 100 to 200 times the reciprocal of effective population size of *S. balanoides* could potentially counteract the homogenizing effects of gene flow. Consider a conservative scenario: Alleles whose effective selection is one order of magnitude greater than their effective migration ($N_e s = 1000$, $N_e m = 100$) could maintain clines between estuarine and coastal sites. The effective population size in barnacles has been estimated to be between 10^5 and 10^7 (A. D. Kern, P. S. Schmidt, and D. M. Rand, unpubl. data). Thus, alleles with selection coefficients of $s = 0.01\text{--}0.0001$ could potentially be locally adapted to distinct habitats in *S. balanoides*. Making a correction for nuclear versus mtDNA will bring these estimates up to 0.04 to 0.0004, which is trivial given the uncertainty about the true effective population size.

As an alternative estimate we can consider the dynamics of clinal variation. Genetic differentiation across an environmental gradient can be characterized by the slope of an allele frequency cline, which is governed by a balance of gene flow and selection. Cline width is proportional to σ/\sqrt{s} , where σ is the variance in the dispersal distance between parent and offspring and \sqrt{s} is the square root of the selection coefficient (Slatkin 1973). If barnacle larvae can disperse 100 km during the larval period (Flowerdew 1983) and we take 50 km as a conservative estimate of the average dispersal distance between parent and offspring, then selection of 4% ($s = 0.04$) would be sufficient to maintain a 10% difference in allele frequency over a 10-km gradient.

Recent studies of *S. balanoides* by Schmidt and Rand (1999) and Schmidt et al. (2000) and other marine invertebrates (McDonald 1991; Johannesson et al. 1995) indicate that selection coefficients this high may in fact be common for enzyme polymorphisms. However, for *Mpi* in barnacles the spatial scale of this selection may be quite small (meters: Holm and Bourget 1994; Schmidt and Rand 1999), making the notion of local adaptation inappropriate for this mosaic system. For other traits where parents and offspring are more likely to experience the same direction of selection each generation (thermal and osmotic stress in the upper estuaries, physical stress of wave action on exposed points of land), the notion of a cline between coast and estuary seems more appropriate. Genes important in thermal stress, osmoregulation, calcium carbonate deposition, and the adhesive barnacles secrete to anchor themselves to rocky substrate come to mind as likely candidate loci for this sort of local adaptation.

With extensive gene flow, however, the scale of local adaptation needs to be considered. We have presented data supporting weak population subdivision between Maine and Rhode Island. This is not surprising given oceanographic and population data suggesting that Cape Cod may serve as a barrier to dispersal (e.g., Perkins et al. 1997). Moreover, the DNA sequence data indicate that samples south of Cape Cod

are more variable than the sequences from Maine. This pattern of variation may be a consequence of a range expansion out of a refugial population of *S. balanoides* that was pushed south of what is now Cape Cod during the last glacial maximum, well after colonization of the Atlantic across the arctic (Vermeij 1991; Dunton 1992). It may be that local adaptations have accumulated at a larger biogeographic scale in *S. balanoides*. Transplant experiments at this scale, coupled with analyses of neutral markers, may prove informative in addressing the important problem of adaptation in marine species with wide larval dispersal (Crisp 1964; Flowerdew and Crisp 1975; Hedgecock 1986; Bertness and Gaines 1993; Hellberg 1996).

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