

Diversification Before the Most Recent Glaciation in *Balanus glandula*

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Abstract. A deep genetic cline between southern populations of the barnacle *Balanus glandula* (from about Monterey Bay southward) and northern populations (from northern California through Alaska) has recently been described. If this pattern is due to historical isolation and genetic drift, we expect it to have formed recently and represent a transient, nonequilibrium state. However, this cline appears to have formed well before the last glacial maximum. Our assays of sequence diversity at a region of mitochondrial cytochrome oxidase I, combined with coalescent estimators of the time of separation for these two regions, suggest that a late Pleistocene event more than 100 thousand years ago may be responsible for the initial separation. This suggests that either strong oceanographic mechanisms or natural selection have maintained the cline, because there has clearly been adequate time for this cline or polymorphism to resolve itself by genetic drift and migration. However, reliance on only a single mitochondrial marker for which the substitution rate has been estimated still limits the resolution of our analysis.

Introduction

Patterns of genetic variation in some coastal species indicate the effects of changing paleoclimate on distribution and abundance (*e.g.*, Edmands, 2001; Dawson, 2001; Hickerson and Ross, 2001; Marko, 2004). These effects may lead to significant levels of population structure, where some regions harbor significantly different frequencies of particular alleles or molecular markers (Grosberg and Cunning-

ham, 2001). Predictions range from extreme divergence of regions, indicated by reciprocal monophyly on a gene tree of regional samples (see Avise, 2000), to gradients in overall genetic diversity. Patterns suggesting divergence between populations currently distributed to the north and south of the glacial margin at the last glacial maximum (LGM)—about 18 thousand years ago (kya)—are often explained as representing populations that survived Pleistocene glaciation in “unglaciated refugia” (Holder *et al.*, 1999; Hickerson and Ross, 2001; Wares, 2002). A finding of lower genetic diversity in populations north of the LGM glacial margin is typically interpreted as post-glacial expansion from a southern refugial distribution (see Marko, 2004). In marine organisms, the location and persistence of paleoceanographic phenomena must also be considered, such as the location of extant currents and their persistence through Pleistocene climate change (Herbert *et al.*, 2001; Wares, 2002).

Sotka *et al.* (2004) characterized the genealogical relationship among individuals of *Balanus glandula* from coastal British Columbia to southern California, using DNA sequence data from two loci (mitochondrial cytochrome oxidase I [mtCOI] and nuclear elongation factor 1 α). The analysis of these data indicates a clinal pattern of allelic class frequencies, suggesting that there are surprisingly low levels of realized migration (gene flow) along the California coast from Point Arena to Monterey Bay. The divergence between the northern and southern lineages does not represent a classical “phylogeographic break” in which the two groups are genealogically distinct (class I phylogeographic status; Avise 2000). Instead, the pattern is consistent with secondary contact between historically isolated lineages, which has proceeded slowly because of selection or physical oceanographic forces. One way to distinguish among the mechanisms that may be maintaining this pattern is to estimate how long ago the cline formed. If the cline is quite

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Abbreviations: kya, thousand years ago; LGM, last glacial maximum; N_e , effective population size; mtCOI, mitochondrial cytochrome oxidase I.

ancient, it becomes less likely that neutral processes could have maintained it.

The effective population size (N_e ; Hudson, 1990) is a parameter that describes the idealized average number of individuals contributing genetic diversity each generation. When estimated from genealogical data using methods based on coalescent theory, this parameter represents the long-term size of the inbreeding population and thus describes the demography of a population over time scales of 10^3 – 10^5 years (Turner *et al.*, 2002). The time of isolation between populations can also be measured in terms of N_e . Here we apply these methods to mitochondrial data taken from *B. glandula* populations between southern Alaska and southern California (see Table 1). Although other molecular data sets are available (*e.g.*, Sotka *et al.*, 2004), estimates of the substitution rate μ have only been calculated for this portion of the mtCOI locus in balanoid barnacles (see Materials and Methods). Using these data, we estimate regional N_e and the range of divergence times between the southern and northern allelic classes of *B. glandula* to determine whether this divergence was associated with the LGM as is commonly predicted, or if it significantly predates the LGM (see Marko, 2004).

Because it is difficult to distinguish between weak selection and physical oceanographic forces in maintaining this genetic cline (Sotka *et al.*, 2004), these demographic parameters may reflect the predictions made by physical oceanographers regarding changes along the continental shelf during the Pleistocene (Gaines and Roughgarden, 1985; Roughgarden *et al.*, 1988; Wing *et al.*, 1995; Lyle *et al.*, 2001) that would create barriers to gene flow, and areas of larval retention. The formation and maintenance of this cline essentially requires such selection or oceanographic structuring, without which it should collapse quickly (Irwin, 2002; Sotka *et al.*, 2004). By narrowing the range of historical demographic scenarios associated with this cline, we can better infer the conditions that are maintaining it.

Materials and Methods

Balanus glandula (Darwin, 1854) is distributed in dense aggregates on rocky shores from northern Alaska to just south of the border between the United States and Mexico (Barnes and Barnes, 1956; Newman and Abbott, 1980) and is readily distinguished from other cirriped species by exoskeletal characters of the operculum, parieties, and basis (Newman and Abbott, 1980). Individuals ($n = 370$) were haphazardly collected in the field (see Table 1 for collection locations) and placed in 95% ethanol prior to DNA isolation. All molecular methods are as in Wares *et al.* (2001); a 710-bp fragment of the mitochondrial cytochrome oxidase I gene (mtCOI) was amplified using the universal primers of Folmer *et al.* (1994), with sequence data deposited in GenBank (Benson *et al.*, 2004), as AF234351-234462 and

AY795085-795281, and the aligned data set available from EMBL (Kulikova *et al.*, 2004), as ALIGN-000504. This mitochondrial data set is entirely independent of the one presented in Sotka *et al.* (2004), using different individuals, mostly collected 2–3 years earlier, and a different fragment of the mtCOI gene; these data include almost three times as many individuals as in Wares *et al.* (2001) and eight newly sampled populations (Juneau, Alaska; Bamfield, British Columbia; Fogarty Creek and Strawberry Hill, Oregon; and Point St. George, Cape Mendocino, Point Arena, and Bodega Bay, California). Phylogenetic analysis of these data was performed using PAUP*4.0b10 (Swofford, 2002). We followed the methods of Wares *et al.* (2001) to obtain the full set of parsimony trees, and we used Arlequin 2.001 (Schneider *et al.*, 1997) to analyze sequence data for geographic associations.

We estimated effective population size (N_e) within populations and pairwise migration among populations, as well as divergence time, using four related but distinct methods. (1) To treat the continuous coalescent description of population relationships, we used the method of Wilkins and Wakeley (2002) to estimate N_e and neighborhood size (N_b ; roughly the per-generation effective gene flow distance) for populations south of the cline (see Fig. 1), within the clinal region, and north of the clinal region. (2) Coalescent estimation of N_e and pairwise migration M was calculated using MIGRATE 1.7.6 (Beerli and Felsenstein, 1999). Replicate analyses were performed using the methodology of Turner *et al.* (2002). (3) Estimates of diversity in terms of $\theta(S)$ and $\theta(\pi)$ were calculated using Arlequin 2.001 (Schneider *et al.*, 1997). These estimates, combined with the estimated substitution rate at mtCOI for balanomorph barnacles (Wares, 2001; Wares and Cunningham, 2001), were used to calculate frequency-based estimates of allelic age (Slatkin and Rannala, 1997). (4) Recent improvements in Markov chain Monte Carlo (MCMC) coestimation of ancestral parameters allow the coordinated estimation of migration parameters between regions as well as the divergence time between those regions. The program IM (Hey and Nielsen, 2004) estimated these parameters, again grouping the “northern” and “southern” regions, both across Monterey Bay and across Cape Blanco, Oregon. This latter comparison is primarily used as a control estimate of divergence; because analyses of divergence time are rarely verified against populations with known divergence using an independent estimate of μ , we estimate this parameter for Cape Blanco because no phylogeographic transition is present in *B. glandula* at this location (Sotka *et al.*, 2004; see Results). Thus, only a minimal divergence is expected relative to the divergence across the observed cline.

Because of computational difficulties with searching and resolving large genealogies for ancestral population parameters, two groups of MCMC analyses were performed using IM: the full data set was separated at Monterey Bay for

analysis with 10^6 chains after discarding the first 10^5 chains. Then, as in Turner *et al.* (2002), the data partitions were randomly sampled for 20 sequences from each regional group to allow the analysis to stabilize parameter results more efficiently. A program, SEQSAMPLER.RB (written in Ruby and available from JPW), generated 40 pseudoreplicate data sets that were each analyzed twice using distinct starting seeds and the same initial parameters as for the whole data set.

Results

Sequences from the southernmost populations of *Balanus glandula* (south of Monterey Bay) are statistically distinct from those to the north. Although phylogenetic analysis shows this distinction to be robust (Wares *et al.*, 2001), the signal can be summarized by looking at only two nucleotide positions (273 and 360 in the aligned data set of 632 bp, of which 61 positions are parsimony informative and 50 are variable but parsimony uninformative; Fig. 1). The southernmost populations are fixed for an adenine residue at these nucleotide positions; the branches supporting the two clades that are limited to northern populations are themselves supported in 100% of maximum parsimony trees (consensus). This trend is significant when measured by Φ_{st} (Wares *et al.*, 2001). No populations from Cape Mendocino northward or Pacific Grove southward are significantly differentiated from each other within regions, but comparisons between regions are highly significant in analysis of molecular variance. Figure 2 shows that this pattern is driven by the secondary contact of genetically distinct populations, as the apparent isolation by distance pattern (Fig. 2a, Mantel test of pairwise F_{st} matrix against pairwise geographic distance, $P < 0.01$) breaks down when clades are analyzed separately, even though overall geographic distribution is comparable (Fig. 2b). Equilibrium isolation by distance (*sensu* Wright, 1943) represents limited dispersal and gene flow, but nonequilibrium dynamics (such as vicariance) can artificially create the same genetic patterns (Peterson and Denno 1998).

Analysis of divergence time between the lineages that dominate either northern or southern populations of *B. glandula* indicates that the initial formation of the phylogeographic discontinuity is more ancient than the last glacial maximum (LGM) by at least an order of magnitude. Here we assume that the substitution rate for cytochrome oxidase I in *B. glandula* is similar to that measured for other barnacles ($\mu \approx 1.55 \times 10^{-8}$ in *Chthamalus*, Wares, 2001; $\mu \approx 2.18 \pm 1.1 \times 10^{-8}$ in *Semibalanus*, Wares and Cunningham, 2001). The method of Wilkins and Wakeley (2002), using the mean of these two estimates, indicates that the maximum likelihood estimation of the effective population size (N_e) in populations to the north of the cline is approximately 251,200 and slightly lower in the populations to the

south of the cline (199,500) and in the clinal region itself (208,900). In a purely panmictic population, the time to the most recent common ancestor is expected to be $2N_e$ (Hudson, 1990); thus the combined set of allelic classes in each region indicates that current diversity has arisen in the past 500 thousand years or so. This same method suggests that the neighborhood size is indistinguishable from infinity for the regions to the north and south of the cline, while populations within the cline have a neighborhood size of less than 1000 m, which corresponds to the results of Sotka *et al.* (2004).

Estimates of $\theta = 2N_e\mu$ for each population sampled ranged from 1.80 to 4.66 in our MIGRATE analyses. However, for some population samples we were unable to get a stable estimate from this method. Estimates of θ were somewhat correlated (0.45) with estimates of θ made using Arlequin, and log plots of this parameter show the same basic trend; because of difficulties with obtaining stable estimates using MIGRATE, however, we report only the results from Arlequin. In Table 1, N_e at each site is estimated using $\theta(\pi)$ and the above substitution rates. Frequency-based methods (Slatkin and Rannala, 1997) of estimating the age of alleles indicate that the “northern” mitochondrial haplotype groups are likely to have arisen well before the LGM, as well. The average $\theta(\pi)$ within each allelic class and the frequency of each class is used in these estimates: southern group $\theta(\pi) = 3.192$ (119–253 kya); northern groups $\theta(\pi) = 2.489$ and 1.600 (93–197 kya and 59–127 kya, respectively).

The mean time of divergence from our two full-data analyses using IM is $t = 4.66$ (4.64, 4.68 were the maximum likelihood estimates from each replicate), where $t = \mu t$ (t is time in years; Hey and Nielsen, 2004). This mean generates a divergence time of 338,000 ($\pm 113,000$) years using a per-gene substitution rate of 1.4×10^{-5} ($\pm 6.9 \times 10^{-6}$; Wares and Cunningham, 2001). Migration across Monterey Bay was limited (estimated as $m_1 = 0.91$ from north to south, and $m_2 = 0.99$ from south to north), consistent with the finding of limited gene flow in Sotka *et al.* (2004) and the restriction indicated by analysis with the Wilkins and Wakeley (2002) method. The same values estimated from the 80 pseudoreplicate data sets were $t = 1.34 \pm 1.47$, and much lower estimates of m_1 and m_2 (0.44, 0.39). From the 82 IM analyses, the minimum divergence time between the northern and southern regions across Monterey Bay is $t = 0.595$ (43.2 ± 14.5 kya). Estimated effective population size in both regions is much higher in the IM analysis (8.7×10^6 in populations north of Monterey Bay, 7.0×10^6 in populations to the south). The pseudoreplicate analyses may be conflated by significantly higher estimates of θ because any small subsample of the complete, highly diverse data set involves long branch lengths between

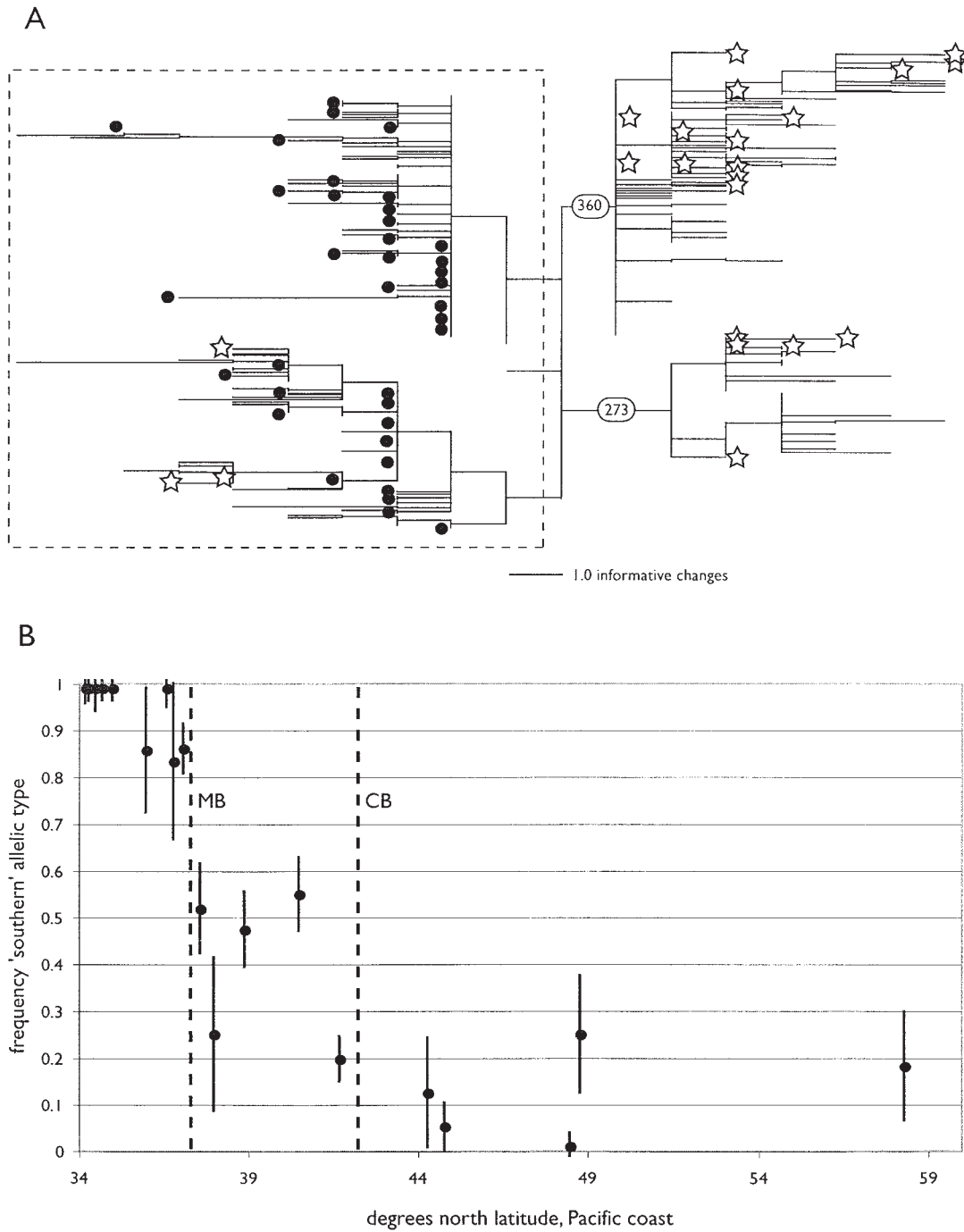


Figure 1. A. maximum-parsimony phylogram representing phylogeographic diversity of parsimony-informative sites in *Balanus glandula* ($n = 363$). For reference, dark circles represent individuals collected from south of Point Conception (34.5°N) and open stars represent individuals collected from the 3 northernmost collection sites. In general, the “northern” clades harbor only a single individual collected from south of Monterey Bay (Kirk Creek). As indicated by the plot in B, the frequency of the “southern” allelic type (see text) decreases rapidly between Monterey Bay (MB; California) and Cape Blanco (CB; Oregon). Here the southern type is defined as those individuals not found in the clades formed by substitutions at sites 273 and 360 from the reported alignment of mitochondrial cytochrome oxidase I. Binomial sampling error of the frequency of the southern type from each population is indicated.

Discussion

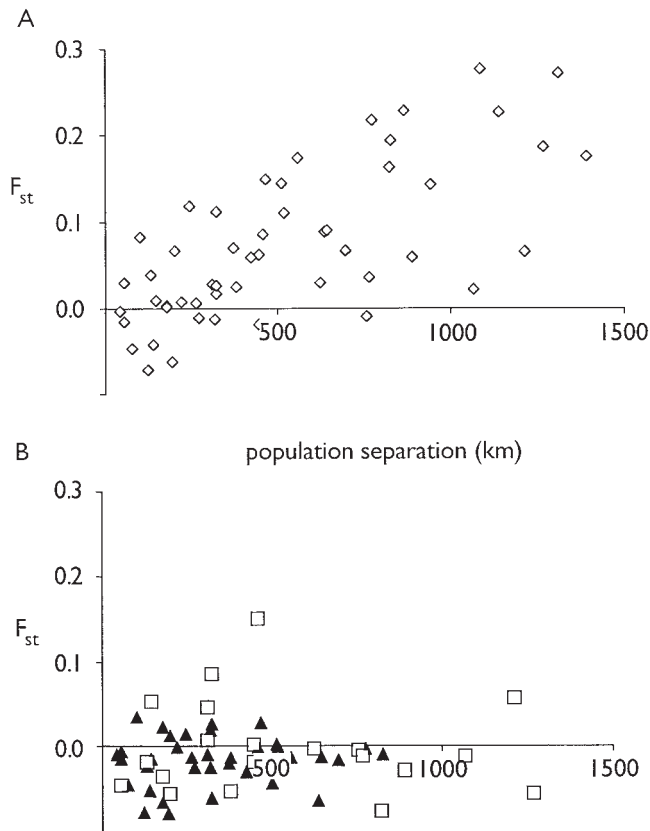


Figure 2. Isolation by distance plots for haplotype data in *Balanus glandula*. (A) All data combined; vertical axis is pairwise F_{st} between populations and horizontal is pairwise distance in kilometers between populations. A Mantel test (1000 matrix permutations) indicated that the relationship between F_{st} and distance is significant ($P < 0.01$). (B) Sequence data from the clades that predominate in northern populations (substitutions 360 and 273 on Figure 1; black) and the clade that predominates in southern populations (white) analyzed separately. Neither relationship is statistically significant.

individuals, perhaps suggesting much more unsampled diversity than exists when the entire data set is available.

Replicate comparisons of populations across Cape Blanco (excluding populations south of the clinal region), despite the lack of phylogeographic signal based on other types of analyses (Wares *et al.*, 2001; Sotka *et al.*, 2004), are not different from the Monterey Bay comparisons. The mean divergence time of pseudoreplicate data sets using IM is in fact higher ($t = 1.67 \pm 1.64$ kya), although the average estimates of migration across Cape Blanco are also much higher ($m_1 = 3.56$ and $m_2 = 4.16$). As with the pseudoreplicate analyses across Monterey Bay, estimates of θ were highly inflated by the subsampling process by nearly an order of magnitude over estimates based on $\theta(\pi)$. The average across- Cape Blanco estimate using the entire data set (excluding populations south of Monterey Bay) is $t = 4.479$ ($m_1 = 3.58$ and $m_2 = 2.72$).

Estimating the age of an evolutionary event by using molecular data is often a circular task. Without adequate fossil or biogeographic evidence, inferences based on genetic diversity must rely on consistency among different analytical methods. Here, calculations of population age and time of separation between populations of *Balanus glandula* on either side of a significant genetic cline suggest that the separate allelic groups likely formed well before the last glacial maximum (LGM). However, these estimates have broad confidence intervals, indicating that in some cases we cannot reject a more recent event. Few molecular markers are available for which the substitution rate μ is known with confidence, and without better cases for which this parameter has been estimated independently of the biogeographic or paleontological hypothesis being tested, it will be difficult to improve such estimates. In this study, we did not obtain significantly different estimates of divergence when comparing regions on either side of the strong genetic cline than when we compared populations on either side of a separate geographic landmark for which there is no evidence of genetic differentiation. Thus, these methods and estimates must be applied with caution.

When there is complete random mating across the samples, the expected time to the most recent common ancestor is $\sim 2N_e$ generations (Hudson, 1990), but there is high variance around this prediction (Hudson and Turelli, 2003) and an inference from a single gene may be misleading. Our estimates of $N_e = \theta/2\mu$ are on the order of 10^5 for each sampled location, similar to estimates of N_e (by the method of Wilkins and Wakeley, 2004) when comparing regional groups (north and south) separated by the multilocus cline between Monterey Bay and Pt. Arena. Frequency-based methods (Slatkin and Rannala, 1997) also suggest that these groups diverged during or prior to marine isotope stage 5 (74–131 kya; Lyle *et al.*, 2001). Under panmixia, we expect similar estimates of diversity at multiple spatial scales (Hudson, 1990; Beerli, 2004). However, methods designed to estimate divergence time (t) and gene flow simultaneously (Hey and Nielsen, 2004) suggest much higher N_e in each region (on the order of 10^6 – 10^7) and a much older divergence. Methods that do not simultaneously account for ongoing migration following the divergence of populations frequently estimate lower separation times (Wares *et al.*, 2002). However, estimates of t are similar for populations across Cape Blanco and Monterey Bay in this study, despite strong differences in estimated migration regimes across these two geographic features. These estimates also vary widely depending on the sample size from each region. If within-region coalescence is not significantly different from among-region coalescence, it should indicate high gene flow and the homogenization of population structure.

The tendency to attribute marine phylogeographic pat-

Table 1

Summary of collection data and estimates of effective population size (N_e) for populations of *Balanus glandulus*

Population	Collection date*	Location	Sample size	N_e †	SE‡
Juneau, AK	Summer 2003	58.3°N 134.5°W	11	131,885	164,715
Bamfield, BC (Canada)	Summer 2002	48.8°N 125.1°W	10	150,360	186,675
Friday Harbor, WA	Summer 1996	48.5°N 123.0°W	12	140,215	172,035
Fogarty Creek, OR	Summer 2002	44.8°N 124.0°W	19	140,940	166,910
Strawberry Hill, OR	Summer 2002	44.3°N 124.1°W	8	134,420	174,965
Point St. George, CA	Summer 1997	41.7°N 124.2°W	76	160,870	179,335
Cape Mendocino, CA	Summer 1997	40.5°N 124.4°W	40	162,320	182,285
Pt. Arena, CA	Summer 1997	38.9°N 123.7°W	47	142,030	163,250
Bodega Bay, CA	Summer 1997	38.3°N 123.0°W	8	144,925	185,944
Pacifica, CA	Spring 1997	37.6°N 122.5°W	17	168,840	196,925
Greyhound Rock, CA	Spring 1997	37.1°N 122.3°W	39	110,505	133,965
Moss Landing, CA	Spring 1997	36.8°N 121.8°W	6	91,665	133,235
Lovers Point, CA	Spring 1997	36.6°N 121.9°W	6	65,215	101,024
Kirk Creek, CA	Spring 1997	36.0°N 121.5°W	7	93,115	131,040
Tenera Point, CA	Summer 1995	35.0°N 121.5°W	14	110,115	138,360
Vandenberg AFB, CA	Summer 1995	34.7°N 121.5°W	14	100,000	128,110
Jalama, CA	Summer 1995	34.5°N 120.5°W	4	90,580	147,145
Arroyo Hondo, CA	Summer 1995	34.3°N 121.0°W	13	138,405	169,105
Mussels Shoals, CA	Summer 1995	34.2°N 120.5°W	10	129,345	163,980

* Collections were made between 1995 and 2003.

† Effective population size estimated by $\theta(\pi)/2\mu$ at each sampled location.‡ Standard error was calculated using Arlequin (Schneider *et al.*, 1997), incorporating error estimates for μ from Wares and Cunningham (2001).

terns to the LGM is probably associated with the overall lack of information on earlier glacial events and the impact each stage had on nearshore geography and oceanographic patterns (Valentine and Jablonski, 1993; Lyle *et al.*, 2001). Nevertheless, it is clear that divergence among populations is often far more ancient than the LGM (Edmands, 2001; Dawson *et al.*, 2001; Hickerson and Ross, 2001; Marko, 2004). The time required for significant divergence between isolated populations is generally on a much longer scale than 20–40 thousand years (Palumbi and Kessing, 1991; Wares, 2002). In *B. glandula*, the presence of similar levels of diversity [$\theta(\pi)$] throughout the studied range (Table 1) suggests long-term persistence in habitats north of glacial margins (*e.g.*, southern Alaska), indicating that unlike other coastal species (Hickerson and Ross, 2001; Hellberg *et al.*, 2001; Dawson, 2001; Jacobs *et al.*, 2004; Marko, 2004), *B. glandula* has not expanded northward from a southern refugial population.

It is important to recognize the difference between primary divergence and secondary contact in this and other systems (Endler, 1982). The statistical signal for isolation by distance in *B. glandula* is driven solely by the divergence of the northern and southern allelic types, because removal of those diagnostic nucleotide sites—or analysis within allelic “type” alone—eliminates any signal of geographic differentiation (Fig. 2). No obvious or statistically significant differences exist between settlement classes at this mitochondrial locus or at several allozyme loci across large spatial scales, different intertidal heights, or among settle-

ment sites (Hedgecock, 1986; Wares, unpubl. data). These patterns are robust across other data types as well—although only a couple of microsatellite loci have been isolated for *B. glandula*, gene flow also appears to be quite high between populations throughout the northern range of this species, including across Cape Blanco (R. J. Toonen and J. P. Wares, unpubl. data).

The potential for the cline between northern and southern allelic types to be maintained by selection, rather than by the introgression of historically differentiated populations of this barnacle, is not yet known (Sotka *et al.*, 2004), which complicates any demographic interpretations of these data on the basis of neutral expectations. It is now recognized that although mitochondrial sequence data have their merits for phylogeographic analyses (Avice, 2000), they may also reflect non-neutral evolutionary patterns (Ballard and Kreitman, 1995; Schizas *et al.*, 2001). The current position of the cline may not reflect the original geographic disjunction, because ancient clines tend to be “attracted” to contemporary discontinuities in gene flow (Endler, 1977; Hare and Avice, 1996; Irwin, 2002). The nearshore oceanography appears to have changed dramatically during late Pleistocene glacial and interglacial periods (Hendy and Kennett, 2000), and the strength and geographic position of the cline could have been similarly affected.

Clinal genetic variation has been identified in only a few marine metapopulations (Gardner, 1996). Although some hybrid zones are known for intertidal and abyssal species (Hilbish, 1996; Won *et al.*, 2003), few represent interactions

among populations of the same species (*e.g.*, Hilbish, 1996)—a set of interactions that may represent either secondary introgression of isolated lineages or primary divergence due to environmental adaptive pressures (Endler, 1982; Nielsen *et al.*, 2003). Sotka *et al.* (2004) argue conservatively that if the northern and southern populations of *B. glandula* separated at the LGM, the rate of introgression, or “rebound,” from this disruption is extremely slow considering the assumed neutral rate of gene flow for a species with broadly dispersing planktonic larvae. Here we show that this cline has been maintained for far longer, either by abiotic factors that limit the dispersal of *Balanus* across the coast of central California, or by natural selection that limits the spread of the historically differentiated lineages, or both.

Physical oceanographic patterns, including currents and gyres, probably play the greatest role in driving larval dispersal in *B. glandula* (Connolly and Roughgarden, 1999; Connolly *et al.*, 2001). If these patterns alone maintain the distinction of northern and southern genetic races of *B. glandula*, the data presented here suggest that these oceanographic mechanisms would have to be stable since well before the LGM. We still know little about coastal geology and physical oceanographic processes prior to the LGM (Lyle *et al.*, 2001; Herbert *et al.*, 2001). Thus, we must be cautious about the precision of our estimates of N_e (in Table 1, error exceeds the estimates) or the age of the separation between these populations based on estimates of μ obtained from other taxa and without the confirmation of fossil data (Marko, 2002, 2004; Clarke and Knight, 2003; Graur and Martin, 2004). Here we simply suggest that the divergence of *B. glandula* populations predates the LGM, and the maintenance of this cline—perhaps by selection, since migration and advection are expected to homogenize these populations—must be considered on this longer time scale.

Since many physical oceanographic structures are believed to be unstable during glacial maxima (Herbert *et al.*, 2001; Graham *et al.*, 2003), the transitions between glacial and interglacial periods are likely to have interrupted or moved any such “selectively neutral” barrier to gene flow. Temperature regimes along the coast also change during climatic cycling, and species are known to extend their range northward during El Niño events (Newman and McConnaughey, 1987; Engle and Richards, 2001; Connolly and Roughgarden, 1999; but see Paine 1986). However, the actual latitudinal distribution and fitness of a species may depend on a number of abiotic variables, including coastal exposure, substrate type, food availability, and other spatial components (Sanford, 2001; Helmuth *et al.*, 2002; Harley and Helmuth, 2003). It is not clear how old *B. glandula* actually is, with little fossil data available (Pleistocene, Oregon coast; Zullo, 1969, 1984), but it has persisted through many climatic changes. Except for species in the *B. amphitrite* species complex (Puspasari *et al.*, 2001), a detailed phylogeny is not available for *Balanus*. The peculiar

nature of this cline, in which allele frequencies differ significantly but most alleles are still present on both sides of the region, adds to the complexity of this pattern. The long-term maintenance of diversity in both regions is important for our understanding of the interaction between abiotic environment and genetic diversity (*e.g.*, Ma *et al.*, 2000) and the extent to which historical demographics contribute to contemporary character evolution (*e.g.*, Marchinko and Palmer, 2003; Marchinko *et al.*, 2004). Further genetic and ecological analysis of *B. glandula* will be necessary to fully explain this intraspecific divergence.

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