

A COMPARATIVE STUDY OF ASYMMETRIC MIGRATION EVENTS ACROSS A MARINE BIOGEOGRAPHIC BOUNDARY

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Abstract.—In many nonclonal, benthic marine species, geographic distribution is mediated by the dispersal of their larvae. The dispersal and recruitment of marine larvae may be limited by temperature gradients that can affect mortality or by ocean currents that can directly affect the movements of pelagic larvae. We focus on Point Conception, a well-known biogeographic boundary between the Californian and Oregonian biogeographic provinces, to investigate whether ocean currents affect patterns of gene flow in intertidal marine invertebrates. The predominance of pelagically dispersing species with northern range limits at Point Conception suggests that ocean currents can affect species distributions by erecting barriers to the dispersal of planktonic larvae. In this paper, we investigate whether the predominantly southward currents have left a recognizable genetic signature in species with pelagically dispersing larvae whose ranges span Point Conception. We use patterns of genetic diversity and a new method for inferring cladistic migration events to test the hypothesis that southward currents increase southward gene flow for species with pelagically dispersing larvae. We collected mitochondrial DNA (mtDNA) sequence data for the barnacles *Balanus glandula* and *Chthamalus fissus* and also reanalyzed a previously published mtDNA dataset (*Strongylocentrotus purpuratus*, Edmands et al. 1996). For all three species, our cladistic approach identified an excess of southward migration events across Point Conception. In data from a fourth species with nondispersing larvae (*Nucella emarginata*, Marko 1998), our method suggests that ocean currents have not played a role in generating genetic structure.

Key words.—Asymmetry, *Balanus*, *Chthamalus*, cytochrome oxidase I, dispersal, gene flow, *Nucella*, oceanography, *Strongylocentrotus*.

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The forces that limit a species' geographic range are of central importance to studies of ecology and evolution. In many nonclonal, benthic marine species, geographic distribution is mediated in part by the dispersal of their larvae. Species range limits in such organisms are therefore found where larval recruits cannot sustain a population. The dispersal and recruitment of marine larvae may be limited by temperature gradients that can affect mortality or by ocean currents that can directly affect the movements of pelagic larvae (Hedgecock 1986; Scheltema 1986; Cowen et al. 1993; Hare and Cowen 1996). Distinguishing between the effects of temperature gradients and currents is difficult because they often co-occur, especially where ocean currents converge at coastal headlands to produce extreme temperature gradients and potential oceanographic dispersal barriers (Gaylord and Gaines 2000).

One way to distinguish between these factors is to compare species with pelagically dispersing (planktonic) and poorly dispersing (brooded or crawlaway) larvae (S. D. Gaines, G. Eckert, C. Blanchette, B. Gaylord, and S. Navarette, unpubl. ms. a; S. D. Gaines, C. Blanchette, S. Navarette, S. Worcester, and B. Gaylord, unpubl. ms. b). Although temperature gradients should equally affect the mortality of both larval types, pelagically dispersing larvae should be most affected by the action of ocean currents. Before we describe the use of DNA sequence data to investigate gene flow between populations within a species—the main focus of this paper—we will discuss whether the distribution of species range limits supports the hypothesis that ocean currents limit the ranges of taxa with pelagically dispersing larvae.

Point Conception (34.5°N, along the coast of California) is a headland where many species range limits are found (Valentine 1973; Briggs 1974; Newman 1979; Doyle 1985). The strong southward flow of the California Current into the Southern California Eddy is responsible for a 5°C temperature gradient (Fig. 1). Although species with pelagically dispersing larvae show a significant clustering of range limits at Point Conception, there is no significant clustering of range limits in species with poorly dispersing larvae (Fig. 2). By itself, this pattern suggests that ocean currents may play an important role in determining range limits at Point Conception. Moreover, consistent with the southward direction of the currents, there are significantly more northern range limits at Point Conception than southern range limits (Roy et al. 1995; Fig. 2). The predominance of pelagically dispersing species with northern range limits at Point Conception suggests that ocean currents may affect species distributions by erecting barriers to the dispersal of planktonic larvae (Gaines et al., unpubl. ms. a,b).

If ocean currents significantly influence species range limits, they may likewise have an effect on gene flow within species. In this paper, we use mitochondrial DNA sequence data to investigate whether the predominantly southward currents have left a recognizable genetic signature in species with pelagically dispersing larvae whose ranges span Point Conception. Asymmetric migration is often described in terms of classical population genetics, with the expectation that the homogenizing force of migration drives the average frequency of an allele toward that of the source population (Bodmer and Cavalli-Sforza 1968; Wright 1969; Felsenstein

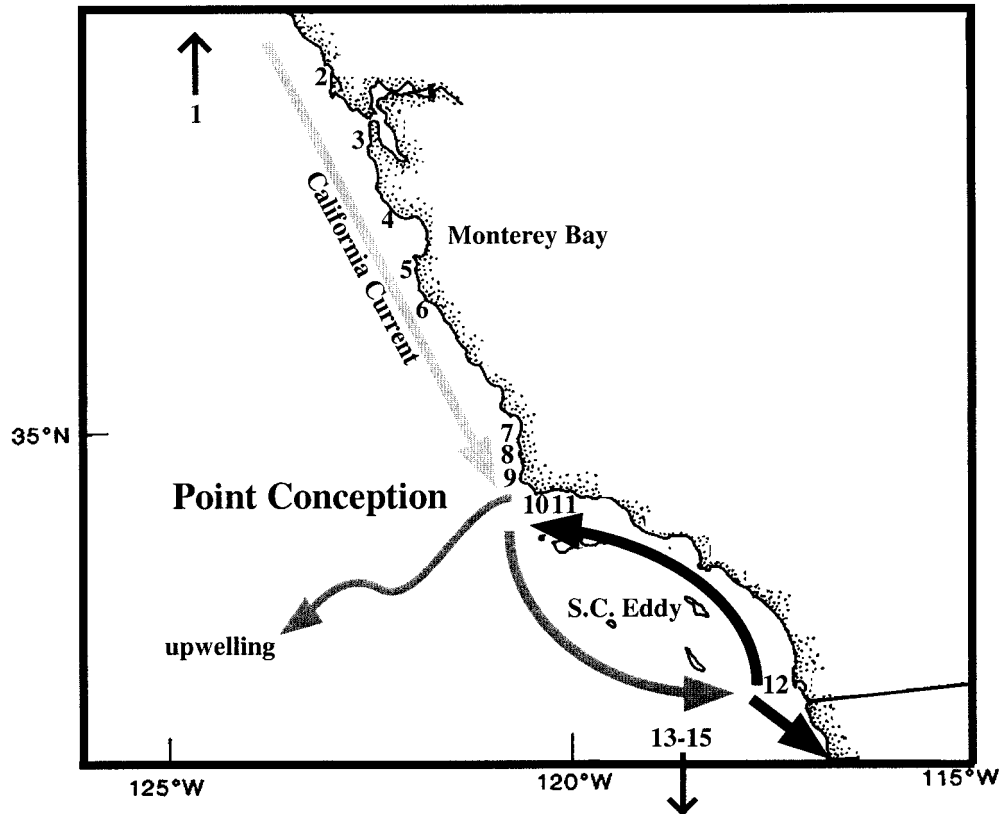


FIG. 1. At Point Conception and populations to the north, intense upwelling transports nearshore water and suspended plankton hundreds of kilometers offshore (Pavlova 1966; Bernstein et al. 1977; Seapy and Littler 1980). The transport of larvae by upwelling is believed to strongly reduce recruitment to nearshore populations (Gaines and Roughgarden 1985; Roughgarden et al. 1988; Ebert et al. 1994). The winds that drive upwelling drive the California Current southward, past Point Conception. South of Point Conception the circulation pattern is markedly different. The current is bent landward by an underwater peninsula (Pavlova 1966; Seapy and Littler 1980). The poleward arm of the current feeds a large cyclonic gyre (Southern California Eddy) during the spring and summer. This flow moves slowly in the shallow waters of southern California and becomes much warmer than the current north of Point Conception (5°C; Owen 1980; Seapy and Littler 1980). At Point Conception, water from the Southern California Eddy is retained in the Southern California Bight (Pavlova 1966; Owen 1980). Thus, Point Conception represents an extreme temperature gradient as well as a headland between very different circulation patterns, which may limit northward dispersal (Haury et al. 1986). Populations sampled: (1) Friday Harbor (WA, not on map); (2) Bodega Bay; (3) Pacifica; (4) Greyhound Rock; (5) Lovers Point; (6) Kirk's Creek; (7) Tenera Point; (8) Vandenberg AFB; (9) Jalama; (10) Arroyo Hondo; (11) Mussels Shoals; (12) La Jolla; (13) Punta Santo Tomas; (14) Punta Santa Rosalita; (15) Guerrero Negro (13–15 are off the map). Exact latitudes of populations given in Table 1.

1976; Hartl and Clark 1989). With DNA sequence data under an infinite-alleles model, this and more recent work focusing on the spatial correlation among related genes (Epperson 1993) suggests that the input from a source population will add to the genetic diversity in a stable recipient population (Palumbi 1995; Dias 1996).

In the case of Point Conception, a southward flow of genotypes should lead to a higher diversity south of Point Conception. Unfortunately, this prediction is complicated by the likelihood that many species along the California coast have extended their ranges to the north since the last glaciation (Hellberg 1994; Roy et al. 1995; Marko 1998). Because only a subset of haplotypes will take part in a northward range expansion, this process will lead to the same prediction as the southward flow hypothesis—higher genetic diversity in the south (Hewitt 1996).

Fortunately, the difference between northward range expansion and southward flow of haplotypes should be discernable with cladistic methods. For example, consider a

clade of haplotypes confined entirely to the area north of Point Conception. If a haplotype of this clade is swept to the south, this should appear as a southward transition on the cladogram (Fig. 3A). Although our method is based on the cladistic method of Slatkin and Maddison (1989), they made generalizable predictions about migration parameters (Nm) using an island model. We simply employ cladistic methods to test whether transitions from one population to another are higher than expected given sample sizes.

The advantage of cladistic analysis is that two likely processes make opposite predictions. As described above, haplotypes carried south by the California Current should result in a preponderance of southward transitions (Fig. 3A). Conversely, a northward range expansion should result in a preponderance of northward transitions on a cladogram (Fig. 3B).

We test these predictions using mitochondrial sequence data collected from two barnacle species with pelagically dispersing larvae whose ranges span Point Conception (*Bal-*

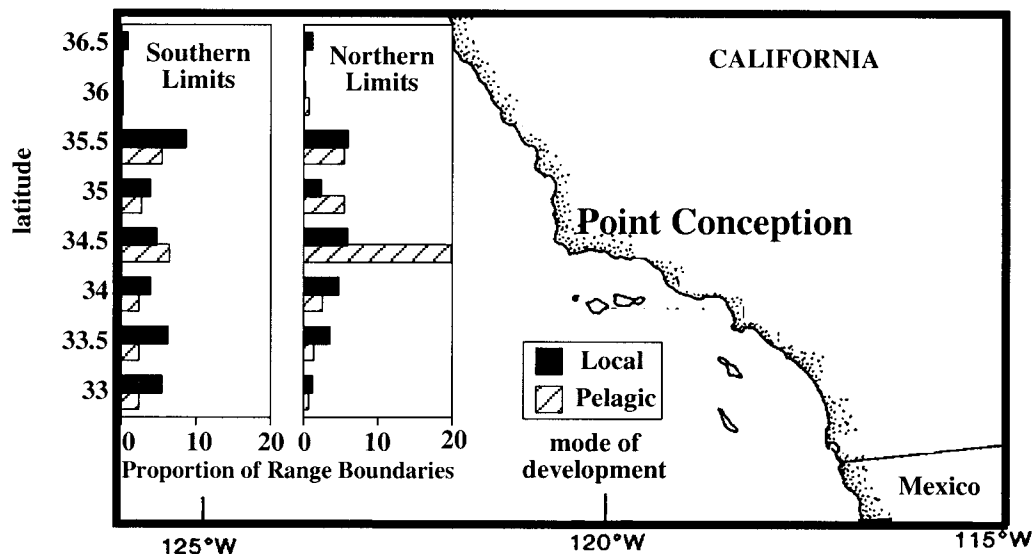


FIG. 2. The distribution of species range limits in southern and central California. Data are from a database of biogeographic distributions of more than 750 species of intertidal and subtidal invertebrates on the west coast of North America (G. Eckert, unpubl. data). Distribution data come largely from Morris et al. (1980), with supplemental information from the literature. Species included in this figure have relatively or completely sessile adults. They are divided into two life-history groups to isolate the effects of ocean currents on range patterns: one group has pelagic larval development, where larvae spend at least a few days in the plankton; the other group has local reproduction, where there is either no pelagic larval stage or larvae are pelagic for less than 1 day. The figure plots the proportions of both southern and northern range limits as a function of latitude for a section of the California coastline, the region spanning Point Conception. The proportion of range boundaries is the proportion of species from the entire dataset of West Coast species with southern/northern range limits in this region. Two contrasts are striking. First, for northern range boundaries, there is a marked difference in the distribution of boundaries for species with pelagic development versus local development. The difference is at Point Conception. The distribution of range boundaries can be compared with a uniform distribution using χ^2 tests. The peak of range boundaries at Point Conception is significantly larger for the pelagically developing species than for the locally developing species (log-likelihood ratio of distributional tests $\chi^2 = 9.98$, $df = 1$, $P = 0.0016$ for comparison of frequency of range boundaries at Point Conception vs. elsewhere for the two life-history groups). Point Conception is a biogeographic boundary for species with pelagic larval development only. Second, considering only the pelagically developing species, there is a clear difference in the distribution of northern versus southern range limits. Point Conception has a disproportionately large number of northern range limits relative to southern range limits (log-likelihood ratio $\chi^2 = 17.25$, $df = 1$, $P < 0.0001$ for comparison of the frequency of range boundaries at Point Conception vs. elsewhere for northern and southern ranges). Therefore, Point Conception is a one-way biogeographic boundary (see Roy et al. 1995).

anus glandula and *Chthamalus fissus*). We perform a thorough analysis of these taxa, along with analysis of sequence data from two published studies: a sea urchin with pelagically dispersing larvae (*Strongylocentrotus purpuratus*, Edmands et al. 1996) and a gastropod species with poorly dispersing larvae (*Nucella emarginata*, Marko 1998). The results for each pelagically dispersing species are consistent with predictions based on the predominant southward flow of the California Current.

MATERIALS AND METHODS

Collections

Specimens of *B. glandula* were collected between 1995 and 1997 from 11 intertidal sites between Friday Harbor, Washington (48.5°N) and Mussel Shoals, California (near their southern range limit, 34.2°N, Table 1). Similar collections were made of *C. fissus* the summer of 1995 from nine Pacific intertidal sites between Tenera Point, California (near its northern range limit, 35.0°N) and Guerrero Negro, Mexico (28.02°N, Table 1). Individuals were collected and placed into 95% ethanol for subsequent identification and molecular analysis.

DNA Extraction and Amplification

DNA was phenol-extracted from each specimen using the protocol outlined in Hillis et al. (1996) and stored at -80°C until analysis. We amplified a portion of the mitochondrial cytochrome *c* oxidase I (COI) gene using the primers LCO14905'–GGTCAACAAATCATAAAGATATGG–3' and HCO2198 5'–TAAACTTCAGGGTGACCAAAAAATCA–3' (Folmer et al. 1994). From initial sequences obtained, a new set of internal primers was designed: BARNIF1 5'–ATTA–TAATTGGGGGTTTGG–3' (upstream) and BARNIR2 5'–TCAAAAAGTTAAAGTTTCTGCTCG–3' (downstream). Amplification was performed using a combination of these primers in tandem, in 50- μl reactions containing 10–100 ng DNA, 0.02 mM each primer, 5 μl Promega (Madison, WI) 10 \times polymerase buffer, 0.8 mM dNTPs (Pharmacia Biotech, Piscataway, NJ), and 1 U *Taq* polymerase (Perkin-Elmer, Branchburg, NJ). We used a Perkin-Elmer 480 thermocycler with a cycling profile of 94°C (60 sec), 40°C (90 sec), 72°C (150 sec) for 40 cycles.

DNA Sequencing

Polymerase-chain-reaction (PCR) products from each individual were sequenced with both primers used in ampli-

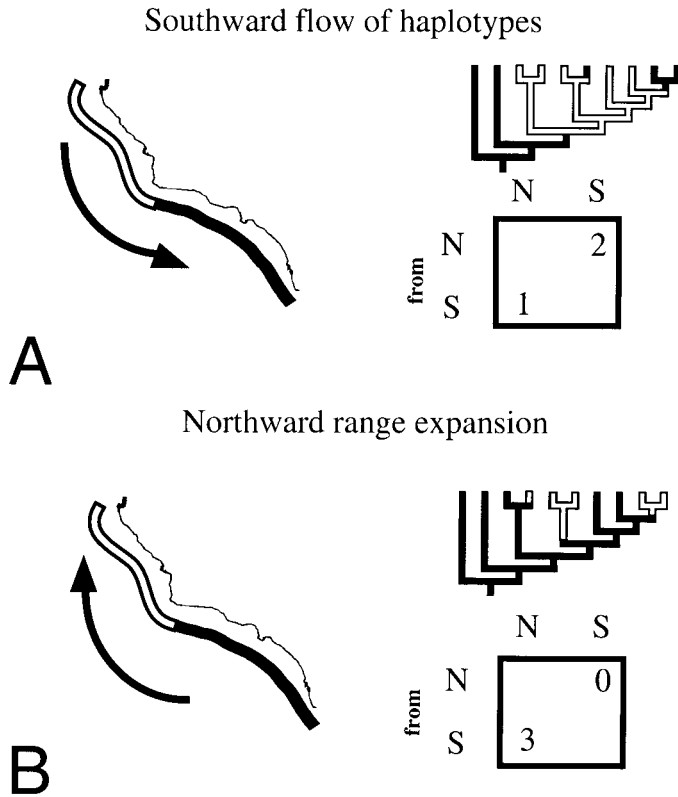


FIG. 3. Cladistic inference of migration events as used in this study. By sampling a large number of equally parsimonious trees, we are able to infer (using MacClade 4.0b13, see Materials and Methods) the average number of transitions from each population to another for a given dataset. This is illustrated for two scenarios: (A) northward range expansion followed by southward migration events mediated by southward ocean currents; in this example, there is an excess of southward migration events; (B) northward range expansion as illustrated by multiple northward transitions between locations on the cladogram; an equilibrium between migration and drift has not been achieved, and the persistent historical signal is indicated by an excess of northward transitions.

fication. We purified double-stranded PCR products using Promega Wizard PCR Preps resin and resuspended the product in 30 μ l ddH₂O. The template was cycle-sequenced using dRhodamine fluorescently labeled dideoxy terminators according to manufacturer's recommended conditions (Perkin-Elmer). Unincorporated dideoxynucleotides were removed using Sephadex G-25 (Sigma, St. Louis, MO) exclusion columns. The products were then electrophoresed on an ABI 373 automated DNA sequencer. All individuals were sequenced from both directions. We aligned the sequence data and checked ambiguities against complementary fragments using Sequencher 3.0 (Genecodes Corp.). Consensus sequences were exported as a Nexus file for subsequent analysis in PAUP* version 4.0 (Swofford 1998).

Datasets from previously published studies were used, including COI from *S. purpuratus* (from Edmands et al. 1996) and COI and 12S from *N. emarginata* (Marko 1998).

Tests of Geographic Subdivision

To test for significant population structure within groups of populations to the north or south of Point Conception, a

TABLE 1. Collection locations for the two barnacle species (*Balanus glandula* and *Chthamalus fissus*) used in this study. Number of individuals sampled is indicated in parentheses. Some individuals were excluded from analysis due to population subdivision (see Results).

Collection site	Latitude	Species collected
Friday Harbor (WA)	48°30'	<i>B. glandula</i> (15)
Bodega Bay (CA)	38°20'	<i>B. glandula</i> (11)
Pacifica (CA)	37°10'	<i>B. glandula</i> (9)
Greyhound Rock (CA)	37°00'	<i>B. glandula</i> (3)
Lover's Point (CA)	36°40'	<i>B. glandula</i> (6)
Kirk's Creek (CA)	36°00'	<i>B. glandula</i> (7)
Tenera Point (CA)	35°00'	<i>B. glandula</i> (18), <i>C. fissus</i> (11)
Vandenberg AFB (CA)	34°50'	<i>B. glandula</i> (14), <i>C. fissus</i> (6)
Jalama (CA)	34°40'	<i>B. glandula</i> (5), <i>C. fissus</i> (4)
Point Conception	34°30'	
Arroyo Hondo (CA)	34°20'	<i>B. glandula</i> (14), <i>C. fissus</i> (4)
Mussels Shoals (CA)	34°15'	<i>B. glandula</i> (10), <i>C. fissus</i> (8)
La Jolla (CA)	32°49'	<i>C. fissus</i> (8)
Punta Santo Tomas (MX)	31°33'	<i>C. fissus</i> (2)
Punta Santa Rosalita (MX)	28°40'	<i>C. fissus</i> (2)
Guerreo Negro (MX)	28°02'	<i>C. fissus</i> (4)

nested analysis of molecular variance (AMOVA) was performed (in Arlequin, Schneider et al. 1997). The AMOVA analysis calculates genetic variance components and hierarchical F -statistic analogs (Φ -statistics) and tests the significance of these observed variances by matrix permutation (Excoffier et al. 1992). Interhaplotype distances for this analysis were calculated using the HKY85 model with estimated proportion of invariant sites (Hasegawa et al. 1985). This model was chosen by likelihood-ratio testing as the model that provided the best fit to the data (Goldman 1993; Huelsenbeck and Rannala 1997; Cunningham et al. 1998).

In several of our datasets we found evidence of significant geographic subdivision between populations not immediately adjacent to Point Conception. Because populations beyond these genetic breaks may not participate in the Point Conception regional larval exchange, which is the main focus of this study, we removed them from further analysis (populations north of Monterey in *B. glandula* and south of Laguna Beach in *S. purpuratus*).

Haplotype (H) and nucleotide diversity (π) were calculated for each species according to Nei (1987, eqs. 8.4 and 10.6). Randomization tests were performed to determine significance as follows: Individual sequences were randomly assigned to populations and 100 permutations of the data matrix were performed for each population while holding sample size constant. Reported P -values are two-tailed and indicate the proportion of null replicates with higher or lower diversity than observed.

Effective population sizes were estimated using the coalescent method of Kuhner et al. (1995) in the program Fluctuate (Kuhner et al. 1998), assuming a growth rate of zero and transition:transversion ratios estimated from the data using an HKY maximum-likelihood model using PAUP* version 4.0b4a (Swofford 1998). All other settings used default parameters (Kuhner et al. 1998).

A Method for Estimating Asymmetric Cladistic Migration Events

Directional migration across Point Conception was assessed using a modification of the cladistic method of Slatkin and Maddison (1989). This cladistic method treats geographic location as a distinct character and uses parsimony to infer the number of changes from one location to another on an intraspecific cladogram (hereafter referred to as cladistic migration events). Slatkin and Maddison (1989) interpreted cladistic migration events in the context of population genetic models that assume an equilibrium between migration and genetic drift. Under the equilibrium assumptions of an island model, Slatkin and Maddison (1989) determined a relationship between cladistic migration events and number of migrants per generation (Nm).

In Slatkin and Maddison's method, migration is assumed to be symmetric and the direction of migration events on the cladogram is not considered. In contrast, our study specifically asks whether there is an excess of southward migration events across Point Conception, and therefore requires that directionality be assessed. Instead of pooling northward and southward cladistic migration events, as in Slatkin and Maddison's method, we simply consider northward and southward cladistic migration events separately.

Unlike Slatkin and Maddison's method, we cannot assume equilibrium because we consider two major alternative hypotheses where an equilibrium between migration and drift has *not* been reached. According to the first alternative, the frequency of migration events is not directly correlated with the effective population size, but is instead mediated by external forces (southward ocean currents, Fig. 3A). In the second alternative hypothesis, the historical signal remains from a recent northward range expansion (Fig. 3B). Instead of attempting to relate inferred events on the cladogram to estimates of Nm under equilibrium assumptions, we will simply determine whether a species shows an excess of northward or southward migration events and compare this result to other species expected to be similarly affected by southward ocean currents.

Our method must consider four major issues: sampling, phylogenetic reconstruction, rooting, and estimation of cladistic migration events.

Haplotype sampling.—We addressed two related sampling issues: identical haplotypes shared between populations and asymmetries in sample size from each population. Shared haplotypes are relevant to estimates of migration because they imply that some migration has taken place (Slatkin and Maddison 1989). But because shared haplotypes are often found many times between populations, it is not clear how to incorporate them into an estimate of migration. Because a useful estimate of directionality must be robust to sampling methods, we used two approaches to shared haplotypes that represent logical extremes. Our first approach simply excluded *all* haplotypes shared between populations, whereas our second approach included all haplotypes, regardless of whether the haplotypes were shared in common. Although we believe the second approach is likely to be more accurate, both methods give very similar results.

Asymmetries in sample size can generate asymmetries in

the direction of migration events, such that the number of migration events toward the rare geographical state (the population with a smaller sample size) will be overestimated (Collins et al. 1994). Like Slatkin and Maddison's (1989) method, we equalized the number of sequences from each population by randomly deleting taxa from the larger populations. Although averaging across the results from only 10 randomly deleted sets gave very consistent results, we averaged across 20 rounds of random deletions.

Phylogenetic analysis.—Because they are asexually inherited, the actual relationships between any set of haplotypes must be fully bifurcating. Recovering this bifurcating phylogeny is problematic because only a subset of bifurcation (coalescent) events is accompanied by a nucleotide substitution. For this reason, many sequences identical to truly ancestral haplotypes are often still present in modern populations (Crandall and Templeton 1996). Because ancestors, by definition, have no autapomorphies, their inclusion greatly increases the number of equally parsimonious, fully bifurcating phylogenies.

Unlike Slatkin and Maddison (1989), we did not constrain identical haplotypes to form monophyletic groups. Because shared haplotypes are often ancestral—deeply nested in the haplotype network—their true relationships to one another and to descendant haplotypes are complex. Instead, we allowed the phylogenetic reconstruction to consider equally parsimonious placements for those haplotypes, as described in the Rooting section.

We expedited the search of tree space by considering a random subset of most-parsimonious trees. For each set of random haplotype deletions, we saved the first 1000 trees from each of 10 random taxon additions (heuristic searches with tree bisection reconnection, using equally weighted maximum parsimony, and zero-length branches not collapsed). Our results changed only slightly when we considered more trees from each of the 10 random additions of taxa, but greatly increased the amount of time during geographic analysis (next section).

Rooting.—Because inferring directionality of migration events requires a rooted phylogeny we used two methods, midpoint rooting and Castelleo and Templeton's (1994) coalescent method, for identifying the most likely root. Because the Castelleo and Templeton method always identified a shared haplotype as the most likely root, this method was only applied for those samples where shared haplotypes were allowed to remain in the datasets. We used outgroup rooting to reflect the most likely root in the following way. First, we designated as members of the outgroup all representatives of the root haplotype from a particular location (e.g., north of Point Conception). This ensured that at least one representative was present in each deleted taxon set. We then repeated the procedure, except that we designated as the outgroup all representatives of the root haplotype from the other locations (e.g., south of Point Conception). Trees from both sets of outgroup rootings were pooled for the MacClade analysis. A PAUP* block that will automatically perform this phylogenetic analysis is included in the Appendix.

Inference of migration events.—We used MacClade version 4.0b13 (Maddison and Maddison 1992) to code each haplotype according to its geographical location. We then used

TABLE 2. AMOVA results for *Chthamalus fissus*. Populations were grouped into the Point Conception region (NPC, populations 7–9 on Fig. 1; SPC, populations 10–12) and Baja California (BAJA, populations 13–15). Baja was considered a geographically distinct region because these populations do not fall within the Southern California Bight.

<i>Chthamalus fissus</i>				
Source of variation	d.f.	Sum of squares	Variance components	% of variation
Among groups	1	2.2	0.01869	0.81
Among populations, within groups	1	1.56	-0.03701	-1.60
Within populations	48	112.083	2.33507	100.79
Population pairwise F_{ST} values (P -values)				
	NPC		SPC	
SPC	-0.01438 (0.85)			
BAJA	-0.00041 (0.40)		-0.02108 (0.73)	

state-changes-and-stasis analysis to infer the average number of reconstructed northward and southward migration events for the 20,000 fully bifurcating rooted trees saved during the searches described above.

To clarify our procedure, the steps of the asymmetric migration analysis can be summarized as follows. (1) Create 20 datasets of equal population samples by random pruning. Shared haplotypes are treated in two ways: deleting them all or including them all. (2) Sample the most-parsimonious tree space for these 20 datasets to find a maximum of 20,000 fully bifurcating trees. (3) Root these phylogenies either by midpoint rooting or by the coalescent method of Castelloe and Templeton. (4) Edit the resulting tree file to fit the MacClade Nexus format. (5) Open a datafile with geographical locations in MacClade, and import the file of rooted trees. Use MacClade to infer the average number of migration events in each direction.

RESULTS

Sequence Data

For both *B. glandula* ($n = 112$) and *C. fissus* ($n = 49$), we obtained 406 bp of the gene encoding mitochondrial COI. All sequences are available in GenBank (*Balanus* AF234351–AF234462; *Chthamalus* AF234463–AF234527). *Strongylocentrotus purpuratus* data were taken from Edmands et al. (1996) and *N. emarginata* data from Marko (1998). Some haplotypes were excluded from genetic diversity and cladistic analyses due to evidence of historical differentiation (see below).

Geographic Subdivision

Because we were specifically interested in the effect of ocean currents on genetic patterns, we identified a set of northern populations and a set of southern populations for which we were unable to reject the null hypothesis of panmixia. Geographic subdivision within regional pools reflects past or ongoing cessation of gene flow between populations, making it possible that apparent asymmetries are just the result of incomplete lineage sorting.

We tested geographic subdivision using a nested AMOVA. We grouped the sampled populations into distinct regions (locations as in Table 1): Washington (WA; population 1), northern California (NC; populations 2–4: all California populations north of Monterey Bay, 36°50'N); north of Point

Conception (NPC; populations 5–9: Lover's Point, Kirk's Creek, Tenera Point, Vandenberg, and Jalama); south of Point Conception (SPC; populations 10–12: Arroyo Hondo, Mussel Shoals, and La Jolla); and Baja California (BAJA; populations 13–15).

In *C. fissus*, no significant geographic subdivision was detected using AMOVA (Table 2), whereas AMOVA identified significant geographic subdivision in *B. glandula*. This genetic break was found, not at Point Conception, but between populations on either side of Monterey Bay (36.5°N, Table 3). Populations north of Monterey Bay have been removed from further analysis.

In *S. purpuratus*, Edmands et al. (1996) used AMOVA to show significant geographic subdivision between two locations south of Point Conception (Laguna Beach, 33.5°N; La Jolla, 32.8°N). Our analysis of the *S. purpuratus* data (kindly provided by R. Burton, also based on a fragment of the COI gene) excluded populations south of and including La Jolla.

Finally, Marko's (1998) analysis of the *N. emarginata* dataset indicated that there is significant geographic subdivision between populations to the north and south of Point Conception. However, this subdivision was not apparent within northern or southern groups, so no populations were excluded in subsequent analyses.

All cladistic analyses for *B. glandula* and *S. purpuratus* were repeated without excluding the aforementioned individuals from the analyses, with little effect on the estimates of asymmetric migration across Point Conception (results not shown).

Genetic Diversity

The results for three measures of genetic diversity are given in Table 4. As predicted both by hypotheses of southward migration mediated by ocean currents (Fig. 3A) and hypotheses of northward range expansion (Fig. 3B), three of the four species show evidence for higher genetic diversity in southern populations.

In *B. glandula*, genetic diversity south of Point Conception is higher than northern populations for all three measures of genetic diversity. Randomization tests showed this increase was highly significant for both haplotype and nucleotide diversity ($P < 0.05$). Both *S. purpuratus* and *N. emarginata* also showed higher genetic diversity south of Point Conception for all three measures of genetic diversity, although this difference was not significant in *S. purpuratus* (Table 4). The

TABLE 3. AMOVA results for *Balanus glandula*. Cladistic analysis (not presented) indicated two distinct clades of individuals from northern California and Friday Harbor (FH) that are separate from all remaining groups. Individuals from these distinct populations (1–4) were placed in one geographic group for comparison with the group of populations south of Monterey Bay (5–11). Within these groups, populations were defined as in the Geographic Subdivision section: NC (populations 5, 6); NPC (7–9); and SPC (10, 11). Pairwise F_{ST} indicates that the excluded clades are significantly differentiated from those used in subsequent analyses (* $P < 0.05$).

<i>Balanus glandula</i> (AMOVA)				
Source of variation	d.f.	Sum of squares	Variance components	% of variation
Among groups	1	15.925	0.379	26.51
Among populations within groups	3	6.252	0.004	-0.78
Within populations	88	176.156	2.002	74.27
Population pairwise F_{ST} (P -value)				
	FH	NC(N)	NC(S)	NPC
NC(N)	-0.00436 (0.20)			
NC(S)	0.24679 (0.002)	0.26024 (0.001)		
NPC	0.27304 (0.001)	0.30331 (0.001)	-0.01477 (0.59)	
SPC	0.20767 (0.001)	0.22369 (0.002)	-0.02394 (0.68)	-0.01495 (0.93)

higher genetic diversity in *N. emarginata* is consistent with Marko's (1998) hypothesis of a northern range expansion. In *C. fissus*, there is no consistent trend of higher diversity south of Point Conception, with different patterns being observed for each of the three measures of genetic diversity.

As discussed above, high genetic diversity in southern populations can be caused either by southward gene flow or by northward range expansion from a southern refuge (Figs. 3A, B). As predicted, for all but *C. fissus* there is at least a weak trend toward higher genetic diversity in the south. To attempt to distinguish between these hypotheses, we turn to our method of cladistic estimates of asymmetric migration.

Cladistic Estimates of Asymmetric Migration Events

We estimated the relative proportion of southward migration events across Point Conception for three taxa with planktonic larvae, the barnacles *B. glandula* and *C. fissus* and the sea urchin *S. purpuratus* (data from Edmands et al. 1996). The gastropod (*N. emarginata*) was included for comparison because, unlike the other three species, it has poorly dispersing (crawling) larvae.

Because we were specifically interested in the effect of ocean currents on genetic patterns in the Point Conception region, we removed populations beyond each of two genetic breaks that were identified by AMOVA (as discussed above; populations north of Monterey in *B. glandula* and south of

Laguna Beach in *S. purpuratus*). *Nucella emarginata*, which has poorly dispersing larvae, does show significant geographic subdivision between population groups north and south of Pt. Conception, and the results of the analysis on this species with poorly dispersing larvae are included for purposes of discussion.

Cladistic estimates of asymmetric migration were carried out under two sampling and two rooting methods. Regardless of which method was used, all three taxa with planktonic larvae (*B. glandula*, *C. fissus*, *S. purpuratus*) showed an excess of southward migration events across Point Conception, with the ratios of southward to northward migration events falling between 1.1 to 1.5 depending on the species and treatment (Table 5). A one-tailed, paired t -test comparing northward and southward migration events across Point Conception provided results that differed depending on how haplotypes were treated and how rooting was carried out: $P < 0.08$ (all haplotypes included, midpoint rooted); $P < 0.04$ (all haplotypes included, coalescent rooted); and $P < 0.01$ (only haplotypes included in a single population included, midpoint rooted).

However, there are concerns about the nonindependence of inferred migration events when using phylogenetic methods. To ensure that the use of a t -test is not a biased approach, we tried two procedures using random data and random trees, keeping sample sizes for each species constant. Because tree shape is important and may be very different between a set

TABLE 4. Diversity analyses for *Balanus glandula*, *Chthamalus fissus*, *Strongylocentrotus purpuratus* and *Nucella emarginata*. NPC, north of Point Conception; SPC, south of Point Conception; BAJA, Baja California, Mexico. Haplotype and nucleotide diversity were calculated according to Nei (1987). The data matrix was resampled 100 times, randomly assigning individuals to populations; P -values represent the proportion of null replicates with higher or lower diversity than observed, where in each case the hypothesis tested in NPC is whether the observed diversity is lower than expected given panmixia, and in SPC the statistic describes the proportion of null replicates with higher diversity.

Species/population	Haplotype diversity	P -value	Nucleotide diversity	P -value	Θ
<i>Balanus</i> NPC	0.907	0.25	0.0072	0.03	0.04436
<i>Balanus</i> SPC	0.966	<0.01	0.0106	0.02	0.05672
<i>Chthamalus</i> NPC	0.947145	0.30	0.0112	0.39	0.04541
<i>Chthamalus</i> SPC	0.953846	0.47	0.0112	0.49	0.03654
<i>Chthamalus</i> BAJA	0.915033	0.87	0.0144	0.15	0.03871
<i>Strongylocentrotus</i> NPC	0.9217	0.58	0.01103	0.10	0.03847
<i>Strongylocentrotus</i> SPC	0.9413	0.16	0.01388	0.10	0.16294
<i>Nucella</i> NPC	0.732	0.01	0.00244	0.01	n/a
<i>Nucella</i> SPC	0.960	0.12	0.01267	0.29	n/a

TABLE 5. Average number of cladistic migration events on the West Coast of the United States estimated by MacClade version 4.0b13 (Maddison and Maddison 1992). NPC, north of Point Conception; SPC, south of Point Conception; BAJA, Baja California, Mexico. Southward and northward migration events are presented side by side to facilitate comparison. Bold face entries represent comparisons between populations immediately to the north and south of Point Conception. The ratio is the proportion of northward/southward events between populations north and south of Point Conception (NPC and SPC). Allhaps, midpoint, all haplotypes were included and midpoint rooting was employed; Allhaps, coalescent, all haplotypes were included and the designated outgroup was a representative of the haplotype deemed the most likely root by the method of Castelleo and Templeton (1994); Uniqehaps, midpoint, only haplotypes found in a single location were included and midpoint rooting was employed. Using the three taxa with pelagically dispersing larvae as independent tests of a hypothesis of southward, current-mediated migration events, a one-tailed, paired *t*-test gave results of $P < 0.08$ (Allhaps, midpoint), $P < 0.04$ (Allhaps, coalescent), and $P < 0.01$ (Uniqehaps, midpoint).

Method	Ratio	NPC-SPC	SPC-NPC	NPC-BAJA	BAJA-NPC	SPC-BAJA	BAJA-SPC
<i>Chthamalus fissus</i> (pelagically dispersing larvae)							
Allhaps, midpoint	1.3	2.25	1.77	1.61	2.4	1.83	3.23
Allhaps, coalescent	1.3	2.29	1.71	1.90	2.13	1.97	3.09
Uniqehaps, midpoint	1.5	2.01	1.33	1.09	1.93	1.19	2.63
<i>Balanus glandula</i> (pelagically dispersing larvae)							
Allhaps, midpoint	1.1	8.75	7.87				
Allhaps, coalescent	1.2	8.98	7.60				
Uniqehaps, midpoint	1.1	6.18	5.65				
<i>Strongylocentrotus purpuratus</i> (pelagically dispersing larvae)							
Allhaps, midpoint	1.3	12.91	10.33				
Allhaps, coalescent	1.2	12.84	11.16				
Uniqehaps, midpoint	1.2	5.27	4.25				
<i>Nucella emarginata</i> (poorly dispersing larvae)							
Allhaps, midpoint	infinite	0	1				
Allhaps, coalescent	infinite	0	1				
Uniqehaps, midpoint	0.08	0.07	0.93				

of “random” trees and trees formed from actual data (Maddison and Slatkin 1991), we first used the set of maximum-parsimony trees saved from initial analyses on the data. Geographic location was randomized (10 randomizations across 100 trees including all haplotypes and using the coalescent rooting) to generate a dataset for each species. Using these true phylogenies with random geographic data and comparing the three species for each replicate results in a $P < 0.05$ with a frequency of 0.026. Alternatively, random trees (in MacClade, random joining and splitting method as in Maddison and Slatkin 1991) were used to obtain another dataset with the sample sizes held constant, resulting in a $P < 0.05$ with a frequency of 0.075. These comparisons suggest that there is some bias introduced due to phylogenetic signal, but it is minor and may not decrease the power of our cladistic method for inferring asymmetric migration. The important observation in this study is that for all three species with pelagically dispersing larvae, there was a consistent bias toward migration events from north to south.

In contrast, the only taxon with crawling larvae (*N. emarginata*) showed an excess of northward migration events across Point Conception. This interpretation is consistent with Marko’s (1998) conclusion (also supported by his allozyme data) that this species underwent a recent northward range expansion across Point Conception (as in Fig. 3A). Because *N. emarginata* has crawling larvae, we would not expect the signature of this northward range expansion to be erased by southward flowing currents.

DISCUSSION

Whereas previous studies have tried to find evidence of genetic subdivision within marine invertebrate species with

ranges that span Point Conception, (reviewed in Burton 1998), we have focused more specifically on whether the predominance of southward currents during most of the year will leave a signature of asymmetric migration events. Because the expected effect is subtle, we have compared patterns in several species across the same geographic region.

For pelagically dispersing species, we expect an excess of southward migration events caused by the predominantly southward flow of ocean currents across Point Conception (Figs. 1, 3A). For all three species with pelagically dispersing larvae, cladistic analyses revealed a slight excess of southward migration events, with the ratio of southward:northward events ranging from 1.1 to 1.5. Despite an admittedly subtle pattern, this excess was statistically significant for two of the three approaches for dealing with multiple haplotypes and rooting (Table 5).

Our repeated discovery of this pattern of southward migration bias is remarkable because we would expect the opposite pattern if any of these species experienced a recent northward range expansion (Fig. 3B). As a case in point, Marko (1998) suggested that *N. emarginata* experienced a recent range expansion across Point Conception after the last glacial episode. Because *N. emarginata* is the only one of the four species in this study with crawling larvae, we would not expect southward ocean currents to counteract this pattern. As expected, *N. emarginata* shows a clear signature of recent range expansion, with all haplotypes collected north of Point Conception forming a clade, that is nested within southern populations, as in Figure 3B (fig. 2 in Marko 1998).

The repeated observation of an excess of southward migration events is also remarkable because occasional northward migration brought about by El Niño Southern Oscil-

lation (ENSO) events would tend to obscure a pattern generated by predominantly southward currents (Glynn 1988; Palumbi 1995). Evidence of northward migration during ENSO events is found in the many reports of species well to the north of their usual ranges and to the north of Point Conception (e.g., Newman and McConnaughey 1987; Stepien and Rosenblatt 1991).

Before accepting our inference of southward gene flow for the three species with pelagically dispersing larvae, we must also consider other processes that could produce the same pattern. In *B. glandula*, whose range only extends to northern Baja California, the region south of Point Conception may represent a marginal, sink habitat that is only maintained by constant infusion from northern populations (Dias 1996). A source-sink process could explain a net southward gene flow without needing to invoke ocean currents. Although a source-sink hypothesis must be considered for *B. glandula*, whose southern range limit is just south of Point Conception, the higher genetic diversity south of Point Conception suggests this relationship does not exist (Table 4). Sink population dynamics would not be expected to apply to *C. fissus* or *S. purpuratus*, whose ranges extend well into Baja California (Smith and Carlton 1975).

Source-sink dynamics have also been invoked to explain why eastern Pacific biogeographic boundaries like Point Conception tend to have more northern than southern range limits (e.g., Fig. 2). Newman (1979) and Roy et al. (1995) have argued that localized upwelling can create pockets of cold water well to the south of a species' usual southern range limit. Thus, occasional migrants from the north may be enough to sustain isolated southern populations. This scenario would explain why Point Conception is a "leaky" southern boundary for species with pelagically dispersing larvae (Fig. 2). Some genetic support for southern extralimital populations has been found in the kelpfish *Gibbonsia montereyensis* and *G. metzi*. Stepien and Rosenblatt (1991) found that disjunct populations of these species from north of Point Conception are very similar genetically to individuals from areas of cold water upwelling off northern Baja California. Although source-sink arguments are worth considering, they fail to explain why Point Conception, with its relatively steep thermal gradient, does not appear to significantly limit the ranges of species with crawling larvae (Fig. 2). It is possible, however, that these direct-developing species are more capable of local adaptation (Behrens Yamada 1989).

In addition to the subtle genetic pattern we report, there is ecological evidence that the California Current system may structure the population dynamics of some species in southern California (Hauray et al. 1996). Ebert et al. (1994) noted that annual recruitment in *S. purpuratus* was less variable overall in the region just south of Point Conception than in the populations to the north of Point Conception or in northern Baja California, Mexico. One possible explanation for this finding is that there is greater retention of water within the Southern California Bight compared to the regions of higher advection to the north and south. Other studies have also noted distinct population genetic responses to coastal eddies, currents, and regions of higher overall water retention (Bucklin 1991; Bertness and Gaines 1992; Rocha-Olivares and Vetter 1999).

Although other studies have reported genetic patterns that appear to be inconsistent with predominant current patterns (Benzie and Williams 1995, 1997; Shulman and Bermingham 1995; Palumbi et al. 1997), these studies have typically relied on estimates of gene flow based on pairwise F_{ST} -type measures. In contrast, the cladistic method we use has the potential for identifying cases of asymmetric migration events even when there has been no significant geographic subdivision. Although the effects are expected to be subtle due to oceanographic fluctuations and stochastic events (Newman 1979; Lynn and Simpson 1987), comparison of multiple species across a region may elucidate patterns that have been previously dismissed.

Finally, although it was not the focus of this study, our cladistic method also allows an analysis of asymmetric migration between subdivided populations on either side of a genetic break. For example in Reeb and Avise's (1990) classic study, members of the Gulf clade were occasionally found in Georgia, whereas the reverse was never seen. This suggests a pattern of asymmetric migration that would have been missed by relying exclusively on pairwise F_{ST} -type measures (Whitlock and McCauley 1999). In conclusion, we feel that other questions of biogeographic pattern or gene flow may be worth revisiting, using data from multiple species to indicate subtle but important abiotic influences.

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APPENDIX

This appendix contains a PAUP* block to perform a series of random searches to explore tree space. Trees are found in a succession of random addition sequences, and only the first 100 are saved from each random addition sequence. Only searches finding minimum-length trees are kept. Trees are midpoint rooted and saved to a larger file, where trees from each of the 20 exclusion sets are found. These trees are then analyzed using MacClade. Comments are given in bold.

The following PAUP block will create two sets of trees, one midpoint rooted, and one rooted by coalescent rooting in which all observed haplotypes have been retained in the analysis. The following taxsets randomly exclude haplotypes from the population with the larger sample size, so that the included sample sizes from the two populations are equal.

```
taxset equal 1 = SPSCI4 SpLb39 SpSri17 splbsr104 SpSri27 SPLB8 SpLb34 SPSCI1 SPLB2 SpSri121 SPSCI31 splbsr108
LBS014 SpLb28 SPLB5 splbsr109 SpSri22 SpLb37 lbs48 SpSCI15 LBS007 LBS150 LBS125 LBS153
LBS004 LBS011 LBS5 SpSCI32 LBS129 splbsr112 SpSri101 LBS012 SRI105 SPSCI9 LBS008 SpSCI36
LBS2 SpSri125 SPLB4 LBS130;
```

```
taxset equal 2 = SpLb37 SPSCI5 splbsr103 splbsr109 SpSCI36 SPSCI31 splbsr110 SpSri12 lbs45 lbs46 LBS006 SpLb33
LBS1 SpSri5 SPLB5 SPSCI4 SPSCI1 SpSri9 SPLB8 splbsr104 SpSri27 LBS125 LBS007 LBS008
splbsr108 splbsr113 LBS3 SpSri6 LBS150 SpSCI16 SpSri106 SpSri8 LBS124 SCI12 SRI14 LBS129
SPSCI9 splbsr112 LBS132 SpSri104;
```

```
pset opt = minF; required for midpoint rooting
```

The following two lines clear trees in memory and restore all haplotypes.

```
cleartrees nowarn;
restore all;
```

delete equal 1; deletes the first random set of haplotypes to create equal sample sizes

```
set increase = no;
set autoclose = yes;
set maxtrees = 1500;
set warntree = no;
set warnrest = no;
```

The following line ensures that only fully bifurcating trees are retained.

```
pset collapse = no;
```

The following line initiates a series of searches that randomize the input order of taxa, retaining the first 100 trees from each search. Because some input orders will not find minimum-length trees, nchuck discards these searches.

```
hsearch addseq = random nreps = 15 nchuck = 100 chuckscore = 1;
savetrees format = altnexus file = temporary.trees replace = yes root = no;
cleartrees nowarn;
```

The following line gets the first 1000 trees from the temporary file.

```
gettrees file = temporary.trees from = 1 to = 1000;
roottrees method = midpoint;
savetrees format = altnexus file = midpoint.root replace = yes root = yes;
deroot;
```

The next few lines designate as outgroups members of the root haplotype found in population 1. Because these haplotypes are all identical, only one is necessary to root the tree, but all are included so that at least one remains after the process or randomly deleting sets of haplotypes.

```
outgroup SpFh8 SPMC6 SPMC8 SpPB22 SPPB13 SPMC19 Fh4/only;
roottrees method = outgroup;
savetrees format = altnexus file = coalescent.root append = yes root = yes;
deroot;
```

The next few lines do the same for the members of the root haplotype found in population 2.

```
outgroup SPSCI5 SpSri 27 SRI34 SpLb32 LBS130 SPLB3 SPLB2 LBS014 SpSri22 SpLb38 LBS2 SpLb39 SpSri9 SpSri107/only;
roottrees method = outgroup;
savetrees format = altnexus file = coalescent.root append = yes root = yes;
```
