

DISCORDANT DISTRIBUTION OF POPULATIONS AND GENETIC VARIATION IN A SEA STAR WITH HIGH DISPERSAL POTENTIAL

Carson C. Keever,^{1,2} Jennifer Sunday,¹ Jonathan B. Puritz,³ Jason A. Addison,^{2,4} Robert J. Toonen,³ Richard K. Grosberg,² and Michael W. Hart^{1,5}

¹Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

²Center for Population Biology, College of Biological Sciences, University of California, Davis, California 95616

³Hawaii Institute of Marine Biology, University of Hawaii, P.O. Box 1346, Kaneohe, Hawaii 96744

⁵E-mail: mwhart@sfu.ca

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Patiria miniata, a broadcast-spawning sea star species with high dispersal potential, has a geographic range in the intertidal zone of the northeast Pacific Ocean from Alaska to California that is characterized by a large range gap in Washington and Oregon. We analyzed spatial genetic variation across the *P. miniata* range using multilocus sequence data (mtDNA, nuclear introns) and multilocus genotype data (microsatellites). We found a strong phylogeographic break at Queen Charlotte Sound in British Columbia that was not in the location predicted by the geographical distribution of the populations. However, this population genetic discontinuity does correspond to previously described phylogeographic breaks in other species. Northern populations from Alaska and Haida Gwaii were strongly differentiated from all southern populations from Vancouver Island and California. Populations from Vancouver Island and California were undifferentiated with evidence of high gene flow or very recent separation across the range disjunction between them. The surprising and discordant spatial distribution of populations and alleles suggests that historical vicariance (possibly caused by glaciations) and contemporary dispersal barriers (possibly caused by oceanographic conditions) both shape population genetic structure in this species.

KEY WORDS: Asterinidae, ATPS, effective population size, GPI, life history, planktotrophy.

Explaining the origin and persistence of large geographical discontinuities in species distributions, such as the antitropical distributions of many temperate-zone animals and plants, is one of the original goals of evolutionary ecology (Darwin 1859; Ekman 1953; Briggs 1987; Wiley 1988; Lindberg 1991). Such range disjunctions may be initiated and maintained by a complex combination of factors, encompassing extrinsic geological and climatic barriers to dispersal and colonization, and intrinsic biological properties of organisms including habitat preferences

and dispersal capabilities (Schwaninger 2008). Dispersal barriers such as mountain ranges or climatic effects associated with Pleistocene glacial cycles (DeChaine and Martin 2006; Knowles and Carstens 2007) are well-known determinants of the geographical distribution both of populations and of genetic variation in animals and plants of the Northern Hemisphere (Hewitt 1996; Riddle 1996; Byun et al. 1997; Knowles 2001; Lovette 2005).

The intrinsic potential of some organisms to traverse physical and geological features that are barriers to dispersal for other species (e.g., Lessios et al. 1998) is arguably greatest among sessile and sedentary marine animals that have prolonged development of feeding planktonic (planktotrophic) larvae. Direct

⁴Current address: Department of Biology, University of New Brunswick, Fredericton, NB E3B 5A3, Canada

estimates of realized dispersal in species with planktrophic development are restricted for logistical reasons to cases in which realized dispersal is limited to small spatial scales (e.g., Knowlton and Keller 1986), but inferences based on oceanography and larval duration suggest that realized dispersal can exceed 10^3 – 10^4 km per generation (Scheltema 1986; Shanks et al. 2003). Numerous lines of evidence suggest a correlation between the evolutionary gain or loss of planktonic larval development and the magnitude or geographical scale of neutral genetic differentiation among marine animal populations (Arndt and Smith 1998; Bohonak 1999; Kyle and Boulding 2000; Grosberg and Cunningham 2001; Hellberg et al. 2002; Kelly and Eernisse 2007; Teske et al. 2007). However, this trend conflicts with a conspicuous minority of other studies that reveal strong phylogeographic breaks in spite of prolonged planktonic larval development, or large differences in population genetic structure between sympatric species with similar larval dispersal potential (Benzie 1999; Swearer et al. 1999; Forward et al. 2003; Marko 2004; Hickerson and Cunningham 2005; Crandall et al. 2008; Hamilton et al. 2008).

The concordance between the geographical distributions of populations (including patterns such as range disjunctions) and of population genetic variation (phylogeographic breaks) can be used to test biogeographic hypotheses (Avice 2000). Range disjunctions that coincide with phylogeographic breaks are consistent with the effects of geological or physical processes (like glaciations) that limit dispersal, and implicate dispersal barriers in the origin of the disjunct range (e.g., Muñoz-Salazar et al. 2005). Alternatively, range disjunctions that do not correspond to phylogeographic breaks suggest gene flow across the disjunction, and implicate ecological effects such as the distribution of suitable habitat, habitat selection by recruiting propagules, juvenile mortality, or recent extirpation (rather than dispersal barriers) in the origin of the disjunct range. Comparative analyses of sympatric species that share the same range disjunction have revealed mixtures of results that match both of these patterns (Bernardi et al. 2003; Ayre et al. 2009).

Here we use a large suite of mitochondrial and nuclear genetic markers to test the strength of the concordance between biogeographic and phylogeographic discontinuities in the bat star *Patiria miniata* (formerly *Asterina miniata*; O'Loughlin and Waters [2004]) from the northeast Pacific. Bat stars are abundant omnivores in intertidal and shallow subtidal marine communities in sheltered waters of southeast Alaska, British Columbia, central and southern California, and Baja California (Fisher 1911; Lambert 2000), but are strikingly rare in or absent from the outer coasts of Washington, Oregon, and northern California between Cape Flattery, WA ($48^{\circ}38'N$, $124^{\circ}71'W$) and Cape Mendocino, CA ($40^{\circ}26'N$, $124^{\circ}24'W$) (Kozloff 1983) (Fig. 1). The northern part of the *P. miniata* range includes the transition between

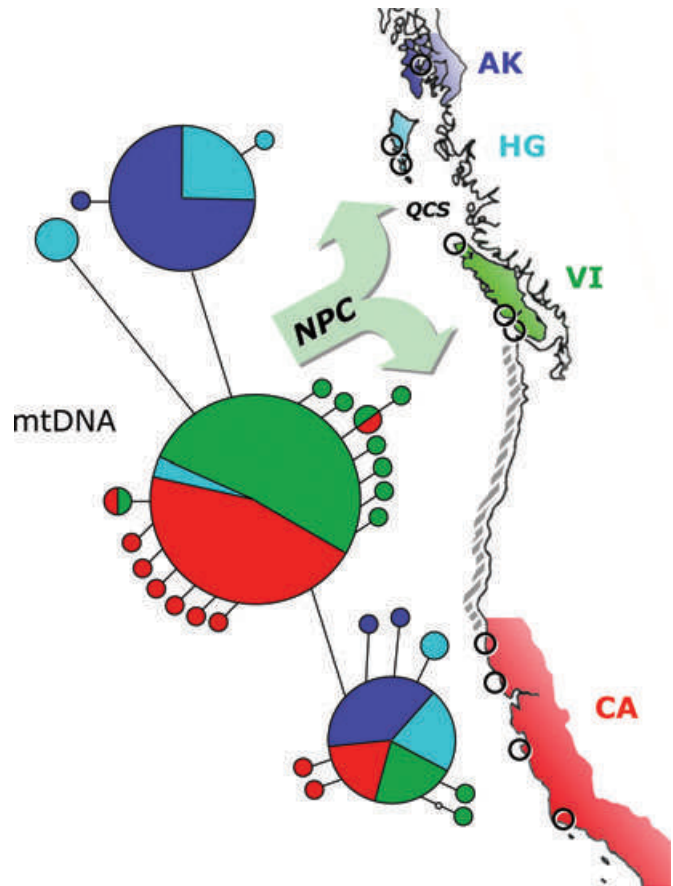


Figure 1. Haplotype network for *Patiria miniata* mtDNA sequences. Each symbol represents a unique sequence (28 total). The area of each circle (and of the segments in each pie diagram) is proportional to haplotype frequency ($n = 1$ – 120). Lines between symbols indicate single substitution differences between haplotypes (the small open symbol indicates one missing haplotype inferred for two sequences that differed by two substitutions), and are not proportional to genetic distance between haplotypes. Color coding indicates the origin of each private haplotype (solid circles) or the frequency of shared haplotypes (pie diagrams) in each region indicated in the map at right: Alaska (AK, dark blue) and Haida Gwaii (HG, cyan) to the north of Queen Charlotte Sound (QCS); Vancouver Island (VI, green) and California (CA, red) to the south. Black rings on the map show approximate locations of sample sites (some indicate >1 locations separated by small distances; see Table 1). The arrows show the approximate location of the divergence of the North Pacific Current. The hatched shading shows the *P. miniata* range disjunction.

the Oregonian and Aleutian zoogeographical provinces (Briggs 1974), and the range disjunction includes the southern extent of the Wisconsin glaciation on the Pacific coast of North America at about $48^{\circ}N$ latitude (Pielou 1991).

Comparative population genetic studies of this community (Hellberg 1996; Arndt and Smith 1998; Burton 1998; Rocha-Olivares and Vetter 1999; Kyle and Boulding 2000; Dawson

Table 1. Sample locations and number of individuals genotyped per location (n) for each marker class. For ATPS and GPI, the number of alleles sequenced = $2n$. Summary statistics for diversity are observed (H_O), and expected (H_E) heterozygosity and standardized allelic richness (R_S) averaged across seven microsatellite loci; and haplotype (h) and nucleotide (π) diversity for each of the three sequenced loci.

Region, sample site	Latitude	Longitude	Microsatellites				mtDNA			ATPS			GPI		
			n	H_O	H_E	R_S	n	h	π	n	h	π	n	h	π
Alaska															
View Cove (VC)	55°05'01"N	133°01'05"W	40	0.48	0.55	3.9	36	0.52	0.0029	29	0.94	0.036	29	0.74	0.055
Dunbar Inlet (DI)	55°04'09"N	132°50'47"W	29	0.43	0.53	3.9	27	0.36	0.0019	–	–	–	–	–	–
Haida Gwaii															
Rennel Sound (RS)	53°24'24"N	132°31'39"W	15	0.28	0.49	3.2	12	0.75	0.0031	–	–	–	–	–	–
Moresby Camp (MC)	53°03'07"N	132°01'32"W	15	0.35	0.58	4.2	13	0.68	0.0036	–	–	–	–	–	–
Louise Narrows (LN)	52°57'07"N	131°53'56"W	50	0.52	0.62	4.2	–	–	–	10	0.99	0.033	9	0.88	0.062
Tanuu (TA)	52°45'55"N	131°36'55"W	48	0.46	0.59	4.2	11	0.60	0.0030	16	0.96	0.033	15	0.87	0.067
Vancouver Island															
Winter Harbor (WH)	50°31'16"N	128°01'31"W	50	0.48	0.60	5.1	18	0.40	0.0017	25	0.99	0.042	24	0.98	0.060
Tofino (TO)	49°08'38"N	125°53'30"W	36	0.34	0.56	5.3	31	0.30	0.0008	–	–	–	–	–	–
Bamfield (BA)	48°49'43"N	125°08'05"W	48	0.41	0.55	4.9	32	0.50	0.0018	29	0.99	0.036	27	0.96	0.051
California															
Fort Bragg (FB)	39°24'32"N	123°48'22"W	20	0.49	0.60	5.2	15	0.13	0.0003	22	0.99	0.036	21	0.98	0.062
Bodega Bay (BOD)	38°19'09"N	123°02'59"W	24	0.48	0.63	5.5	23	0.62	0.0020	27	0.99	0.035	26	0.99	0.047
Monterey (HOP)	36°37'15"N	121°54'16"W	–	–	–	–	5	0.70	0.0027	–	–	–	–	–	–
Carmel (CAR)	36°32'39"N	121°54'12"W	–	–	–	–	10	0.53	0.0016	–	–	–	–	–	–
Santa Barbara (SB)	34°27'02"N	119°42'05"W	33	0.38	0.55	5.5	20	0.36	0.0012	22	0.99	0.035	21	0.98	0.055

2001; Edmands 2001; Hellberg et al. 2001; Hickerson and Ross 2001; Marko 2004; Sotka et al. 2004; Hickerson and Cunningham 2005; Cassone and Boulding 2006; Harley et al. 2006; Wilson 2006; Kelly and Eernisse 2007; Lee and Boulding 2007; Polson et al. 2009) have typically revealed evidence of either (1) strong north-south genetic differentiation only in species that lack a prolonged planktonic larval stage (with low intrinsic dispersal potential); or (2) broad genetic homogeneity, often in species with long-lived planktonic larvae, but sometimes in species lacking long-distance dispersal potential (reviewed by Marko [2004]). The first pattern has usually been interpreted as evidence of north-south vicariance, with persistence in northern and southern ice-free refuges during the Pleistocene (Byun et al. 1997; Holder et al. 1999; Smith et al. 2001; Hetherington et al. 2003). The second pattern likely arises from either long-term gene flow across these geological and oceanographic boundaries, or a short recent history of northern populations following northern extirpation and subsequent Holocene range expansion out of southern refuges (Edmands 2001).

We use our population genetic data to test two working hypotheses about the factors responsible for the range disjunction in *P. miniata*. Coincidence of the *P. miniata* distributional gap with both the southern extent of the last glacial maximum and a significant phylogeographic break would be consistent with a history of vicariant isolation of bat star populations north and south

of the range disjunction, and would be consistent with slow and incomplete range expansion into the formerly glaciated areas as the cause of the range disjunction. In contrast, population genetic homogeneity across the range disjunction would be consistent with larval dispersal and gene flow across the disjunct part of the range. Such a result would implicate benthic ecological factors, such as the distribution or larval selection of suitable benthic habitat, as the cause of the distributional gap. The results allow us to disentangle the different roles of past vicariant events and contemporary ecological processes in establishing and maintaining a striking range disjunction in the sea.

Methods

POPULATION SAMPLING

We obtained tissue samples (tube feet) preserved in 70–95% ethanol from 423 individual sea stars collected from 14 locations in southeast Alaska, Haida Gwaii (the Queen Charlotte Islands), Vancouver Island, and California (Table 1, Fig. 1). Based on preliminary results from analysis of mtDNA sequences for populations sampled in 2004 (BA, BOD) and 2005 (VC, DI, RS, MC, TO, FB, HOP, CAR, SB), we focused our analysis of microsatellite and nuclear intron sequence variation on a subset of those populations plus additional populations from Vancouver Island (WH) and Haida Gwaii (LN, TA) sampled in 2006. In total,

we genotyped 253 individuals from 13 populations for mtDNA, 408 individuals from 12 populations for microsatellites, and 172–180 individuals from eight populations for the nuclear introns (Table 1).

GENETIC DATA COLLECTION

Microsatellites

We extracted DNA from tube feet using either a CTAB protocol (Grosberg et al. 1996) or a simple proteinase K digestion of a single tube foot in water (Addison and Hart 2005a). We used these DNA extractions in PCR to amplify and characterize allele size variation at ATG or CAG microsatellite loci. We used PCR cocktails, thermal cycling conditions, electrophoresis methods, and allele size estimation software to obtain genotypes for seven loci (B11, B201, B202, C8, C204a, C113, and C210) as described previously (Keever et al. 2008).

mtDNA

We used the same CTAB protocol noted above to extract genomic DNA. We used primers from Colgan et al. (2005) to amplify part of the mitochondrial genome that contains five transfer RNA genes and the 5' end of the cytochrome *c* oxidase subunit I (COI) gene (Hart et al. 1997). Thermal cycling conditions were 90° (2:00), 55° (0:40), 72° (2:00) for one cycle; 90° (0:30), 55° (0:30), 72° (1:40) for 30 cycles; and 90° (0:40), 55° (0:40), 72° (7:00) for one cycle. Amplicons were checked by agarose gel electrophoresis, purified by sodium acetate precipitation, and directly sequenced with the forward primer (Hart et al. 1997). Sequences were proofread in 4Peaks v. 1.6 (A. Griekspoor and T. Groothuis, mekentosj.com), aligned in Clustal W using the default settings (Thompson et al. 1997), and trimmed to standard length (369 bp; GenBank accession numbers EF165733-EF165790; EF165792-EF165971; FJ939314-FJ939328).

Introns

Genomic DNA used for nuclear DNA sequence analysis was extracted using the Qiagen DNeasy kit (Qiagen, Valencia, CA). We used primers from Jarman et al. (2002) to amplify and sequence an intron in the alpha subunit of the ATP synthetase gene (ATPS). From these preliminary sequences, we made and used two *P. miniata*-specific primers PMATPSF9 (5'-TAAGGCCGTGGATAGTCTGG) and PMATPSR669 (5'-TGATGGTGCAATGGCTACAG) that were designed to amplify a ~670 bp fragment. Thermal cycling conditions for these primers were 95° (3:00) for one cycle; 94° (0:30), 58.5° (0:30), 72° (0:30) for 40 cycles; and 72° (20:00) for one cycle. Amplicons were then cleaned using the QIAquick PCR Purification Kit (Qiagen) and directly sequenced in both directions. Amplicons from heterozygous individuals were cloned into the pZero plasmid vector using a custom TA cloning system, and 5–8 clones per individual

were then sequenced and compared directly to the heterozygous genotype sequence to ascertain each allele and correct all cloning errors. All sequences were edited in Sequencher v. 4.8 (Gene Codes), aligned using Clustal W in MEGA v. 3.0 (Kumar et al. 2004), then aligned again by eye and trimmed to standard length (635 bp; GenBank accession numbers FJ850593-FJ850958).

We amplified and sequenced a second intron from the glucose-6-phosphate isomerase gene (GPI). We used degenerate primers designed from an alignment of genomic GPI sequences from *Strongylocentrotus purpuratus*, *Danio rerio*, *Canis familiaris*, and *Bufo melanostictus* to obtain preliminary sequence data. From these preliminary sequences, we designed two asterinid-specific primers GPIFN4 (5'-GCCAAGCACTTTGTBGCCT) and GPIR28M (5'-TCCCARAAVGGAAACATRTTWCCTTGT). Thermal cycling conditions for these primers were 95° (3:00) for one cycle; 94° (0:30), 54–57° (0:45), 72° (0:30) for 40 cycles; and 72° (20:00) for one cycle. Amplicons (~240–480 bp) were then cleaned using the QIAquick kit and directly sequenced in both directions. Alleles of some heterozygous individuals could be inferred parsimoniously from comparison to known alleles previously sampled from homozygous individuals. Amplicons of all other heterozygotes were cloned using the CloneJet (Fermentas, Burlington, ON, Canada) blunt end cloning kit and compared directly to the heterozygous genotype sequence. All sequences were edited and aligned as described above (476 bp; GenBank accession numbers FJ850243-FJ850592).

QUANTITATIVE ANALYSIS

Polymorphism

For each population sample (Table 1), we calculated allelic frequencies, expected and observed heterozygosity, linkage disequilibrium (for microsatellites), and haplotype and nucleotide diversity (for mtDNA and introns) using Arlequin v. 3.11 (Excoffier et al. 2005). For the microsatellites, we calculated allelic richness by standardizing to the smallest sample size ($n = 15$; Table 1) using the rarefaction method in FSTAT v. 2.9.3 (Goudet 1995). Differences in allelic richness between groups of populations identified by clustering analysis in STRUCTURE (see below) were tested by a whole-sample permutation test. For the three sequenced loci, we used Arlequin to calculate Tajima's *D* and Fu and Li's *F* to test for departures from neutral variation in allelic diversity relative to the number of segregating sites (Tajima 1989) or number of alleles (Fu and Li 1993; Simonsen et al. 1995).

Heuristic measures of population structure

We used the clustering method in STRUCTURE v. 2.1 (Pritchard et al. 2000) with or without geographical priors to identify genotype groupings that minimize Hardy-Weinberg disequilibrium and linkage disequilibrium by assigning individual multilocus genotypes to *k* groups. We analyzed the microsatellite genotypes alone

($n = 408$) and the combined microsatellite and GPI genotype data for individuals genotyped for both marker classes ($n = 138$); we did not include ATPS in this combined analysis because of the very high allelic diversity at this locus and the limited scope for measuring linkage with alleles at other loci). For each data set, we estimated the number of populations sampled (k) under an admixture model without geographical priors, with allele frequencies correlated between populations. Admixture proportions were estimated from the dataset and fixed for all populations. We ran Bayesian MCMC searches of 1,000,000 steps with a burn-in of 250,000. For each analysis, we carried out seven independent runs for each value of k up to $k = 5$ populations. We used the method of Evanno et al. (2005) to find the best-fit value of k . These analyses identified two significant genotype clusters ($k = 2$) that were clearly segregated into a northern (Alaska, Haida Gwaii) and a southern (Vancouver Island, California) group.

We also examined spatial associations within haplotype networks based on the ancestor–descendant relationships among mtDNA and intron sequences. The very high haplotype diversity in ATPS and GPI samples included many apparent recombinants. Because recombination obscures ancestor–descendant relationships among sequences, we used DnaSP v. 4.20 (Rozas et al. 2003) to identify the largest contiguous block of nucleotide sites for each intron alignment that satisfied the four-gamete test and was therefore assumed to experience no recombination (e.g., Woerner et al. 2007). For ATPS, this largest nonrecombining block consisted of nucleotide sites 202–314 (~18% of the original sequence alignment); for GPI, we analyzed nucleotide sites 85–427 (~72% of the original sequence alignment). We used median-joining networks constructed by the algorithm of Bandelt et al. (1999) implemented in Network v. 4.5 using the default parameter values (equal character weighting = 10; epsilon = 0; distance criterion = connection cost) and simplified by using the MP algorithm of Polzin and Daneshmand (2003). We resolved one ambiguity (a closed circuit) in the resulting mtDNA network in favor of ancestor–descendant relationships between common haplotypes and singletons.

Population differentiation

We calculated fixation indices (F -statistics) among all populations, between pairs of populations (F_{ST}), and among individuals within populations (F_{IS}), using Arlequin. We used Mantel tests in Arlequin to characterize isolation by distance as the correlation between population differentiation (pairwise F_{ST}) and straight-line geographical distance. All F -statistics were computed by the method of Weir and Cockerham (1984), and F -values significantly different from zero were identified by comparison to results from 10,000 permutations of the data (Raymond and Rousset 1995). We tested the statistical significance of pairwise F_{ST} values by genotypic permutation using a G -test implemented in FSTAT.

We adjusted the critical P -values for these tests using sequential Bonferroni corrections.

We asked whether microsatellite allele size contributed significant additional information to estimates of population structure (R_{ST}) compared to estimates based on allele frequencies alone (F_{ST}). We calculated R_{ST} for each locus and compared the results to an expected R_{ST} distribution generated by 10,000 allele-size permutations in SPAGeDi v. 1.2.1 (Hardy and Vekemans 2002).

To test for regional subdivision of sequence and microsatellite diversity, we used analysis of molecular variance (AMOVA) in Arlequin. We used 10,000 permutations of the data to identify measures of differentiation (Φ) significantly different from zero. We partitioned this variance into differences between northern and southern population groups (Φ_{CT}) and differences among populations within each group (Φ_{SC}). We carried out two of these analyses: one based on population groups north (Alaska, Haida Gwaii, Vancouver Island) and south (all California sites) of the range disjunction; and a second based on the STRUCTURE results, which strongly suggested a phylogeographic break between population groups north (Alaska, Haida Gwaii) and south (Vancouver Island, California) of Queen Charlotte Sound (see Fig. 1).

Gene flow and effective population size

We carried out coalescent analyses of migration rate (m) and effective population size (N_e) for the mtDNA and intron sequences using the Bayesian method in MIGRATE-N v. 2.4 (Beerli and Felsenstein 2001; Beerli 2006). We tried to avoid the confounding effects of recombination on each analysis of intron sequences by using only the largest nonrecombining block of nucleotide sites identified by DnaSP (see above). Because our STRUCTURE and haplotype network analyses identified an unexpected and strong phylogeographic break at Queen Charlotte Sound in northern British Columbia, we were specifically interested in contrasting gene flow across Queen Charlotte Sound relative to gene flow across the range disjunction. We therefore organized our MIGRATE analyses to match our AMOVA analyses. We pooled all population samples from California, Vancouver Island, Haida Gwaii, and Alaska, into four regional samples, and estimated the 16 corresponding parameter values (four effective population sizes, 12 pairwise migration rates). For this number of parameters, it was not possible to run MIGRATE using our full population samples because the gene genealogies were prohibitively large. Consequently, we limited the analysis to the 102 individuals from Alaska ($n = 17$), Haida Gwaii ($n = 21$), Vancouver Island ($n = 30$), and California ($n = 34$), for which we had characterized all loci. The MCMC search was based on a chain of 20,000,000 (ATPS, GPI) or 50,000,000 (mtDNA) steps sampled every 40 or 100 steps for a total of 500,000 samples (with a burn-in of 50,000 samples). We used exponential prior distributions for migration rate (0, 1000) and effective population size (0, 0.01) because such

priors often explore parameter space much more efficiently than other prior distributions. We used adaptive heating of three additional chains to search more effectively the genealogy space for the highly polymorphic ATPS and GPI data (but not for mtDNA). We repeated this intron analysis using uniform (rather than exponential) priors to test for any bias caused by the exponential priors, and because parameter estimates under uniform (but not other) prior distributions can also be considered maximum likelihood estimates. We assessed convergence by comparing results from multiple runs. We analyzed the two introns separately and in parallel to obtain a single two-locus estimate of parameter values. We characterized effective population size as $\theta = 4N_e\mu$, and expressed the magnitude of gene flow estimated from these analyses as the number of migrants per generation $M = \theta m/\mu$. We used the “estimate” option in MIGRATE to infer the mutation rate parameter μ that scales both θ and M (Beerli 2006).

An alternative approach to accounting for the effects of recombination on such coalescent analyses is to estimate the recombination rate as part of the MCMC process using the whole sequence alignment (rather than analyzing a smaller portion of the alignment that is inferred to be nonrecombining). We used LAMARC v. 2.1.3 (Kuhner 2006) to estimate simultaneously recombination rate (the ratio of recombination events per site per generation to mutations per site per generation), migration, and effective population size for both the mtDNA sequences (which presumably were nonrecombining) and the full intron sequence alignments. Estimating recombination rate greatly increased the computational time for these MCMC analyses, so we further trimmed the data set (and the allelic genealogies) to a randomly selected group of 10 individuals (20 alleles) from each of the four pooled population samples (80 alleles total). We used a short chain of 1,250,000 steps sampled every 50 steps for a total of 25,000 samples (with a burn-in of 2500) and a long chain of 25,000,000 steps sampled every 100 steps for 250,000 samples (with a burn-in of 25,000). We used logarithmic prior distributions for recombination rate (0.001, 10), migration rate (0.01, 3000), and effective population size (0.00001, 10). We assessed convergence by monitoring the effective sample size for each parameter and by confirming similar results from multiple runs.

Results

POLYMORPHISM

We found 3–12 microsatellite alleles per locus (summed across all 12 populations), with standardized allelic richness of 3.9–5.5 (averaged across loci; Table 1). Observed and expected heterozygosities were ~ 0.3 to ~ 0.6 across all populations. We found homozygote excesses (high F_{IS}) significantly greater than zero in most populations ($0.13 < F_{IS} < 0.44$) and at most loci ($0.09 < F_{IS} < 0.70$), a characteristic of many broadcast-spawning

marine invertebrates (Addison and Hart 2005b). Five of 2856 total single-locus genotypes were null homozygotes. We found one case of a pair of loci (B11 and B202) in one population (Louise Narrows) in linkage disequilibrium; no other loci were in linkage disequilibrium within any other populations.

The mtDNA sequence alignment included 28 unique haplotypes that differed by up to five substitutions. There were 2–11 haplotypes per population, with haplotype diversities of 0.13–0.75 (Table 1). Nucleotide diversity was low ($\pi < 0.004$) for these slow-evolving tRNA sequences but varied about four-fold among populations.

We found 143 GPI alleles among 172 individuals, with 9–36 alleles per population that differed by up to 14 substitutions. Allele size ranged from 242 to 415 bp. The sequence alignment (Table S1) included 318 gap sites and was consistent with 70 insertion–deletion events of 15.0 bp average length. Both haplotype (0.74–0.99) and nucleotide (0.047–0.067) diversity were correspondingly high.

The ATPS sequences exhibited the greatest overall diversity: 252 alleles among 180 individuals, and 19–50 alleles per population, with up to 30 substitution differences. Allele size varied from 584 to 616 bp. The alignment (Table S2) included 158 gap sites, consistent with 59 insertion–deletion events of 4.2 bp average length. Most individuals in most populations were heterozygotes ($h \gg 0.9$) in part due to the many short insertion–deletion differences. Nucleotide diversity was slightly lower (0.033–0.042) than GPI diversity in the same samples.

Although there was no detectable association between latitude and mitochondrial diversity, the nuclear markers were generally less diverse in northern compared to southern populations. The sample site latitude (Table 1) was negatively correlated with average allelic richness for microsatellites ($r = -0.79$, $P = 0.002$) and with haplotype diversity for GPI ($r = -0.71$, $P = 0.047$). For the very high ATPS haplotype diversities, the correlation was slightly similar and nonsignificant ($r = -0.58$, $P = 0.135$), and there was no association between latitude and mitochondrial diversity. A permutation test suggested that Haida Gwaii and Alaska samples had significantly lower mean microsatellite allelic richness (4.0) than samples from California and Vancouver Island (5.3; $P = 0.003$). These differences in diversity appeared to be consistent with neutral variation (or at least not the product of large and easily detected nonneutral effects). After correction for 58 simultaneous tests of neutrality (D or F) across three loci, eight or 13 populations, and two methods, four values of F (from -5.56 to -22.04) for ATPS from Bodega Bay and for mtDNA from Louise Narrows, Tofino, and Bodega Bay, were significantly different from zero (neutral expectation). No values of D were significantly different from zero. The bias toward a few significant F (rather than D) values could reflect the information from invariant sites incorporated into the F statistic (329/635 bp for

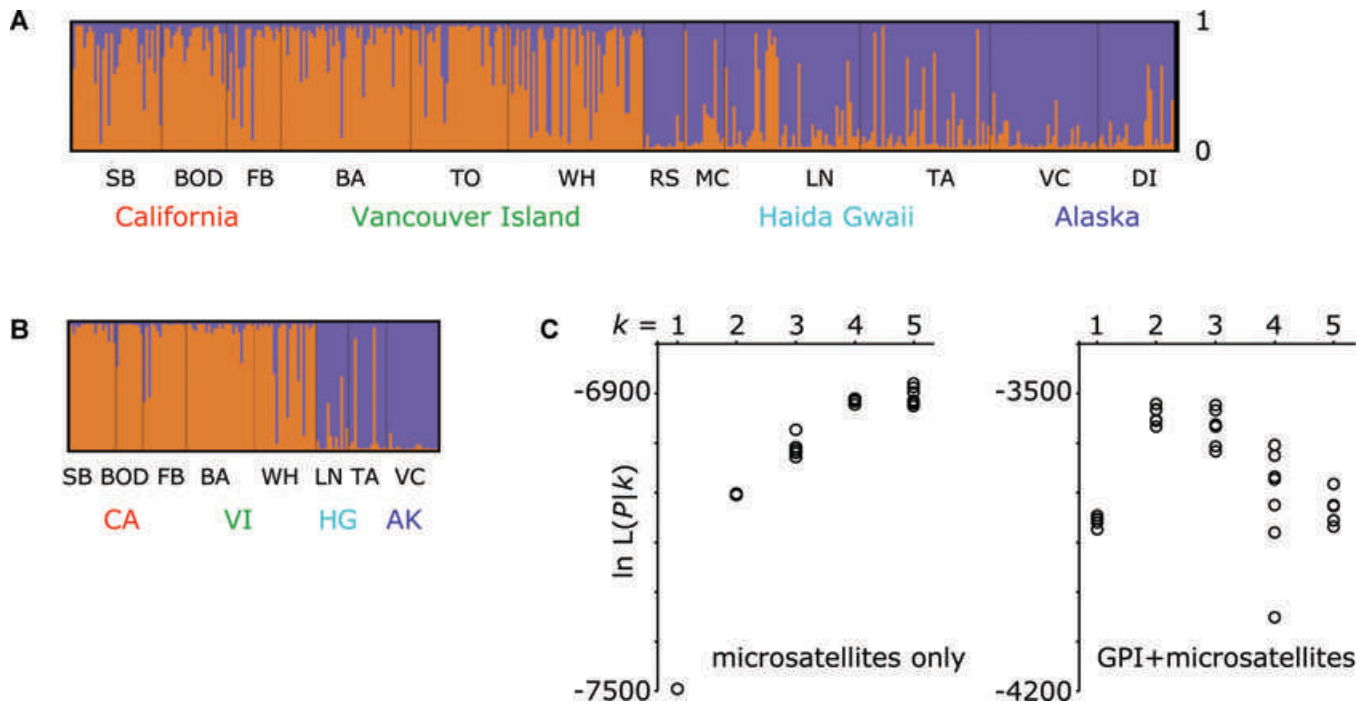


Figure 2. STRUCTURE clustering of *Patiria miniata* microsatellite (A) and GPI + microsatellite (B) genotypes. Both data sets suggest a strong phylogeographic break between samples north (Alaska, Haida Gwaii) and south (Vancouver Island, California) of Queen Charlotte Sound. The heights of individual bars in each panel show the probability of assignment to one of $k = 2$ empirically identified groups indicated by color (orange or purple). Sample site abbreviations (two- or three-letter acronyms) as in Table 1. Microsatellite data alone could be fitted to $k = 2$ groups (C, left) with a nonsignificant improvement in log-likelihood scores up to $k = 4$; the combined data (C, right) strongly suggested a best fit with $k = 2$ groups. Both data sets had a modal value of Δk (a measure of the best-fit number of genotype clusters) at $k = 2$.

ATPS, 273/476 bp for GPI, 346/369 bp for mtDNA). The result suggests some isolated cases of nonneutral deficit of haplotypes relative to segregating sites or number of alleles consistent with recent population expansion.

POPULATION STRUCTURE

Replicated analysis with the admixture model in STRUCTURE identified two significant clusters of microsatellite genotypes with very different frequencies in two geographic regions. One group of samples (shaded orange in Fig. 2A) occurred mainly in California and Vancouver Island, and the other (shaded purple in Fig. 2A) was mainly restricted to Haida Gwaii and Alaska. The marginal likelihood improvements with higher values of k (Fig. 2C) were not significant: the Δk statistic of Evanno et al. (2005) showed a strong mode at $k = 2$ that was about two orders of magnitude higher than at $k = 3$. Adding the GPI genotypes to the microsatellite data set confirmed the existence of just two significant clusters of genotypes with the same pronounced geographical difference in their distribution (Fig. 2B, C).

The geographical distribution of the mtDNA clades exhibited a similar north–south grouping. Most individuals (88%) had one of three haplotypes that differed from each other by one or

two substitutions; four other shared haplotypes occurred in 2–5 individuals. Of the three most common haplotypes, only one ($n = 42$) appeared in all four geographical regions from Alaska to California (Fig. 1). The single most common haplotype ($n = 120$) was entirely absent from our large sample of sea stars from Alaska and was rare in Haida Gwaii. The second most common haplotype ($n = 59$) was restricted to these two northern regions, as were two other shared haplotypes. Altogether, 70 of 96 DNA sequences from Alaska and Haida Gwaii samples were unique to those two regions north of Queen Charlotte Sound. South of Queen Charlotte Sound, the Vancouver Island and California populations shared two of the three most common haplotypes as well as two of the rarest haplotypes.

Networks of ATPS and GPI alleles based on analysis of the complete alignment (with recombination) or based on the largest nonrecombining block of nucleotide sites were considerably more complex (data not shown). Most alleles were relatively rare and separated from each other by large numbers of substitutions and insertion–deletion changes. Some geographical structure similar to the mtDNA results was evident in the form of shared alleles found only in Alaska and Haida Gwaii, or only in Vancouver Island and California samples.

Table 2. *Patiria miniata* population structure from analysis of molecular variance (AMOVA) in allele frequencies.

	Source of variation	d.f.	Sum of squares	% of variance	Fixation index	P-value
Microsatellites	Among populations	10	101.4	4.7	$F_{ST}=0.04$	<0.001
	Within populations	396	993.7	22.5	$F_{IS}=0.24$	<0.001
	Within individuals	6	632.0	72.7	$F_{IT}=0.27$	<0.001
mtDNA	Among populations	12	32.9	25.5	$F_{ST}=0.25$	<0.001
	Within populations	240	87.1	74.4		
ATPS	Among populations	7	178.6	2.4	$F_{ST}=0.02$	0.004
	Within populations	172	2257.7	15.5	$F_{IS}=0.16$	<0.001
	Within individuals	180	1716.1	82.1	$F_{IT}=0.18$	<0.001
GPI	Among populations	7	403.6	6.4	$F_{ST}=0.06$	<0.001
	Within populations	164	3582.4	73.4	$F_{IS}=0.78$	<0.001
	Within individuals	172	453.9	20.2	$F_{IT}=0.79$	<0.001

POPULATION DIFFERENTIATION

Global measures of deviation from equilibrium genotype and allele frequencies differed significantly from zero for all loci and suggested a considerable history of genetic drift within populations and changes in allele frequencies among some populations (Table 2). We used F_{ST} rather than R_{ST} in these comparisons for the microsatellite data because results from the allele-size permutation test (data not shown) suggested that allele size did not add useful information for any of the seven loci.

Population pairwise F_{ST} values were typically lower in comparisons among populations north or south of Queen Charlotte Sound, and considerably higher in comparisons between populations separated by Queen Charlotte Sound (see Table S3). Mantel tests identified weak signals of isolation-by-distance for microsatellites ($r = 0.26, P = 0.057$) and mtDNA ($r = 0.32, P = 0.022$), but not for ATPS ($r = 0.05, P = 0.325$), or GPI ($r = 0.21,$

$P = 0.131$). None of these correlations were significantly different from zero after Bonferroni correction for four simultaneous tests.

In an AMOVA that grouped populations into regions north and south of the range disjunction, differentiation between groups was small ($0.01 \leq \Phi_{CT} \leq 0.07$), was not significantly different from zero, and accounted for 0.1–7.5% of molecular variance (Table 3) for all marker types. A large proportion of the molecular variance (up to 21% for mtDNA) was due to differences among populations within these two groups, in particular to the many large pairwise differences between populations from Vancouver Island and those from Alaska and Haida Gwaii.

In contrast, when we grouped populations north and south of Queen Charlotte Sound (the population grouping found in our STRUCTURE analysis above), we found considerably greater differentiation between regional groups (as high as $\Phi_{CT} = 0.33$;

Table 3. *Patiria miniata* population structure from analysis of molecular variance (AMOVA) among population groups north (Alaska, Haida Gwaii, Vancouver Island) and south (California) of the geographic range disjunction (left) or north (Alaska, Haida Gwaii) and south (Vancouver Island, California) of the phylogeographic break at Queen Charlotte Sound.

Grouping by	Source of variation	Range disjunction			Phylogeographic break (QCS)		
		% of variance	Fixation index	P-value	% of variance	Fixation index	P-value
Microsatellites	Among groups	0.2	$\Phi_{CT} < 0.01$	0.286	5.2	$\Phi_{CT}=0.05$	<0.001
	Among populations groups	4.6	$\Phi_{SC}=0.04$	<0.001	1.6	$\Phi_{SC}=0.02$	<0.001
	Among individuals	22.4	$\Phi_{IS}=0.23$	<0.001	21.9	$\Phi_{IS}=0.23$	<0.001
mtDNA	Among groups	7.5	$\Phi_{CT}=0.07$	0.137	33.9	$\Phi_{CT}=0.33$	<0.001
	Among populations groups	21.1	$\Phi_{ST}=0.28$	<0.001	3.6	$\Phi_{ST}=0.37$	<0.001
	Within populations	71.3	$\Phi_{SC}=0.22$	<0.001	62.4	$\Phi_{SC}=0.05$	0.005
ATPS	Among groups	0.1	$\Phi_{CT}=0.01$	0.184	3.1	$\Phi_{CT}=0.03$	0.019
	Among populations groups	2.0	$\Phi_{SC}=0.02$	<0.001	0.8	$\Phi_{SC}=0.01$	0.030
	Among individuals	15.4	$\Phi_{IS}=0.15$	<0.001	15.2	$\Phi_{IS}=0.15$	<0.001
GPI	Among groups	2.6	$\Phi_{CT}=0.02$	0.179	11.1	$\Phi_{CT}=0.11$	0.016
	Among populations groups	5.0	$\Phi_{SC}=0.05$	<0.001	0.6	$\Phi_{SC}=0.01$	0.211
	Among individuals	72.6	$\Phi_{IS}=0.78$	<0.001	69.2	$\Phi_{IS}=0.78$	<0.001

Table 3). After correction for simultaneous comparisons for four markers, only the estimate of between-group differentiation for the very highly polymorphic ATPS ($\Phi_{CT} = 0.03$) was not significantly different from zero. For all four markers, this alternative grouping improved the among-groups sum of squares by a factor of ~ 2 (for highly polymorphic introns) to ~ 5 (for microsatellites and mtDNA).

GENE FLOW AND EFFECTIVE POPULATION SIZE

The analyses above based on allele frequencies and genetic distances consistently revealed one major phylogeographic break in *P. miniata*. We used the coalescent analyses to explore two aspects of this population genetic structure: (1) demographic differences among populations manifested as variation in effective population size; and (2) asymmetries in gene flow that might underlie patterns of allele frequency similarities between populations.

Some of the MIGRATE results suggested large differences in effective population sizes (Fig. 3), consistent with the frequency-based observations of genetic diversity (Table 1). In particular, the most probable estimates of θ based on introns from Alaska samples were one to three orders of magnitude lower than those for introns from all populations to the south (Fig. 7B). Among the latter populations, θ values for Vancouver Island and California populations were significantly higher than for Haida Gwaii. We found similar parameter estimates using uniform priors (Fig. 3) and using exponential priors (data not shown). The analyses of much less polymorphic mtDNA did not reveal such significant differences among population sizes (Fig. 3A).

Analysis of gene flow from the mtDNA data did not identify any significant differences in migration rates among these populations. In general, the MCMC search converged on coalescent patterns that emphasized widespread ancestral polymorphisms within populations to account for allele or haplotype sharing rather than high and variable rates of migration between populations (Fig. 3A).

In contrast, the considerably greater allelic diversity and higher divergence among alleles of the two introns allowed MIGRATE to identify several significant differences in migration rates, including higher M from Vancouver Island to California than from California to Alaska or Haida Gwaii, or from Vancouver Island to Alaska (Fig. 3B). We found similar results in separate analyses of the two intron alignments individually (data not shown). We obtained these more informative results despite the relatively short sequence alignments (113–343 bp) that could be identified as nonrecombining blocks suitable for MIGRATE analysis. The MIGRATE analysis of ATPS and GPI variation included low nonzero estimates of gene flow across Queen Charlotte Sound, most notably as north-to-south gene flow from Haida Gwaii to Vancouver Island (Table S4).

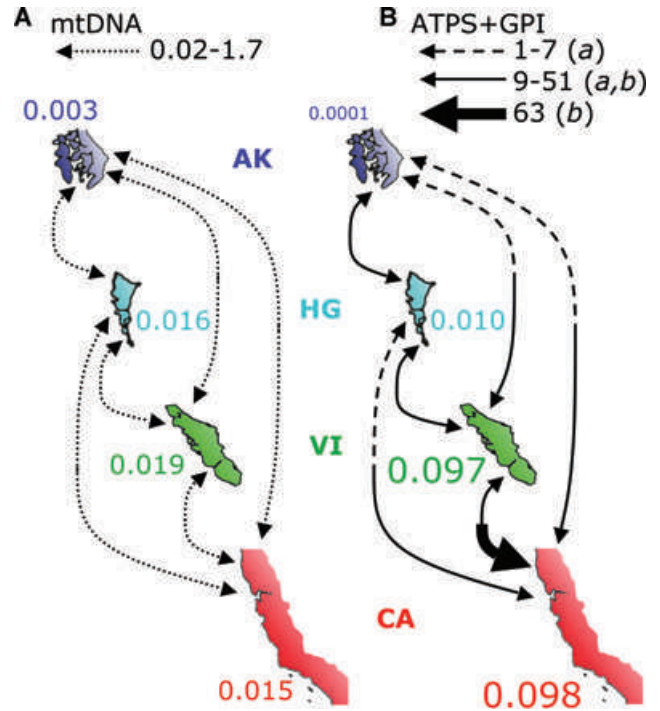


Figure 3. Asymmetrical gene flow (as migrants per generation, $M = \theta m/\mu$) and effective population sizes ($\theta = 4N_e\mu$) estimated in MIGRATE for mtDNA (A) and combined results from ATPS and GPI intron polymorphisms (B) in four regional population samples (see Fig. 1). Arrows show asymmetrical gene flow from one region to another, numbers show effective population size for each region. Estimates of θ for mtDNA were multiplied by 4 for comparison to diploid biparental nuclear markers. Within each panel, similar line weights indicate groups of migration rates with overlapping 95% confidence intervals in post hoc comparisons (see the legend for each panel); significant differences between migration rates in panel B are indicated by migration rates that do not share the same italic letters *a* or *b*. Asymmetries are shown as differences in line weight for the same arrow (e.g., between Vancouver Island and California for introns). Within each panel, different font sizes for θ show significant differences between regions. See the Supporting Information (Table S4) for most probable estimates and confidence intervals for θ and M values for each population, population pair, data set, and method.

The most probable estimates (and confidence intervals) of recombination rates from LAMARC analysis of the full GPI ($R = 0.46$, 0.31–0.58) and ATPS ($R = 1.21$, 0.91–1.68) alignments were high and repeatable in a second MCMC analysis (e.g., for GPI $R = 0.50$, 0.32–0.72). Because the ATPS alignment (635 bp) was based on longer sequences (584–616 bp) and included fewer indels than the 476 bp alignment of GPI alleles (242–415 bp), the very high ATPS recombination rate might be the more accurate estimate of the two in spite of the lower precision implied by the broader confidence interval. As expected, recombination rate estimated for mtDNA was low ($R < 0.001$) and not different from zero.

Parameter value estimates from these coalescent analyses were positively correlated across methods and genetic markers. For example, MIGRATE (Fig. 3A) and LAMARC converged on four similar pairs of θ estimates for the same mtDNA alignment ($r = 0.52$; see the Supporting Information). Similarly, the LAMARC analysis converged on 12 similar pairs of M estimates for ATPS and GPI alignments that differed in recombination rate, total length, and overall levels of polymorphism ($r = 0.90$).

Discussion

Previous reviews of empirical studies across oceans and taxa (Bohonak 1999; Grosberg and Cunningham 2001; Hellberg et al. 2002) have clearly articulated the expected patterns of population genetic variation for marine animals such as *P. miniata* with sedentary benthic adults and long-lived planktonic larvae. These patterns include (1) genetic homogeneity, perhaps with isolation-by-distance across the extremes of the range (Hellberg 1996); (2) genetic differentiation corresponding to present-day biogeographic features including physical oceanographic structure, range disjunctions, or boundaries between zoogeographic provinces that represent past or continuing barriers to dispersal (Burton 1998); or (3) population genetic signals of disjunction or vicariance that do not correspond to obvious contemporary barriers to gene flow (Benzie 1999). Identifying such patterns, documenting their relative frequencies, and understanding their causes is an important goal of evolutionary ecology and contributes to a general understanding of the opportunities for local adaptation, extirpation, and speciation in the seas (Foltz et al. 2008).

POPULATION GENETIC PATTERNS IN *P. MINIATA*

Two consistent and strong population genetic patterns emerged from our multilocus analysis of genetic structure in the bat star. First, most loci and methods of analysis showed strong and highly significant genetic differentiation between *P. miniata* populations north and south of Queen Charlotte Sound, including populations from southern Haida Gwaii and northern Vancouver Island separated by just a few hundred kilometers. Second, there was no evidence from any locus or method for genetic differentiation across the majority of the bat star geographic range from Vancouver Island to southern California that includes the broad distributional gap in Washington, Oregon, and northern California.

Our success in identifying these patterns from extremely variable intron sequence data depended on the method of analysis. Under infinite allele models based on allele and genotype frequencies (such as AMOVA) these data sometimes failed to identify the major phylogeographic break that was clearly and consistently evident in data from less polymorphic markers (such as mtDNA; Table 3). However, the introns were considerably more informative than other markers under coalescent models that em-

phasize allelic genealogies (such as MIGRATE; Fig. 3) because such methods use the magnitude and history of allelic divergence to estimate demographic parameters. Coalescent analysis of the intron data suggested large and highly significant differences in the effective population size that were consistent with severe population bottlenecks in Alaska and Haida Gwaii. We also found 10-fold higher rates of gene flow across the range disjunction in comparison to some rates of gene flow across Queen Charlotte Sound. Neither of these patterns was evident in similar analyses of the mtDNA data, probably due to the very shallow coalescent depth of the mtDNA phylogeny (Fig. 1). Such differences in information content across markers and models highlight the specific utility of adding highly variable nuclear sequences to phylogeographic studies (in coalescent analyses) and the importance of comparing such data against less variable markers (in frequency-based analyses).

In the absence of genetic information, the coincidence of the *P. miniata* geographic range disjunction and the southern extent of the last North American glaciation on the Pacific coast might imply that bat star populations gradually expanded out of northern and southern glacial refuges, leaving descendants that established the extant populations north and south (respectively) of the range disjunction. However, the discordance between the location of the phylogeographic break and the geographic range disjunction—and the genetic affinity between Vancouver Island and California populations—allows us to reject this simple prediction. Our data and analyses are consistent with either high gene flow across the range disjunction or recent colonization of Vancouver Island by California migrants. A third scenario (which would be difficult to reject on the basis of genetic data alone) is recent local extirpation of bat star populations in Washington, Oregon, and northern California that fragmented a formerly more continuous species range. However, under any of these scenarios, the origin and maintenance of the range disjunction seems to require an ecological rather than a geological or historical explanation.

The origin and maintenance of the phylogeographic break at Queen Charlotte Sound may be more easily associated with geological and oceanographic dispersal barriers. A coalescent estimate of population divergence time between bat stars from Alaska and Vancouver Island using mtDNA and six anonymous nuclear DNA sequence markers (T. McGovern, C. Keever, M. Hart, C. Saski, and P. Marko, unpubl. data) is $\sim 80,000$ years ago. This divergence time is consistent with population isolation due to changes in climate, sea level, and oceanographic circulation associated with the last glacial episode (or with other ecological or geological processes operating on the same time scale but not associated with glacial cycles). The phylogeographic break that we observed coincides with a west-to-east surface current (the North Pacific Current) that diverges at about the same latitude

to form the northward Alaska and southward California current systems (Cummins and Freeland 2007). High-resolution models of density- and wind-driven circulation in the northeast Pacific (Foreman et al. 2008) suggest that exchange of *P. miniata* larvae across Queen Charlotte Sound may be limited. This physical current structure could contribute to the maintenance of a phylogeographic break established by Pleistocene climate change in spite of the prolonged 6- to 10-week period of planktonic larval growth and development in bat stars (Strathmann 1987; Rumrill 1989; Basch 1996).

COMPARISON TO CLOSE NEIGHBORS AND CLOSE RELATIVES

Bat stars share a similar range disjunction with two other rocky intertidal species, the kelp *Eisenia arborea* and the turban snail *Astraea gibberosa*. Future phylogeographic studies of these species across the shared range disjunction could test the hypothesis of a shared ecological cause for this unusual distribution (if all three species show the same population genetic pattern across the disjunction; Bermingham and Moritz 1998; Avise 2000).

Bat stars also share a similar phylogeographic break with a taxonomically heterogeneous group of snails, fishes, and sea cucumbers (see Marko 2004; Hickerson and Cunningham 2005) that all lack a long-lived planktonic larval stage in the life cycle. Our study is the first from this well-known zoogeographic transition zone to show strong population genetic differentiation in a species with long-lived feeding larvae and high intrinsic dispersal potential. Among members of the same community with planktotrophic development, our results contrast with those for the abundant keystone predator *Pisaster ochraceus*. Harley et al. (2006) found no significant mtDNA differentiation in this sea star species at Queen Charlotte Sound (or at any other well-known phylogeographic breaks such as Point Conception in California; Dawson 2001). *Pisaster ochraceus* and *P. miniata* have broadly overlapped geographical distributions but occur in different habitats and on different substrates (Lambert 2000). If the phylogeographic break between bat star populations was initiated by late Pleistocene glaciations and maintained by the North Pacific Current (as suggested above), then these two species may also differ in their response to Pleistocene climate change (and population history) or in their response to present-day ocean currents and the tendency to cross Queen Charlotte Sound during larval development. The nature of such differences is unknown.

Bat stars are members of a clade of shallow-water sea stars (Family Asterinidae) in which highly derived mating systems and modes of dispersal have evolved in parallel in several genera (Hart et al. 1997; Byrne 2006; Keever and Hart 2008). As expected, strong population genetic structure has been found in asterinid species with benthic development of brooded larvae (Hunt 1993; Matsuoka and Asano 2003; Waters et al. 2004; Baus et al.

2005; Colgan et al. 2005; Sherman et al. 2008). These results suggest that evolutionary changes in mode of larval development are important determinants of population genetic structure in asterinids. However, the discovery of unexpected and strong phylogeographic breaks in *P. miniata* and another asterinid (from New Zealand; Waters and Roy 2004) with planktotrophic development suggests that extrinsic physical or geological barriers and historical processes might also have significant effects on population genetic variation in asterinids. Phylogenetic comparative analyses (e.g., Kyle and Boulding 2000; Collin 2001; Kelly and Eernisse 2007; Teske et al. 2007; Sherman et al. 2008) can complement community-level analyses of the contribution of biogeographic history, mode of development, and ecological process to the evolution of population genetic structure. Such phylogenetic comparative analyses using multiple asterinid lineages and genetic markers could be used to estimate the phylogenetic correlations between evolutionary changes in life history and population genetic structure, and the residual contributions of other (geological or ecological) processes in shaping the evolution of population genetic structure.

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Supporting Information

The following supporting information is available for this article:

Table S1. Sequence alignment for GPI alleles.

Table S2. Sequence alignment for ATPS alleles.

Table S3. Population pairwise F_{ST} values for seven microsatellites (plus the average across loci), mtDNA, ATPS, and GPI. Two- or three-letter population identifier acronyms as in Table 1.

Table S4. Effective population size (θ) and number of migrants per generation (M) for mtDNA, ATPS, and GPI (estimated in MIGRATE and in LAMARC).

Supporting Information may be found in the online version of this article.

(This link will take you to the article abstract).

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