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COLONIZATION, DISPERSAL, AND HYBRIDIZATION INFLUENCE PHYLOGEOGRAPHY OF NORTH ATLANTIC SEA URCHINS (*STRONGYLOCENTROTUS DROEBACHIENSIS*)

JASON A. ADDISON[1] AND MICHAEL W. HART[2]
Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada

Abstract.—We used frequency-based and coalescent-based phylogeographic analysis of sea urchin (*Strongylocentrotus droebachiensis*) mitochondrial DNA (mtDNA) sequences and previously published microsatellite data to understand the relative influence of colonization and gene flow from older (north Pacific) and younger (northeast Atlantic) sea urchin populations on genetic variation in the northwest Atlantic. We found strong evidence of survival of northwestern Atlantic populations in local Pleistocene glacial refugia: most haplotypes were the same as or closely related to Pacific haplotypes, with deep gene genealogies that reflect divergence times within the northwestern Atlantic that are much older than the last glacial maximum. We detected gene flow across the North Atlantic in the form of haplotypes shared with or recently descended from European populations. We also found evidence of significant introgression of haplotypes from a closely related species (*S. pallidus*). The relative magnitude of gene flow estimated by coalescent methods (and the effective population size differences among oceanic regions) depended on the genetic marker used. In general, we found very small effective population size in the northeastern Atlantic and high trans-Arctic gene flow between the Pacific and northwestern Atlantic. Both analyses suggested significant back-migration to the Pacific. However, microsatellites more strongly reflected older Pacific migration (with similar effective population sizes across the Arctic), whereas mtDNA sequences appeared to be more sensitive to recent trans- Atlantic dispersal (with larger differences in effective population size). These differences across marker types might have several biological or methodological causes, and they suggest caution in interpretation of the results from a single locus or class of markers.

Key words.—Coalescent analysis, cytochrome *c* oxidase subunit I, Echinoidea, introgression, microsatellites, mitochondrial DNA, range expansion.

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Quaternary sea level fluctuations and climate change have influenced the genetic structure and evolutionary history of many North Atlantic marine organisms (e.g., van Oppen et al. 1995; Dahlgren et al. 2000; Wares and Cunningham 2001; Väinölä 2003). Following the opening of the Bering Strait some 7.4 to 4.8 million years ago (mya; Marincovich and Gladenkov 1999), many Pacific taxa invaded the Arctic and North Atlantic Oceans (e.g., Vermeij 1991). Fossil evidence suggests that sea urchins in the genus *Strongylocentrotus* moved into the Atlantic at least 3.5 mya (Durham and MacNeil 1967). Following these invasions, eustatic sea level changes during the Pleistocene ice ages (2.4 my) are thought to have periodically closed these migratory routes causing widespread isolation and vicariance in the north Atlantic (Hewitt 1996; Cunningham and Collins 1998). These glacial cycles of about 0.1 my have dominated the climate of the northern hemisphere over the last 0.7 my (Hewitt 1996). The last glacial maximum (about 0.02 mya) was thought to have severely affected the population biology of obligate rocky intertidal species of the northwestern (NW) Atlantic because glaciers likely covered the full extent of their rocky habitat (Ingolfsson 1992). For example, a comparative phylogeographic analysis revealed that several species in the NW Atlantic were extirpated and subsequently recolonized from glacial refuges in the northeastern (NE) Atlantic (Wares and Cunningham 2001). Estimated times of colonization calculated using coalescent methods were concordant with a range expansion after the last glacial maximum. However, the much longer coalescent times for a barnacle (*Semibalanus balanoides*) and a mussel (*Mytilus edulis*) were consistent with patterns of glacial refugia in the NW Atlantic (rather than extirpation and recent recolonization). Wares and Cunningham (2001) suggest that NW Atlantic populations of these species persisted because their long-lived planktonic larvae were capable of quickly dispersing into suitable habitat during periods of rapid environmental change such as fluctuations in glacial extent and the appearance or elimination of suitable rocky habitats.

Population genetic studies can be used to assess the relative contributions of different sources of population genetic variation such as early Quaternary trans-Arctic range expansion versus post-Pleistocene trans-Atlantic gene flow. Such comparisons may be useful in predicting the potential for local adaptation to environmental change following range expansion or the limits on local adaptation imposed by larval dispersal between different environments (Helmuth et al. 2002; Marko 2004). Our study of microsatellite variation in green sea urchins (*Strongylocentrotus droebachiensis*) suggests that populations in the NW Atlantic are more closely related to those in the Pacific than to populations in the NE Atlantic (Addison and Hart 2004). The biogeographic history of NW Atlantic populations of sea urchins is further complicated by a relatively recent trans-Arctic connection to ancestral Pacific populations. Although there is evidence of trans-Atlantic gene flow inferred from sea urchin microsatellites, the pattern of population genetic variation in this species is consistent with either glacial refugia in the NW Atlantic or extinction followed by extensive recolonization from the Pacific (rather

[1] Present address: Department of Ecology and Evolutionary Biology, Earth and Marine Science Building, University of California, Santa Cruz, California 95064; E-mail: addison@biology.ucsc.edu.
[2] Present address: Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada; E-mail: mike_hart@sfu.ca.

than recolonization from refuges in the NE Atlantic). Recent gene flow from the Pacific has been detected for several marine species (Meehan et al. 1989; Taylor and Dodson 1994; Rawson and Hilbish 1995; van Oppen et al. 1995; Väinölä 2003) including *Strongylocentrotus droebachiensis* (Palumbi and Wilson 1990) and *S. pallidus* (Palumbi and Kessing 1991). These studies indicate that climate warming during interglacial periods promoted both trans-Arctic and trans-Atlantic dispersal of sea urchins, but the relative influence of these events on population genetic structure in the NW Atlantic is unknown.

We compiled mtDNA (cytochrome *c* oxidase subunit I COI) sequence data for a subset of the samples used in our previous microsatellite DNA study (Addison and Hart 2004). We used these sequences to characterize patterns of genetic diversity within the NW Atlantic and compare traditional measures of population genetic structure with those previously reported for microsatellites. We also used a coalescent-based analysis to estimate the effective population sizes and migration rates among the three oceanic regions (Pacific, NW Atlantic, NE Atlantic). An attractive feature of this analysis is that it is suitable for both allelic data (microsatellites) and DNA sequences, and can be used to compare inferences from these commonly used classes of genetic markers concerning the influences of Pleistocene climate change and trans-Arctic and trans-Atlantic migration on the patterns of genetic diversity within the NW Atlantic. We were specifically interested in examining four different hypotheses that could explain patterns of genetic variation relative to these biogeographical processes. (1) If glacial cycles dramatically affected populations of sea urchins on both coasts of the Atlantic Ocean, then estimates of effective population size should show a dramatic reduction when compared to the ancestral Pacific populations. (2) If the last glacial maximum caused the extirpation of sea urchins in the NW Atlantic, followed by a trans-Arctic or a trans-Atlantic migration event, then these geologically young populations should exhibit relatively low haplotype diversity compared to the source population and should have a high frequency of haplotypes that are either identical to or recently descended from haplotypes in the founding population (Zink et al. 2000; Wares and Cunningham 2001). (3) If the Quaternary biogeography of *S. droebachiensis* is similar to some other north Atlantic rocky habitat marine invertebrates, then parsimony and maximum likelihood analyses should produce haplotype networks or phylograms in which NW Atlantic haplotypes are nested within a set of NE Atlantic haplotypes and reflect a strong influence of recent trans-Atlantic gene flow from east to west (Wares 2001; Wares and Cunningham 2001). (4) Alternatively, if North Atlantic *Strongylocentrotus* biogeography is more strongly influenced by trans-Arctic gene flow (Palumbi and Wilson 1990; Palumbi and Kessing 1991), then haplotypes in NW Atlantic populations may be derived from (and shared in common with) the older and more diverse Pacific populations.

Materials and Methods

Sampling

Samples of *Strongylocentrotus droebachiensis* were obtained as described in Addison and Hart (2004). Samples incorporated in this analysis include individuals from the San Juan Islands (Pacific Ocean), Vestfjorden (Norway), Hvalfjordur (Iceland), and six NW Atlantic populations (Table 1). Two specimens identified on the basis of morphological characters as *Strongylocentrotus pallidus* collected from the Pacific population were also included as a potential outgroup.

DNA Extraction, Polymerase Chain Reaction, and Sequencing

Genomic DNA was extracted from ethanol preserved gonad tissue using a standard cetyltrimethylammonium bromide (CTAB) protocol (Grosberg et al. 1996). Polymerase chain reaction (PCR) products for cytochrome *c* oxidase subunit I (COI) were obtained using the forward primer COIC 5′-TC GTCTGATCCGTCTTTGTCAC-3′ and reverse primer COIJ 5′-CAATACCTGTGAGTCCTCCTA-3′ described by Edmands et al. (1996). These primers amplified a 476 bp section of the COI gene corresponding to base positions 6357–6832 of the *S. purpuratus* genome (Jacobs et al. 1988; Edmands et al. 1996). Amplifications were performed under oil in 12.5 μl volumes containing 10 ng of template DNA, 10 mM Tris-HCL, 50 mM KCl, 0.1% TritonX-100, 0.2 mM of each dNTP (Fermentas, Burlington, Ontario), 2 mM $MgCl_2$ (MBI), 0.3 pmol of each primer, and 0.25 units of *Tsg* Polymerase (Biobasic, Toronto, Canada). The thermal cycling protocol consisted of 37 cycles of 30 sec denaturation at 95°C, 60 sec annealing at 55°C, and 60 sec elongation at 72°C.

Amplified products were visualized in 1.5% agarose (1X TBE) using ethidium bromide. Approximately 75 ng of each product was ethanol precipitated and one strand was sequenced with the COIC primer using Li-Cor (Lincoln, NE) IRD-700 Dye Terminators following the manufacture's protocol. The cycle sequencing profile consisted of an initial denaturation at 95°C for 3 min followed by 32 cycles of 30 sec at 94°C, 30 sec at 50°C, and 60 sec at 72°C. Excess dye terminators were removed using Sephadex G-50 columns (Sigma-Aldrich, Oakville, Ontario) and the reaction products were resolved in 6% (25 cm, 0.2 mm thick) denaturing polyacrylamide gels using a Li-Cor DNA 4200L-2. Sequences from two *S. pallidus* individuals and 181 *S. droebachiensis* individuals were aligned and edited using the image analysis software provided by Li-Cor.

Statistical Analysis

MtDNA sequence variation and patterns of genetic diversity

Edited sequences were aligned using ClustalX (Thompson et al. 1997) and translated using Gene Jockey (Ferguson, MO). Mean sequence divergence (number of differences) within and among populations was calculated using MEGA version 2.1 (Kumar et al. 2001). Haplotype and nucleotide diversity, tests of selective neutrality (Tajima's *D*; Tajima 1989), analysis of molecular variance (AMOVA; Excoffier et al. 1992), pairwise *F*-statistics, and mismatch distributions were calculated using Arlequin 2.001 (Schneider et al. 2000). For the AMOVA, populations were partitioned into the three separate oceanic regions (Pacific, NW Atlantic, NE Atlantic) that corresponded to the scale of geographic subdivision previously detected using nuclear markers (Addison and Hart

TABLE 1. Sea urchin (*Strongylocentrotus droebachiensis*) sampling location number and name (abbreviation), sample size (*n*), number of segregating sites (*S*), number of haplotypes observed (*H*), identifiers of haplotypes present (IDs), diversity measures (with standard deviations), and tests of neutrality (Tajima's *D*) calculated using 418 bp of COI. Haplotypes found only in the Pacific, NE Atlantic, or NW Atlantic have identifiers beginning with P, E, or W, respectively; shared haplotypes are labeled PW or EW, respectively. Sampling location numbers and abbreviations, and haplotype identifiers are the same as those in Table 2 and Figures 2 and 3.

Sampling location	*n*	*S*	*H*	IDs	Haplotype diversity	Nucleotide diversity	Tajima's *D*
1. San Juan Islands, Washington (SJI)	22	23	12	P2, P3, P4, P5, P6, P7, P8, P9, P10, P11, P12, PW1	0.761 (0.099)	0.014 (0.008)	−0.089
2. Conception Bay, Newfoundland (CBY)	7	24	4	W18, W22, W27, PW1	0.809 (0.129)	0.024 (0.014)	0.172
3. Bonne Bay, Newfoundland (BBY)	7	10	3	W18, PW1, EW23	0.666 (0.159)	0.008 (0.005)	−0.608
4. Harve St. Pierre, Quebec (HSP)	7	12	4	W19, W20, PW1, EW23	0.809 (0.129)	0.009 (0.006)	−0.998
5. Miramichi, New Brunswick (MBY)	21	14	6	W18, W19, W20, W25, PW1, EW23	0.761 (0.068)	0.012 (0.006)	1.056
6. East Jeddore, Nova Scotia (JED)	42	17	10	W13, W15, W16, W17, W18, W19, W20, W21, PW1, EW14	0.724 (0.065)	0.005 (0.003)	−1.371
7. Bear Cove, Nova Scotia (BCV)	48	26	10	W16, W17, W18, W19, W20, W24, W26, W28, W29, PW1	0.670 (0.072)	0.009 (0.005)	−1.168
8. Vestfjorden, Norway (NOR)	15	2	3	E30, EW14, EW23	0.590 (0.077)	0.001 (0.001)	0.220
9. Hvalfjordur, Iceland (ICE)	12	3	4	E30, E31, EW14, EW23	0.560 (0.154)	0.002 (0.001)	−0.128
Regional total:							
Pacific	22	23	12		0.761 (0.099)	0.014 (0.008)	−0.089
NW Atlantic	132	30	18		0.716 (0.039)	0.009 (0.005)	−0.858
NE Atlantic	27	3	4		0.592 (0.081)	0.001 (0.001)	0.024

2004). In Tajima's test of selective neutrality, negative *D*-values result from a higher number of segregating sites compared to pairwise distance, and may be indicative of selection or changes in population size (Tajima 1989). The significance of Tajima's *D* was tested by generating 1000 random samples under the hypothesis of selective neutrality and population equilibrium.

Phylogenetic Analysis

Neighbor-joining (NJ) trees were constructed for haplotypes within each oceanic region using Kimura two-parameter distances using MEGA. We constructed a 95% statistical parsimony network using TCS 1.18 (Clement et al. 2000). A maximum likelihood (ML) phylogenetic tree for all unique haplotypes was estimated using PAUP* (Swofford 2002) with a two-parameter substitution model including among-site rate variation and some invariable sites (K80 + I + G substitution model), tree bisection-reconnection branch swapping, and zero branch lengths collapsed. The proportion of invariable sites (I) was 0.848, the transition/transversion ratio (Ti/Tv) was 4.103, and the gamma distribution parameter (α) was 0.774. The model and parameters were estimated using hierarchical likelihood ratio testing in Modeltest 3.06 (Posada and Crandall 1998). We estimated nodal support from 100 bootstrap replicates of the ML analysis in PAUP*.

Effective Population Size and Migration Rates

We used a coalescent approach to calculate the effective population size (Θ) and asymmetrical migration rates between the major oceanic regions from the genealogical structure of both the mtDNA haplotypes and the alleles from the three microsatellite loci characterized in our previous study (Addison and Hart 2004). For this analysis we used the computer program MIGRATE 1.7.6.1 (Beerli and Felsenstein 2001; Beerli 2002), which employs Markov chain Monte Carlo (MCMC) sampling of gene trees to estimate Θ and asymmetrical migration rates among populations. To circumvent the extremely long computational time required to analyze our three-locus microsatellite DNA dataset (n = 962; Addison and Hart 2004), we pooled populations within each oceanic region and randomly sampled 40 individuals from each (n = 120). Because the locus *Sd76* appears to be fixed for null alleles in NE Atlantic (but not other) populations, we did not include it in this analysis. Each locus was analyzed separately using a stepwise mutation model. The MCMC searching strategy employed a series of 10 short chains (5000 gene trees) and three long chains (50,000 gene trees). Initial parameters were estimated using the default settings and the first 10^4 trees were ignored to ensure parameter stability. We repeated this analysis three times and the integrated estimates were used as input for a more exhaustive search using eight heated chains {static:1:1, 1.1, 1.3, 1.5, 2, 3, 5, 10}. A similar approach was used for the analysis of mtDNA sequences and we used the same ML model parameters above. The searching strategy was replicated five times for the microsatellites and 10 times for the mtDNA sequences. Parameter estimates were integrated across the replicates (and loci) and the analyses were repeated twice more to ensure stability. For both marker types, results from all three analyses were similar and we

TABLE 2. Pairwise F_{ST} (above diagonal) and their *P*-values (below diagonal) calculated using 1023 permutations of the mtDNA data. *P*-values in bold are significantly different from zero ($P < 0.05$). Population abbreviations are the same as in Table 1.

Region	Sampling location	Pacific	NW Atlantic						NE Atlantic	
		SJI	BBY	CBY	HSP	MBY	JED	BCV	ICE	NOR
Pacific	SJI		0.265	0.213	0.219	0.236	0.255	0.286	0.325	0.317
NW Atlantic	BBY	**<0.001**		−0.033	0.022	0.029	−0.017	−0.030	0.394	0.379
	CBY	**<0.001**	0.635		−0.043	−0.057	0.013	0.009	0.169	0.192
	HSP	**<0.001**	0.437	0.793		−0.067	−0.038	0.005	0.259	0.261
	MBY	**<0.001**	0.217	0.902	0.925		0.013	0.037	0.203	0.220
	JED	**<0.001**	0.547	0.276	0.797	0.220		−0.005	0.335	0.316
	BCV	**<0.001**	0.692	0.295	0.301	0.065	0.536		0.369	0.360
NE Atlantic	ICE	**<0.001**	**<0.001**	**0.025**	**0.004**	**0.001**	**<0.001**	**<0.001**		0.049
	NOR	**<0.001**	**<0.001**	**0.025**	**0.008**	**<0.001**	**<0.001**	**<0.001**	0.182	

report only one of them here. Effective population sizes are reported as Θ, which translates to $4N_e\mu$ for nuclear DNA and $N_e\mu$ for maternal haploid mtDNA, assuming an equal sex ratio. Similarly, we report migration rates as m/μ, calculated as $4N_em/\Theta$ and N_em/Θ for nuclear and mitochondrial markers, respectively.

RESULTS

Haplotype Diversity

We obtained 418 bp COI sequences (positions 6415–6832 of Jacobs et al. 1988) for all 183 individual sea urchins. There were 32 variable sites and a total of 33 unique haplotypes (GenBank accession numbers: AY504479–AY504511). Mean haplotype and nucleotide diversity were highest for Pacific samples and lowest for NE Atlantic samples (Table 1). Sequence divergence (number of differences) was high between the outgroup *S. pallidus* and *S. droebachiensis* (up to 5.5%), but was highest between two samples that were identified on the basis of morphological traits (Jensen 1974; Kozloff 1987) and microsatellite genotypes (Addison and Hart 2002, 2004) as *S. droebachiensis* (5.7%). Overall mean sequence divergence among the haplotypes was 2.2 ± 0.4%.

Values of Tajima's *D* calculated within populations and across regions ranged from −1.371 to 0.220 (Table 1). Negative results indicate (directional) selection or recovery from a population bottleneck. However, no values of Tajima's *D* were significantly different from zero ($P > 0.05$, 1000 random samples). This result indicates that the segment of COI included in our analyses is evolving under a model of selective neutrality or that sea urchin populations sampled at this spatial scale show little evidence of recent population expansion.

Population Structure

The AMOVA of the mtDNA haplotypes indicated a moderate amount of genetic differentiation among the three oceanic regions ($\phi_{CT} = 0.287$, $P = 0.002$). All trans-Arctic pairwise-F_{ST} values were significant and ranged from 0.213 to 0.325 (Table 2). Larger pairwise values were detected in some trans-Atlantic comparisons (e.g., East Jeddore, Bear Cove, Bonne Bay, Newfoundland and Labrador), all of which were statistically significant ($P \leq 0.025$; Table 2). One interpretation of this difference is greater gene flow across the Arctic than across the Atlantic. There was a small but significant amount of genetic differentiation among populations within the NW Atlantic or NE Atlantic oceanic regions ($\phi_{SC} = 0.006$, $P < 0.001$), but tests for pairwise population differentiation (F_{ST}) revealed no significant differences. These results are qualitatively similar to those reported using microsatellites: significant genetic structure among regions ($F_{ST} = 0.087$, $P < 0.05$) and genetic homogeneity within the NW Atlantic ($F_{ST} = 0.001$, $P > 0.05$; Addison and Hart 2004).

Mismatch Distribution

The mismatch distribution for populations in the NE Atlantic was unimodal and reflects the shallow haplotype phylogeny (Fig. 1) and low nucleotide diversity (Table 1). However, the Pacific and NW Atlantic populations showed multimodal mismatch distributions and deeper haplotype phylogenies (Fig. 1). This reflects a low frequency of highly divergent haplotypes (i.e., smaller peaks in the right-hand part of the distribution) corresponding to either gene flow across the North Atlantic or introgression of haplotypes between species. Multimodal curves such as these are also expected when subdivided populations expand with limited gene flow (e.g., $Nm < 20$) among regions (Ray et al. 2003).

Phylogenetic Analysis

The maximum likelihood analysis converged on eight equivalent trees with a −ln(L) score of 932.7 (Fig. 2). The equivalent trees differed in relationships near the tips, and none of the differences were strongly supported by bootstrapping (one tree is shown in Fig. 2). The deepest divergence between samples distinguished a clade of six haplotypes that included both individuals that were identified as *S. pallidus* (haplotypes Sp32 and Sp33; Fig. 2). Four other *pallidus*-like haplotypes in this clade (P3, P6, W24, W28) came from individuals from Pacific or NW Atlantic populations that had *droebachiensis*-like morphological and microsatellite markers.

The remaining *S. droebachiensis* haplotypes did not form monophyletic groups with respect to sampling location: geographically diverse populations shared identical COI haplotypes, and haplotypes collected from both the Pacific and NW Atlantic oceanic regions were widely distributed across the phylogeny. The majority of the haplotype diversity in the NW Atlantic was descended from a single haplotype shared with the Pacific population (Figs. 2, 3), and the shared hap-

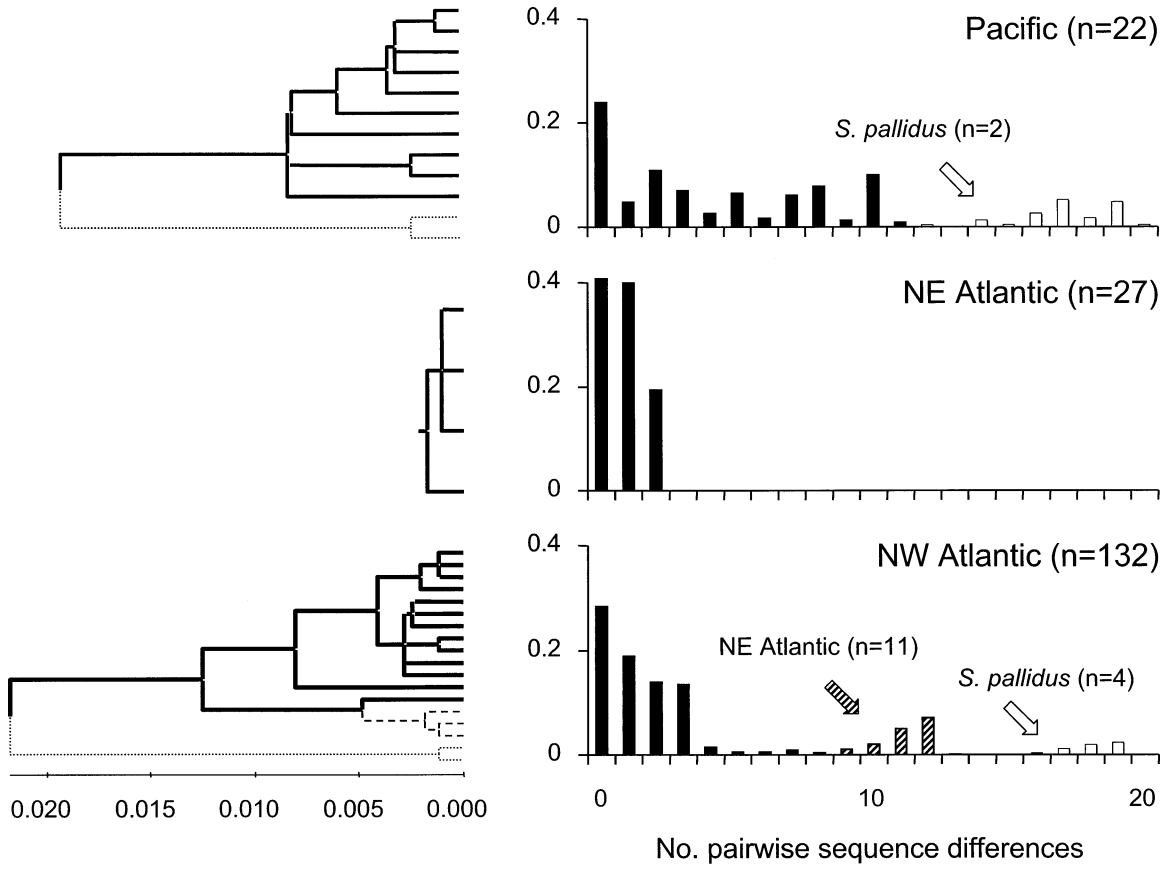

FIG. 1. Divergence and structure of COI haplotypes from *Strongylocentrotus droebachiensis* populations in the Pacific, NE Atlantic, and NW Atlantic. For each biogeographic region we show on the left the phylogenetic relationships among the haplotypes (linearized neighbor-joining tree, Kimura two-parameter distances), and on the right the frequency of pairwise nucleotide differences (mismatch distribution). The haplotype trees were rooted with the *S. pallidus* sequences (Sp32, Sp33; not shown) and are drawn to the same scale. Pairwise genetic distances in the NW Atlantic that involve haplotypes derived from the NE Atlantic (haplotypes W26, EW14, EW23 in Figs. 2 and 3) are indicated by dashed lines in the neighbor-joining tree and hatched bars in the histogram; distances in the Pacific and NW Atlantic that involve haplotypes derived from *S. pallidus* by hybridization (haplotypes P3, P6, W24, W28) are indicated by dotted lines in the neighbor-joining trees and open bars in the histograms.

lotype (PW1) was widespread among NW Atlantic populations. This result is consistent with a major colonization event from the Pacific to the NW Atlantic following which many haplotypes descended from the colonizer but without lineage sorting and extinction of the colonizing haplotype. Alternatively, the shared haplotype could have originated in the NW Atlantic and entered the Pacific more recently, a pattern that is consistent with the location of PW1 in the ML phylogeny (Fig. 1) and with our coalescent analysis (see below). These results are comparable to our analysis of microsatellite allele frequencies across the Arctic (Addison and Hart 2004), which indicate that the history of connection between the Pacific and the NW Atlantic is one of either repeated gene exchange or massive historical migration followed by little genetic drift and lineage sorting in NW Atlantic populations.

Haplotypes in the NE Atlantic were related to a different set of Pacific sequences than those in the NW Atlantic. The TCS analysis left some relationships between Pacific and NE Atlantic haplotypes as ambiguities (Fig. 3) and these relationships were largely unresolved in the ML phylogram (Fig. 2). For example, four Pacific haplotypes (P4, P5, P8, P11) can be related to haplotypes common in the NE Atlantic (EW14, EW23), but these four haplotypes are highly divergent from other Pacific haplotypes and the TCS algorithm linked them (and several others) by a relatively large number of unobserved intermediates to other Pacific haplotypes via a NW Atlantic haplotype (W27; Figs. 2, 3). This linkage seems biogeographically unrealistic, and might reflect our undersampling of a much greater haplotype diversity within Pacific populations.

Haplotype diversity was lower in the NE Atlantic than in the NW Atlantic. Three of the four NE Atlantic haplotypes were found in both Norway and Iceland, and two of these haplotypes were also found at low frequency in the NW Atlantic (Fig. 3). One shared haplotype (EW23) was found only in western Newfoundland and the Gulf of St. Lawrence, and the other (EW14) was found only in Nova Scotia. There was one unique haplotype in the NW Atlantic (W26) that was descended from a NE Atlantic ancestor. The widespread occurrence of these shared haplotypes in NW Atlantic populations suggests substantial trans-Atlantic gene flow into the NW Atlantic; the difference in haplotype diversity across the north Atlantic may be maintained in spite of this gene flow by a severe historical bottleneck in the NE Atlantic (so that

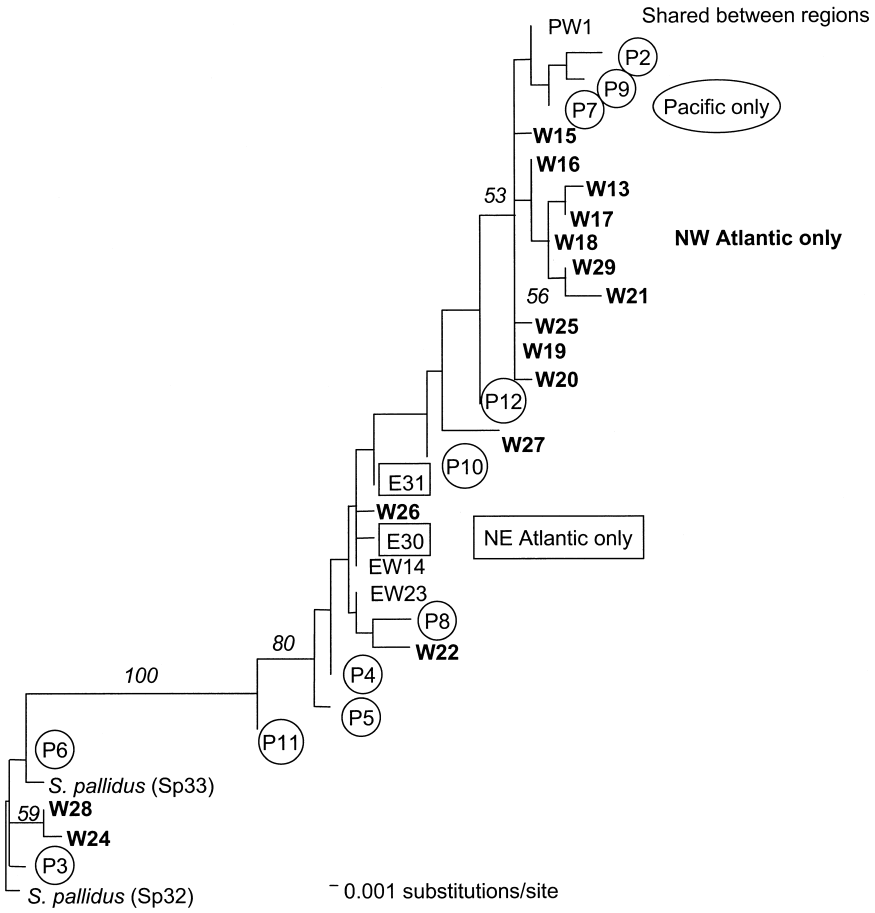

FIG. 2. Maximum likelihood phylogram of the 33 *Strongylocentrotus* COI haplotypes (this is one of eight trees of equivalent −ln(L) score). Numbers in italics next to nodes show bootstrap percentages >50%. Thirty-three unique haplotypes are indicated by identifiers (P1-Sp33) at branch tips. Those haplotypes found only in the Pacific Ocean are labeled P and outlined with circles, NW Atlantic are labeled W in bold, NE Atlantic are labeled E and outlined with squares, and those shared between regions are labeled PW or EW in plain text. Outgroup sequences are *S. pallidus* and labeled Sp.

few haplotypes are available to be exported to the NW Atlantic) and by contributions to the NW Atlantic from Pacific sources. The rarity of new haplotypes descended from these two shared haplotypes suggests that this trans-Atlantic gene flow was relatively recent in comparison to colonization from the Pacific.

Effective Population Size and Migration Rates

The effective population sizes estimated for all three regions using three-locus microsatellite genotypes indicate similar values for the Pacific and NW Atlantic while populations of sea urchins in the NE Atlantic were smaller by a factor of 15 (Table 3). In contrast, mtDNA sequences showed a fivefold reduction in effective population size from the Pacific to the NW Atlantic, and an additional fourfold reduction from the NW to the NE Atlantic (Table 3).

Patterns of gene flow inferred from the two classes of markers were also different, and included some gene flow in unexpected directions. The microsatellite analysis indicated that gene flow out of the Pacific to either Atlantic region was relatively high, and that gene flow out of the NE Atlantic was relatively low (either across the Arctic or across the Atlantic). Surprisingly, gene flow out of the NW Atlantic was also inferred to be relatively high, including substantial gene flow to NE Atlantic populations and back-migration of alleles to ancestral Pacific populations. In contrast, only three of six mtDNA estimates of gene flow were substantial, including trans-Arctic gene flow in both directions between the Pacific and NW Atlantic. The largest mtDNA gene flow we inferred was a relatively massive contribution of NE Atlantic haplotypes to NW Atlantic populations (about fourfold greater than Pacific contributions to NW Atlantic haplotypes), compared to the relatively small magnitude of this vector inferred from microsatellites.

DISCUSSION

Phylogeographic History

Despite first arriving in the NW Atlantic 3.5–3 mya (Durham and MacNeil 1967), the current population genetic structure of *Strongylocentrotus droebachiensis* has been strongly shaped by recent trans-Arctic and trans-Atlantic migration events. Dispersal (possibly by long-lived planktonic larvae) has resulted in regional panmixia without local differentiation

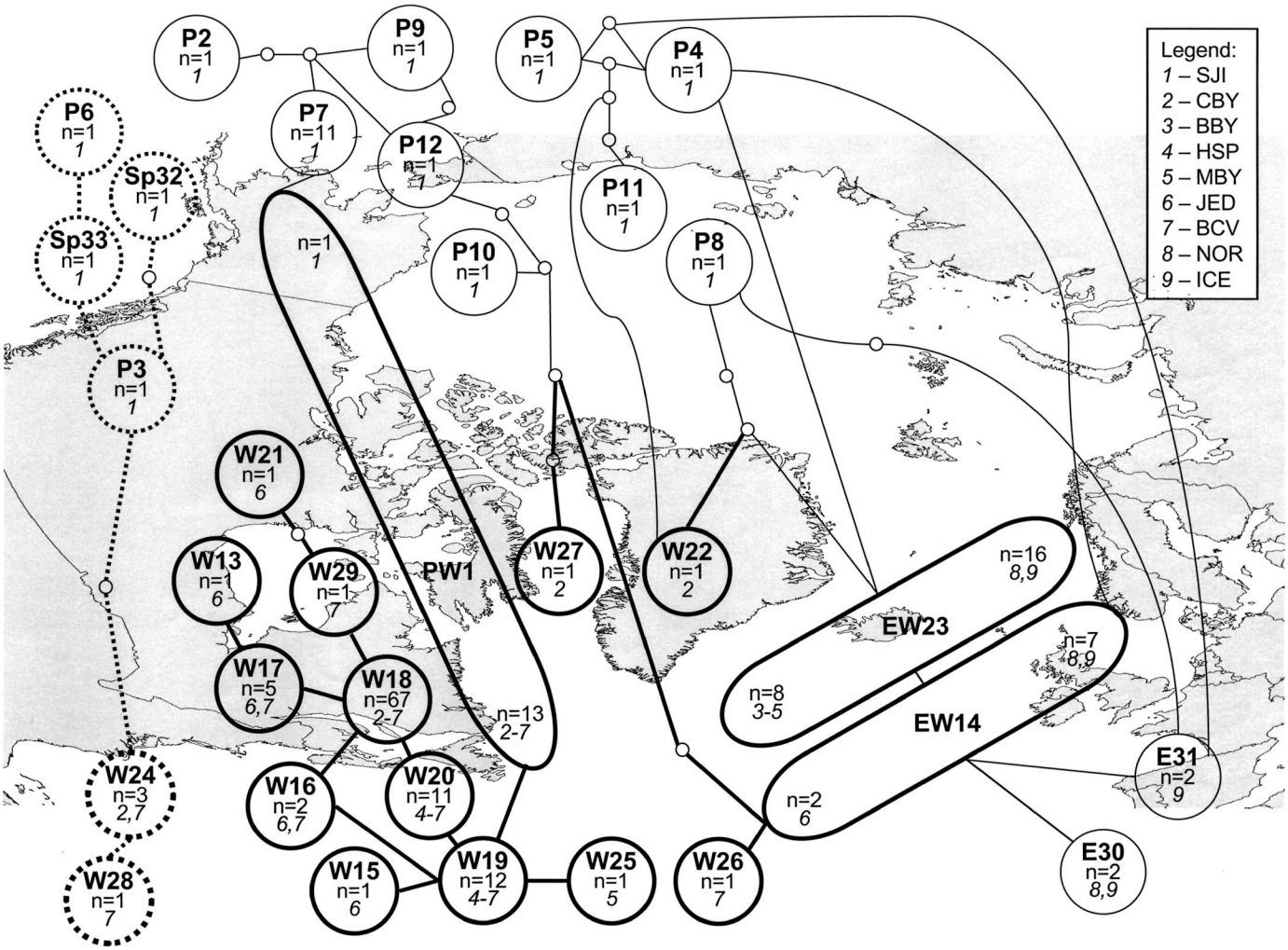

FIG. 3. 95% plausible networks for *Strongylocentrotus* COI haplotypes. Bold lines surround and join haplotypes found in the NW Atlantic; those shared among oceanic regions (PW1, EW14, EW23) are represented by ovals. Haplotype identifiers (as in Table 1 and Fig. 2) are in bold; n is the haplotype sample size; numbers in italics indicate the population in which the haplotypes were detected (SJI, Pacific; NOR, ICE, NE Atlantic; for detailed resolution of NW Atlantic populations see fig. 1 in Addison and Hart 2004). Lines connect haplotypes that differ by one mutation; small open circles indicate missing intermediate haplotypes. Samples of *S. pallidus* (Sp32, Sp33) and four haplotypes from *S. droebachiensis* (P3, P6, W24, W28) form a separate network in the Pacific and NW Atlantic that is indicated by heavy dotted lines.

at either nuclear (Addison and Hart 2004) or mitochondrial loci. However, the analysis of molecular variance (AMOVA) revealed a significant genetic discontinuity among the three oceanic regions (ϕ_{CT} = 0.287, P = 0.002). This result is consistent with our previous analysis using microsatellite DNA (Addison and Hart 2004). In light of the known glacial history of the Arctic and north Atlantic, the polyphyletic nature of the Pacific and Atlantic COI haplotypes indicates that a history of sporadic migration has prevented any complete isolation of populations throughout the species range. The small amount of divergence among regions and the sharing of haplotypes between the Pacific and one (but not both) Atlantic region suggests that the populations on both coasts of the north Atlantic share a long, and independent, history of colonization from the Pacific.

Most of the sea urchin mtDNA diversity in the NW Atlantic is identical or closely related to a single Pacific haplotype (PW1), and these findings are consistent with the pattern described by Palumbi and Wilson (1990). This similarity to previous results by Palumbi is striking because we increased the sampling of individuals within the NW Atlantic more than sixfold and increased our sampling of nucleotide sites using sequences (rather than restricted fragment-length polymorphisms). Because we only detected one shared haplotype between the two oceans, it is unlikely that gene exchange is continuous. However, our previous results using microsatellite DNA indicate that the magnitude of gene exchange across the Arctic was large enough to produce broad allelic similarity between the two oceans, with small pairwise F_{ST} values between Pacific and NW Atlantic populations of 0.019 to 0.032 (based on data in Addison and Hart 2004). Our estimates of coalescent-based migration rates presented here confirm this observation. One possible explanation for this observation is that lineage sorting has resulted in the com-

TABLE 3. Effective population size ($\Theta = 4N_e\mu$ for nDNA, $\Theta = N_e\mu$ for mtDNA) and migration rates ($m/\mu = 4N_em/\Theta$ for nDNA, N_em/Θ for mtDNA) (with upper and lower 95% profile confidence limits in parentheses) calculated for nuclear and mitochondrial DNA markers based on the full MIGRATION-n model. Parameter estimates integrated across microsatellite loci, and estimates from mtDNA sequences, are highlighted in bold for ease of comparison. Results were obtained using a maximum likelihood estimator based on the coalescent in the computer program MIGRATE 1.7.6.1 (Beerli and Felsenstein 2001).

Region	Marker	n	Locus	Effective population size	Migration rates		
					Pacific to:	NW Atlantic to:	NE Atlantic to:
Pacific	nDNA	37	Sd156	9.963	—	15.655	2.685
		39	Sd121	7.054	—	13.536	2.561
		40	Sd63	7.573	—	17.829	4.836
			All	**6.706** (5.788–8.254)	—	**14.990** (12.494–20.770)	**3.614** (2.354–4.694)
	mtDNA	22	COI	**0.036** (0.022–0.062)	—	**128.688** (35.909–369.921)	**<0.001** (<0.001–0.004)
NW Atlantic	nDNA	40	Sd156	4.957	16.655	—	7.009
		40	Sd121	4.258	17.454	—	5.358
		40	Sd63	4.985	21.279	—	8.930
			All	**5.374** (4.613–6.285)	**17.100** (13.818–28.226)	—	**6.801** (5.290–8.674)
	mtDNA	132	COI	**0.005** (0.004–0.007)	**84.280** (15.441–264.255)	—	**333.736** (108.701–678.085)
NE Atlantic	nDNA	40	Sd156	0.405	8.628	15.925	—
		40	Sd121	0.426	9.869	10.463	—
		40	Sd63	0.397	10.281	15.535	—
			All	**0.381** (0.342–0.437)	**11.472** (6.726–14.346)	**14.167** (11.042–18.306)	—
	mtDNA	27	COI	**0.001** (<0.001–0.002)	**<0.001** (<0.001–339.875)	**<0.001** (<0.001–83.625)	—

plete loss of all but three colonizing mtDNA haplotypes (PW1, W22, W27) in the NW Atlantic, of which one (PW1) and its descendents now dominate the NW Atlantic. However, we may have failed to detect other shared haplotypes because of our limited sampling in the Pacific.

The haplotype phylogram (Fig. 2) and the most parsimonious ancestor-descendant relationships among haplotypes (Fig. 3) can be used together to examine the ages of colonization events from the Pacific to the NW Atlantic. If the shared haplotype PW1 (Fig. 3) originated in the Pacific and colonized the NW Atlantic, then we can use the mean divergence between PW1 and its NW Atlantic descendants (by counting substitution differences between 418 bp sequences in Fig. 3) with the method of Saillard et al. (2000) to estimate the mean age (and variance) of that colonization event. COI divergence rate calibrations for different sea urchin genera of 1.6% and 3.5% my^{-1} (Lessios et al. 1999, 2001; McCartney et al. 2000) give mean (variance) ages for the colonization of the NW Atlantic by PW1 of 0.41 (0.20) mya and 0.19 (0.09) mya, respectively. This timeline is slightly older than the previous estimation of migration 0.3 mya (Palumbi and Wilson 1990), and much more recent than the opening of the Bering Strait (>3.0 mya). Both estimates are consistent with gene flow during the interglacial periods in the late Pleistocene, and like other studies of the trans-Arctic exchange (e.g., Ortí et al. 1994) they suggest that recent gene flow has largely replaced the original genetic contribution of early colonizers that reached the NW Atlantic shortly after the opening of the Bering Strait. This calculation assumes that none of the NW Atlantic haplotypes that appear to be descended from PW1 are in fact shared with Pacific populations via more recent gene flow events, an assumption that may be dubious in light of our coalescent inference of relatively frequent trans-Arctic gene flow.

Two other NW Atlantic haplotypes (W22, W27; Fig. 3) were nested deeply in the phylogram (Fig. 2), which suggests that these might be remnants of one or more older colonization events that preceded the invasion of the NW Atlantic by PW1 and could be dated to much older nodes in the haplotype phylogram (possibly as old as the earliest trans-Arctic range expansion from the Pacific to the NW Atlantic). A scenario of multiple colonizations of different ages would be consistent with our coalescent estimates of surprisingly high rates of gene flow between the two oceans, and it seems likely to us that several (perhaps many) independent gene flow events from the Pacific have contributed to NW Atlantic genetic variation. However, the low bootstrap support for deep nesting of W22 and W27 in the phylogram (Fig. 2), and the ambiguous ancestor-descendant relationships between W22, W27, and other haplotypes (the closed circuits in Fig. 3), prevented us from estimating the age(s) of the older colonization(s) in the same way that we estimated the age of the colonization of the NW Atlantic by PW1. Both W22 and W27 are ambiguously connected by only two to four substitutions either to haplotypes from the Pacific (P8, P10, P11) or to other haplotypes that are shared across the north Atlantic (EW14, EW23). In the former case, W22 and W27 might represent descendants of other trans-Arctic gene flow events; in the latter case, these haplotypes might represent trans-Atlantic gene flow.

The patterns and time lines summarized above are not consistent with a hypothesis of local extirpation of sea urchins in the NW Atlantic followed by recent recolonization from the NE Atlantic after the last glacial maximum, and suggest instead the persistence of *S. droebachiensis* in glacial refugia in the NW Atlantic during more recent glacial cycles (see Holder et al. 1999). Two of the haplotypes detected in the NE Atlantic (EW14, EW23) are found in the NW Atlantic, and we found just one unique haplotype (W26) in the NW Atlantic that was descended from a NE Atlantic ancestor by a mutation event following gene flow from east to west. Wares and Cunningham (2001) found similar patterns in other NW Atlantic species that they interpreted as evidence of local extirpation and recent trans-Atlantic recolonization (with little time for the subsequent accumulation of mutations in descendants of these recent colonizers), in contrast with evidence for persistence through Pleistocene glacial cycles in other species that must have survived in refugial populations in the NW Atlantic. Our results for *S. droebachiensis* are more similar to the latter patterns. A larger effective population size in the NW Atlantic than in the NE Atlantic based on the coalescent analysis of mtDNA sequence data (Table 3) also argues against recent establishment of NW Atlantic populations from Pleistocene glacial refugia in the NE Atlantic.

Based on microsatellite analyses, genetic exchange between the Pacific and NE Atlantic appears to be low. Although the estimated magnitude of migration from the Pacific into the NE Atlantic is similar to that estimated for the NW Atlantic, both allele diversity (Addison and Hart 2004) and COI haplotype diversity are lowest in NE Atlantic populations of sea urchins. These results are consistent with either a recent founding event or a long history of isolation and low effective population size. Evidence for the former hypothesis is weak because we found no shared COI haplotypes between the Pacific and NE Atlantic (Castelloe and Templeton 1994; Wares 2001), though founder haplotypes should be shared between regions if there has been little time for lineage sorting to occur. It is clear that finer resolution of the historic relationship among NE Atlantic and Pacific sea urchins requires more sampling throughout the Canadian Arctic archipelago, Scandinavia, Russia, and Greenland.

Effective Population Size and Migration Rates

Effective population sizes calculated for the three oceanic regions based on coalescent analyses of both mtDNA and microsatellites are consistent with a Pleistocene population bottleneck on both coasts of the Atlantic. Both types of marker showed lower Θ in Atlantic than in Pacific sea urchins. The effect of a bottleneck appears to have been much more severe in the NE Atlantic (where allele and haplotype diversity are low) than in the NW Atlantic (where mtDNA and microsatellite Θ were relatively high compared to the NE Atlantic, and microsatellite Θ was only slightly lower than in the Pacific). The observation that sea urchins in NE Atlantic populations are fixed for one or more null alleles at the microsatellite locus *Sd76* is also consistent with the expected effects of bottleneck events.

If the relative influence of trans-Arctic larval dispersal to the NW Atlantic was greater than trans-Atlantic dispersal, then the relative magnitude of these two gene flow vectors calculated using coalescent analyses is expected to be similar for nuclear and mitochondrial loci. Instead, we found evidence of greater trans-Atlantic gene flow from mtDNA, but greater trans-Arctic gene flow from microsatellites. This difference does not appear to be caused by selection acting on the mitochondrial genome (Table 1). One possible scenario that could explain this difference is based on the pattern of shared COI haplotypes between regions. The microsatellite estimate of gene flow from the Pacific to the NW Atlantic may be greater than NE to NW Atlantic gene flow because nuclear allele similarity between Pacific and NW Atlantic populations reflects the influence of multiple colonization events described above, whereas the trans-Atlantic distribution of nuclear alleles is influenced only by recent gene flow that contributed a small fraction of the standing variation in the NW Atlantic. In contrast, the mtDNA estimate of trans-Atlantic gene flow may be greater than gene flow from the Pacific because we found two COI haplotypes (EW14, EW23) shared across the Atlantic but only one (PW1) shared across the Arctic. Furthermore, the two trans-Atlantic haplotypes are abundant and widespread in the NE Atlantic (two of four haplotypes, in 23 of 27 individuals), so the vector from the NE to NW Atlantic is large because so much of the (relatively modest) variation among NE Atlantic sea urchins is also present in NW Atlantic populations. This is not true for the trans-Arctic comparison, where the shared haplotype PW1 is rare in a much larger pool of Pacific haplotype variation. If this explanation holds, then it suggests caution in the interpretation of coalescent-based migration estimates among populations with very different demographic histories. A second possible explanation for the discrepancies between microsatellite and mtDNA results could involve differences between the mutational models for mtDNA and microsatellite loci (and the correspondence between these models and real sequence or allelic evolution in sea urchins). If other comparisons among these loci in other organisms confirm such a difference, then this result would argue for the development and use of improved mutation models and for caution in the use of coalescent-derived migration rates based on mtDNA sequences or nuclear loci alone. A third plausible explanation, which would be consistent with differences in gene flow directions inferred from mitochondrial and nuclear markers (but for which we have no evidence), is some major cytonuclear genetic incompatibility that limits the establishment of NE Atlantic nuclear alleles in the NW Atlantic and prevents the invasion of NW Atlantic haplotypes into the NE Atlantic. Riginos et al. (2004) proposed such incompatibility as the basis for selection against trans-Atlantic gene flow of paternally inherited (but not maternally inherited) mtDNA sequences in mussels. Our estimates of Tajima's *D* argue against such selection influencing haplotype frequencies, but there are few alternative scenarios that could explain these results and do not involve selection.

The Arctic Ocean as a Barrier to Dispersal

The patterns of local genetic homogeneity among *S. droebachiensis* populations with significant genetic differentiation

across the Arctic are consistent with other population genetic studies of sea urchins with dispersing planktonic larvae. Temporary barriers to dispersal caused by sea level changes (and concomitant changes in the speed and direction of surface ocean currents for larval dispersal) likely caused significant genetic differentiation within several species of tropical sea urchins in the Indo-West Pacific (*Diadema*: Lessios et al. 2001; *Eucidaris*: Lessios et al. 1999). This effect of historic dispersal barriers, caused by surface currents that facilitate gene flow in some directions but not others, is also evident in many other studies of marine invertebrates with an Indo-West Pacific range (Benzie 1999). The temporally variable dispersal barriers across the Arctic and North Atlantic formed during the Pleistocene glaciations have had a similar effect on the population genetics of *S. droebachiensis*. Other global barriers to dispersal detected in sea urchins include the Isthmus of Panama, the long stretches of ocean that separate eastern and western regions of both the Atlantic and Pacific, cold water upwelling off the tip of South Africa, and the freshwater plume of the Orinoco and Amazon rivers (Lessios et al. 2003).

Several studies of sea urchins suggest that population genetic patterns are often not concordant among species with similar larval dispersal abilities. One interpretation of these patterns is that some biogeographic barriers are differentially porous to dispersal by larvae of different species. For example, gene flow is limited between Caribbean and Brazilian populations of the sea urchins *Tripneustes* (Lessios et al. 2003), *Diadema* (Lessios et al. 2001), and *Echinometra* (McCartney et al. 2000), but not of *Eucidaris* (Lessios et al. 1999). Across the Indo-Pacific ranges of these genera, *Tripneustes* species show genetic homogeneity at this scale, but *Diadema* and *Eucidaris* do not. The discordant population genetic patterns shown by species with similar high larval dispersal ability suggest that population genetic structure across a broad geographic range is likely the result of the stochastic nature of larval dispersal and colonization (Palumbi 1996; McCartney et al. 2000), and that patterns of allele and haplotype frequency variation may be difficult to interpret clearly in terms of contemporary gene flow alone. Analogous differences between species without planktonic larval dispersal (Marko 2004) also suggest that dispersal ability alone may not be a good predictor of the scale and magnitude of genetic differentiation between populations.

Genetic studies of marine organisms with circum-Arctic ranges also suggest that both trans-Arctic and trans-Atlantic dispersal is sporadic but effective. For example, phylogenetic analyses of clam (*Macoma balthica*) and mussel (*Mytilus edulis*) species complexes reveal strikingly similar histories of recent trans-Arctic contact between populations (Rawson and Hilbish 1995; Väinölä 2003). The divergence between members of both of these taxa began soon after the original introductions of *Macoma* and *Mytilus* to the North Atlantic following the opening of the Bering Strait. Their population genetic patterns have been interpreted in the context of repeated invasions of the Atlantic by larval dispersal from Pacific populations, which resulted in secondary contact between divergent Pacific and Atlantic lineages on both Atlantic coasts. Recent or repeated trans-Arctic dispersal has also been described for other organisms including fishes (Ortí et al. 1994) and marine algae (van Oppen et al. 1995). However, in other species the trans-Arctic colonization events were thought to have occurred prior to the major glaciation events of the Middle Pleistocene (Reid 1990; Zaslavskya et al. 1992; Taylor and Dodson 1994).

Although populations in the Pacific and NW Atlantic are differentiated from each other at both mtDNA and nuclear loci, we found surprising evidence that gene flow across the Arctic may occur in both directions. The biogeographic history of marine animals that formed the trans-Arctic exchange is usually interpreted in terms of movement from ancestral Pacific to descendant Atlantic populations (Vermeij 1991), but both mtDNA and microsatellite coalescent analyses suggested that back-migration to the Pacific is similar in magnitude to gene flow in the other direction (Table 3). We suspected that this result for mtDNA might reflect our much more intensive sampling of haplotype diversity in the NW Atlantic, but the confirmation of this pattern for three unlinked microsatellite loci (for which we subsampled similar numbers of individuals in each region) suggests that the pattern may be real and that the biogeographical history of trans-Arctic organisms may be much more complex than we expected. Although this pattern has not been widely considered, the remarkably high population density and fecundity of *S. droebachiensis* in the NW Atlantic (e.g., Meidel and Scheibling 2001) may provide an unanticipated source of propagules and genes that can be exported back to the Pacific.

The North Atlantic as a Barrier to Dispersal

The previous comparative studies by Wares and Cunningham (2001) of trans-Atlantic mtDNA phylogeography revealed that the NW Atlantic rocky substratum communities include a mixture of species that were locally extirpated and re-established by recent range expansion from glacial refuges in Europe and other species that must have survived with relatively large effective population sizes in NW Atlantic refuges. Other analyses have expanded the list of species that probably persisted in NW Atlantic refuges (F. Harper, unpubl. data). Westheide et al. (2003) suggest that trans-Atlantic dispersal by polychaete larvae might be frequent and effective. Our results suggest that the views of both Palumbi and Wilson (1990) and Wares and Cunningham (2001) are essentially correct. Trans-Atlantic dispersal of sea urchin larvae has transported a few mtDNA haplotypes from Europe to North America (Wares and Cunningham 2001), but in the context of apparently greater and older colonization from the Pacific (Palumbi and Wilson 1990) and survival of those original NW Atlantic populations during the Quaternary glaciations, more recent trans-Atlantic gene flow has had a less substantial influence on nuclear (Addison and Hart 2004) and mitochondrial population genetic structure (Fig. 3).

Nevertheless, our coalescent analyses indicate gene flow in both directions across the north Atlantic. As in our trans-Arctic comparisons, we found large differences in migration rates for the two marker types, for which there are several possible explanations (discussed above). Mitochondrial gene flow appears to be relatively large from the NE Atlantic to the NW Atlantic (as suggested by Wares and Cunningham 2001) in comparison to our other mtDNA gene flow esti-

mates, but microsatellite gene flow is inferred to be much larger from the NW Atlantic to the NE Atlantic in comparison to other microsatellite gene flow estimates. The absence of amplifiable alleles at a fourth microsatellite locus (*Sd76*) in all NE Atlantic sea urchins that we sampled (and the ubiquity of such alleles in the NW Atlantic) suggests to us that there is not much significant introduction of NW Atlantic nuclear alleles into NE Atlantic populations, or that there is selection against the introgression of alleles at some loci (perhaps *Sd76*) but not others (e.g., Riginos et al. 2004).

Combining Nuclear and Mitochondrial Markers

One useful outcome of our combination of mtDNA haplotypes and nuclear microsatellite allelic markers was the ability to identify the striking contribution of introgression to haplotype variation. Interspecific hybridization with *S. pallidus* was detected throughout the range of *S. droebachiensis* in the NW Atlantic and Pacific. However, the overall contribution to haplotypic diversity in the NW Atlantic was small (two haplotypes in four individuals). The patterns of hybridization detected in this study are not the patterns that would be predicted from the results of Strathmann (1981). In controlled laboratory crosses, Strathmann (1981) concluded that the eggs of *S. droebachiensis* were readily fertilized by the sperm of *S. pallidus*, but fertilization rates were very low in the reciprocal cross. We found *S. pallidus* mtDNA haplotypes in individuals morphologically identified as *S. droebachiensis*, a pattern that is only likely to be produced when *S. pallidus* eggs are fertilized by *S. droebachiensis* sperm. These same individuals had *droebachiensis*-like microsatellite genotypes. The two species' nuclear genomes could be qualitatively distinguished because the microsatellites described in Addison and Hart (2002) failed to amplify in any known *S. pallidus* samples that we tested (suggesting that *S. pallidus* populations are fixed for null alleles at these microsatellite loci). The combination of *pallidus*-like maternal markers and fully *droebachiensis*-like nuclear and morphological markers suggests that these four individuals were descendants of female hybrids by one or more generations of matrilineal backcrossing with male *S. droebachiensis* (and not male *S. pallidus*). Such differential backcrossing would be consistent with Strathmann's (1981) hybridization results.

Second, the combination of haplotypes and microsatellites for the same set of sea urchin populations allows us to put the measures of microsatellite population differentiation into an independent context. The biological significance of allozyme or microsatellite F_{ST} estimates that are numerically small but statistically significantly different from zero is sometimes questionable. Because the microsatellite loci have been used only in *S. droebachiensis*, the microsatellite results cannot be put into the context of previous allozyme studies of other sea urchins. However, our measures of population differentiation based on COI haplotypes can be put into the more general context of previous sea urchin studies using mitochondrial protein-coding genes. For example, Edmands et al. (1996) used COI haplotypes to measure population differentiation as $F_{RT} = 0.064$ between groups of *S. purpuratus* north and south of Los Angeles, California; the corresponding *F*-statistic based on allozymes was small and not significantly different from zero. We found pairwise F_{ST} values of 0.21–0.28 based on COI haplotype comparisons between Pacific and NW Atlantic *S. droebachiensis* populations (Table 2); the corresponding measure of microsatellite differentiation among 11 Atlantic and Pacific populations was $F_{ST} = 0.087$ (Addison and Hart 2004). The significant haplotype differentiation in *S. droebachiensis* (comparable to haplotype differentiation detected in other sea urchins using homologous sequences) suggests that our previous estimates of numerically small levels of population differentiation using microsatellites may be biologically highly significant.

Finally, the disparate results obtained using the two different classes of markers highlight the importance of using multiple loci in phylogeographic analyses. The different mutation rates and effective population sizes of mtDNA and microsatellites enabled the finer resolution of historic migration and reduction in effective population sizes among the oceanic regions. As a consequence, the two classes of markers appear to be differentially sensitive to relatively recent or ancient sea urchin dispersal events. Analyzed independently, neither dataset would have provided a complete picture of the temporal and spatial patterns of gene flow influencing patterns of genetic diversity within the NW Atlantic.

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Corresponding Editor: G. Ortí