

INTROGRESSION VERSUS IMMIGRATION IN HYBRIDIZING HIGH-DISPERSAL ECHINODERMS

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Phylogeographic studies designed to estimate rates and patterns of genetic differentiation within species often reveal unexpected and graphically striking cases of allele or haplotype sharing between species (introgression) via hybridization and backcrossing. Does introgression between species significantly influence population genetic structure relative to more conventional sources of differentiation (drift) and similarity (dispersal) among populations within species? Here we use mtDNA sequences from four species in two genera of sea urchins and sea stars to quantify the relative magnitude of gene flow across oceans and across species boundaries in the context of the trans-Arctic interchange of marine organisms between the Pacific and Atlantic oceans. In spite of the much smaller distances between sympatric congeners, rates of gene flow between sympatric species via heterospecific gamete interactions were small and significantly lower than gene flow across oceans via dispersal of planktonic larvae. We conclude that, in these cases at least, larvae are more effective than gametes as vectors of gene flow.

KEY WORDS: *Asterias*, fertilization, gene flow, larval dispersal, phylogeography, speciation, *Strongylocentrotus*, trans-Arctic interchange.

Gene flow from conspecific populations (immigration) and from other species (introgression) can each contribute to local genetic diversity, limit differentiation among populations, moderate effects of inbreeding, and affect rates of local adaptation, speciation, and extinction (Avice 2004; Arnold 2006). The relative significance of these two sources of genetic diversity has rarely been compared in a direct and quantitative way. Some recent studies suggest that introgression can be a significant source of genetic

variation in hybridizing species groups (e.g., Kronforst et al. 2006) in which individual species also show evidence of substantial long-distance gene flow by dispersal (Kronforst and Fleming 2001; Davies and Bermingham 2002). Vollmer and Palumbi (2007) noted that distinguishing hybridization from gene flow may be important in some conservation contexts because the geographically widespread introgression of heterospecific alleles or haplotypes into an endangered species can produce spatial

patterns of genetic variation in that species that resemble the effects of large-scale gene flow even where propagule dispersal is geographically restricted.

The comparison of these sources of genetic diversity may be complicated because the effects of high or low rates of contemporary gene flow among conspecific populations via dispersal can be blurred by differences in the historical or ecological forces that shape patterns of population genetic similarity or differentiation over time (Grosberg and Cunningham 2001). In contrast, the effects of gene flow from other species via hybridization and introgression are often graphically and visually striking, for example, as incongruities between gene- and species-trees (Sang and Zhong 2000), unexpected patterns of clustering in parsimony networks (Gómez-Zurita and Vogler 2006), or strong right-shifted peaks in mismatch distributions (Addison and Hart 2005). These qualitative manifestations of introgression can appear to be most impressive in cases in which hybridizing species meet in zones of secondary contact following a period of divergence and lineage sorting in allopatry, or in other settings in which average pairwise genetic divergence between individuals of different species is much greater than divergence among conspecific individuals in different geographic populations.

One such setting is the trans-Arctic interchange of marine organisms between the northern Pacific and Atlantic oceans following the Pliocene submergence of the Bering land bridge (Vermeij 1991). Many of the organisms involved in this exchange were closely related pairs or groups of Pacific species that made parallel invasions of the Atlantic, or single lineages that invaded the Atlantic and subsequently gave rise to groups of endemic Atlantic species. Species with amphi-Atlantic (and perhaps trans-Arctic) distributions and a sympatric congener can be used to compare gene flow via hybridization (based on short-distance movements of sperm and eggs of different species during spawning) relative to gene flow via dispersal among conspecific populations (based on long-distance movements of planktonic larvae across or between ocean basins). On the time scale of the trans-Arctic interchange, is such introgression a significant force affecting population structure in the north Atlantic, or is it quantitatively trivial (although graphically impressive) compared to the effects of dispersal or other demographic processes that influence within-species genetic differentiation?

We analyzed mitochondrial DNA (mtDNA) gene flow via trans-Atlantic dispersal in two amphi-Atlantic species, the sea star *Asterias rubens* and the sea urchin *Strongylocentrotus droebachiensis*, relative to gene flow via hybridization with sympatric congeners found only in the northwest Atlantic (*A. forbesi*) or in the Atlantic and the Pacific (*S. pallidus*). All four species are dioecious broadcast spawners with long-lived planktonic larval development. Within each congeneric species pair, eggs can be fertilized by heterospecific sperm, hybrids are fertile, and hybrid

adult morphology can be either intermediate between parental phenotypes or characteristic of one parental form (Strathmann 1981; Biermann et al. 2004; Harper and Hart 2005, 2007; Nakachi et al. 2006). The sea stars occur only in the Atlantic (*A. amurensis* occurs in the Pacific): populations of their common ancestor probably diverged in allopatry in the eastern (*A. rubens*) and western (*A. forbesi*) Atlantic followed by a Holocene range expansion of *A. rubens* into the northwest Atlantic (Wares 2001; Wares and Cunningham 2001) where the two species hybridize in a zone of secondary contact from Long Island Sound to the Gulf of St. Lawrence (Harper and Hart 2005). This study contrasts gene flow between species against trans-Atlantic gene flow within *A. rubens*. The sea urchins are broadly sympatric in the Pacific, Arctic, and (unlike other Pacific *Strongylocentrotus* species) Atlantic oceans, probably due to parallel invasions by each species from the Pacific (Palumbi and Wilson 1990; Palumbi and Kessing 1991). This study focused on population genetic structure of *S. droebachiensis* in the Atlantic (Addison and Hart 2005) but sampled both species in the Pacific as well. Here we contrast gene flow between species against both trans-Atlantic and trans-Arctic gene flow within *S. droebachiensis*.

Both genera showed asymmetrical introgression in different directions relative to the analysis of among-population variation in one species in each genus: *rubens*-like haplotypes in *A. forbesi* in the northwest Atlantic (but no *forbesi*-like haplotypes in *A. rubens*); and *pallidus*-like haplotypes in some but not all *S. droebachiensis* populations (but no *droebachiensis*-like haplotypes in *S. pallidus*). We ask whether introgression has affected the relative magnitude of within- versus between-species genetic differentiation in each genus. In *Asterias*, has gene flow between these species substantially eroded the expected between-species genetic differences in a quantitatively similar way to the expected homogenizing effects of gene flow between *A. rubens* populations (across the Atlantic)? In *Strongylocentrotus*, has gene flow between these species substantially enhanced population differentiation in *S. droebachiensis* relative to genetic drift among populations on different coasts or in different oceans?

Materials and Methods

Mitochondrial DNA variation was sampled among 63 *Asterias* individuals from 10 locations (Table 1), including four in the northwest Atlantic (two in Nova Scotia, and one in Maine, one in Prince Edward Island) where *A. rubens* and *A. forbesi* are sympatric. Diagnostic morphological characters (Clark and Downey 1992) were used to identify species phenotypes. Previously described methods (see Addison and Hart 2005) and conserved PCR primers (Smith et al. 1993) were used to amplify and sequence the highly variable control region between the 12S and 16S rRNA genes. The amplified fragment includes the glutamine tRNA gene; from

Table 1. Sampling locations, sizes (N), and haplotype (h) and nucleotide (n) diversity indices (standard deviations in parentheses) for *Asterias* and *Strongylocentrotus* spp. Geographic regions are indicated in parentheses for species (*A. forbesi*, *S. pallidus*) that were sampled in only one region. Sample location details are given for individual *Asterias* sites off Newfoundland (NL), Quebec (PQ), Prince Edward Island (PEI), Nova Scotia (NS), and Maine (ME); see Addison and Hart (2005) for sea urchin sample location details. Voucher specimens for intertidal samples (approximate locations only) are in the collection of C. Cunningham, Duke University. Vouchers for all other *Asterias* samples have been deposited in the collections of the Nova Scotia Museum of Natural History.

Species/region	Sample location	Depth (m)	N	h	n
<i>A. rubens</i> , northeast Atlantic				0.977	0.016
NE Atlantic	Ireland (53°N 10°E)	Intertidal	4	(0.054)	(0.009)
	Norway (64°N 10°E)	Intertidal	2		
	Iceland (64°N 22°E)	Intertidal	1		
	Faeroe Islands (62°N 7°W)	Intertidal	3		
<i>A. rubens</i> , northwest Atlantic				0.812	0.008
NW Atlantic	Bonne Bay, NL (49°31'N 57°33'W)	10	2	(0.055)	(0.005)
	Havre-St-Pierre, PQ (50°14'N 63°36'W)	10	6		
	Bras d'Or Lake, NS (45°83'N 60°83'W)	10	3		
	Bear Cove, NS (44°32'N 63°33'W)	3–10	6		
	Isle of Shoals, ME (42°59'N 70°36'W)	3–10	14		
<i>A. forbesi</i> (northwest Atlantic)				0.982	0.086
	Savage Harbour, PEI (46°42'N 62°85'W)	5–8	4	(0.018)	(0.044)
	Bras d'Or Lake, NS		2		
	Bear Cove, NS		9		
	Isle of Shoals, ME		7		
<i>S. droebachiensis</i> , northeast Atlantic			27	0.592	0.001
				(0.081)	(0.001)
<i>S. droebachiensis</i> , northwest Atlantic			132	0.716	0.009
				(0.039)	(0.005)
<i>S. droebachiensis</i> , Pacific			22	0.761	0.014
				(0.099)	(0.008)
<i>S. pallidus</i> (Pacific)			22	0.632	0.003
				(0.103)	(0.002)

preliminary sequence data for this gene we designed and used an internal sequencing primer (5'-TTTCATGTTATAGGTTTAGG-3') to sequence the adjacent control region from one strand of each PCR product. Sequences were aligned with indels in ClustalX using the default alignment parameters (Thompson et al. 1997) and trimmed to standard length (309 bp; GenBank accession numbers EF179717-EF179779).

Sea urchin populations were sampled on a similar scale in the northeast and northwest Atlantic, plus a single *S. droebachiensis* population in the northeast Pacific (Table 1; see Addison and Hart 2005 for sampling details). We analyzed protein-coding COI sequences from a previous study (Addison and Hart 2005) of gene flow among *S. droebachiensis* populations. That previous study identified hybridization on the basis of two COI sequences from *S. pallidus* phenotypes collected from the same Pacific sampling location (near Friday Harbor, Washington) where Pacific *S. droebachiensis* were collected. For these new analyses, the original COI sequence alignment of 181 *S. droebachiensis* and two *S. pallidus* (AY504479–AY504511) was supplemented with 20

additional *S. pallidus* (EF108346–EF108365) collected from Friday Harbor and identified using standard diagnostic morphological markers (Jensen 1974).

Arlequin (Excoffier et al. 2005) was used to calculate nucleotide and haplotype diversity and pairwise population differentiation (Φ_{ST}) based on pairwise sequence differences. For specific comparisons of Φ_{ST} values between species that differed in within-population haplotype diversity (Hedrick 2005), standardized Φ'_{ST} values were calculated using the method of Meirmans (2006) in GenoDive 2b1. Maximum parsimony phylogenies (with gaps coded as a fifth base) were estimated for unique haplotypes within each genus in PAUP* 4.0b10 (Swofford 2002). Hierarchical analyses of molecular variance (AMOVA) in Arlequin were used to characterize genetic differentiation between congeneric species (Φ_{CT}) and among populations within species (Φ_{SC}). Finally, we directly contrasted rates of gene flow between congeneric species and among populations in coalescent simulations (see, e.g., Kronforst et al. 2006) in Migrate 2.1.3 (Beerli and Felsenstein 2001; Beerli 2006). These analyses used the Bayesian

A. forbesi phenotypes (Fig. 1A); the second included all *A. rubens* phenotypes plus three *A. forbesi* phenotypes that shared two *rubens*-like haplotypes (different by one indel) with each other and with 10 *A. rubens* individuals from the northwest Atlantic (there were no *forbesi*-like haplotypes in the larger sample of *A. rubens* phenotypes). This pattern is consistent with recent introgression of mtDNA from western *A. rubens* into the phenotypic background of its western Atlantic congener (F. M. Harper, pers. obs.). This pattern was also reflected in estimates of genetic diversity within these three groups: haplotype diversity was high (0.812–0.982; Table 1) with broadly overlapping confidence intervals among groups, but nucleotide diversity was significantly (5–10 times) higher in *A. forbesi* due to the presence of highly divergent *rubens*-like haplotypes in a few individuals. Pairwise population differentiation was consistent with this pattern: *A. rubens* population groups on either side of the Atlantic were significantly differentiated ($\Phi_{ST} = 0.131$, $P < 0.001$) but much less so than differentiation between *Asterias* species ($\Phi_{ST} = 0.763$ –0.842).

Strongylocentrotus sequences formed a clade of *S. droebachiensis* phenotypes (Fig. 1B) and a mixed clade that included all *S. pallidus* phenotypes plus six *S. droebachiensis* phenotypes from the Pacific (2) and northwest Atlantic (4) that shared four *pallidus*-like haplotypes (different by one to four substitutions from each other and from haplotypes of individuals with *S. pallidus* phenotypes). Although there was no introgression of *droebachiensis*-like haplotypes into the small sample of *S. pallidus* phenotypes, these results are consistent with unpublished (J. Addison and G. Pogson, pers. comm.) sequence data from several nuclear loci that indicate a deep history of asymmetrical hybridization between these two species. These new data allow a fuller characterization of mtDNA variation in one *S. pallidus* population relative to *S. droebachiensis* populations that experienced introgression. This pattern resembles the differences among *Asterias* groups: haplotype diversity was similar among all four groups (0.592–0.761), but nucleotide diversity was significantly higher (0.009–0.014) in *S. droebachiensis* populations from the northwest Atlantic and the Pacific that included a few individuals with *pallidus*-like haplotypes than in either *S. pallidus* or northeast Atlantic *S. droebachiensis* (0.001–0.003) in which introgression was not detected. This introgression into some, but not all, *S. droebachiensis* populations was reflected in patterns of pairwise population differentiation: all six differences among these four groups were highly significant ($P < 0.001$), but smaller between Pacific and northwest Atlantic *S. droebachiensis* (with some *pallidus*-like haplotypes; $\Phi_{ST} = 0.316$), larger between these two groups and northeast Atlantic *S. droebachiensis* (without evidence of introgression; $\Phi_{ST} = 0.618$ –0.701) or between these two groups and *S. pallidus* ($\Phi_{ST} = 0.776$ –0.829), and largest between *S. pallidus* and northeast Atlantic *S. droebachiensis* ($\Phi_{ST} = 0.937$).

Hierarchical AMOVA suggested that differentiation between *Asterias* species ($\Phi_{CT} = 0.855$) accounted for the large majority of haplotype variation, whereas differences between *A. rubens* from two different geographic regions were in comparison very small (–0.1% of variation) and associated with a negligible fixation index ($\Phi_{SC} = -0.007$). There were no cases of *Asterias* haplotypes shared across the north Atlantic, but many pairs of individuals from the northeast and northwest Atlantic differed by just one or two mutations compared to many much larger pairwise sequence divergences within each of these regions. This result might be caused by repeated parallel distribution of divergent haplotypes across the north Atlantic by larval dispersal, but the absence of shared haplotypes between eastern and western populations more strongly suggests shared ancestral polymorphisms (during the Holocene range expansion) followed by limited mutation and lineage sorting (including the extinction of the ancestral haplotypes) in western populations. Relative to the questions posed above, this result suggests that, although introgression between *Asterias* is graphically striking (Fig. 1A), the quantitative effect of gene flow between these species within the zone of secondary contact has not eroded between-species differentiation to any extent comparable to the genetic similarity between *A. rubens* populations across the Atlantic (Table 2) in spite of the much greater distances between these *A. rubens* populations (>10⁶ m) than between sympatric *A. rubens* and *A. forbesi* individuals (often <10² m).

Differentiation between sea urchin species ($\Phi_{CT} = 0.567$) accounted for only about half of haplotype divergence (Table 2), and differences among *S. droebachiensis* from three different geographic regions were associated with a slightly higher fixation index ($\Phi_{SC} = 0.601$). This population differentiation could be due in part to the six individuals of hybrid origin (Fig. 1): the presence of four *pallidus*-like haplotypes in some (Pacific, northwest Atlantic) but not all (northeast Atlantic) *S. droebachiensis* populations, or differences among populations in which *pallidus*-like haplotypes are present, could inflate among-*droebachiensis* population differentiation (e.g., Vollmer and Palumbi 2007). However, similar AMOVA results ($\Phi_{CT} = 0.596$; $\Phi_{SC} = 0.679$) with slightly lower nucleotide diversity in Pacific and northwest Atlantic populations (0.007–0.009) were obtained when the six individuals of hybrid background were removed from the analysis. This consistent result with and without hybrids suggests that much of this effect is due to sampling of strong differentiation between *S. droebachiensis* on different coasts and in different oceans (Addison and Hart 2004, 2005). Relative to the questions posed above, the graphically conspicuous introgression between *Strongylocentrotus* species (Fig. 1B) does not appear to have dramatically affected overall population structure in *S. droebachiensis*.

Pairwise population differentiation (Φ_{ST}) across the north Atlantic appeared to be considerably greater in *S. droebachiensis*

Table 2. Hierarchical analyses of molecular variance within and among *Asterias* and *Strongylocentrotus* species.

Source of variation	df	Percentage of variation	Fixation index
Among <i>Asterias</i> species	1	85.56	$\Phi_{CT} = 0.855$
Among populations within species	1	-0.11	$\Phi_{SC} = -0.007$
Within populations	60	14.54	$\Phi_{ST} = 0.854$
Among <i>Strongylocentrotus</i> species	1	56.71	$\Phi_{CT} = 0.567$
Among populations within species	2	26.06	$\Phi_{SC} = 0.601$
Within populations	199	17.23	$\Phi_{ST} = 0.827$

($\Phi_{ST} = 0.701$) than in *A. rubens* ($\Phi_{ST} = 0.131$) on the same spatial scale. However, this apparent difference between species seems to be due to the higher rate of mutation of the control region in sea star mtDNA compared to COI sequences in sea urchins and corresponding higher levels of control region haplotype variability within *A. rubens* populations rather than to any differences in rates of mtDNA gene flow across the Atlantic (or differences in other demographic processes). Haplotype diversity was considerably higher in *A. rubens* populations than in *S. droebachiensis* (Table 1), and this difference will tend to cause lower between-population F_{ST} or Φ_{ST} values in *A. rubens* (Hedrick 2005). Standardized Φ'_{ST} values (Meirmans 2006) were very high (1.00 in *A. rubens*; 0.92 in *S. droebachiensis*) largely because both species showed little haplotype sharing across the Atlantic (one common and one rare haplotype in *S. droebachiensis*; no shared haplotypes in *A. rubens*). Values of $\Phi_{ST} \sim 1.0$ can be qualitatively interpreted as evidence that in both species the observed between-population differentiation is about as large as possible given the level of within-population genetic diversity (Hedrick 2005).

Coalescent analyses in Migrate also suggested overall low rates of gene flow between species relative to gene flow between congeneric populations. Point estimates of population size were lower ($\theta = 0.009$) in relatively young northwest Atlantic *A. rubens* populations than in northeast Atlantic *A. rubens* ($\theta = 0.027$) or *A. forbesi* ($\theta = 0.023$), but the 95% confidence intervals around θ values were broadly overlapping. Gene flow between species was considerably less ($M = 7.0$ – 10.7) than trans-Atlantic gene flow between *A. rubens* populations ($M = 120.5$ – 193.0). Both vectors of gene flow between species had narrow posterior frequency distributions, modal values ($M = 2.5$) the same as the smallest bin in the posterior distribution, and 95% confidence intervals that included the smallest bin in the posterior distribution. In contrast, both M estimates between *A. rubens* populations had broad posterior distributions but were significantly greater than zero.

Gene flow estimates in *Strongylocentrotus* were somewhat less precise, perhaps because of the larger and uneven sample sizes. For six of nine parameters, $M = 61.7$ – 147.1 but with 95% confidence intervals that included zero (including some values that were expected to be not different from zero, such as gene flow from *S. pallidus* to northeast Atlantic *S. droebachiensis*). Two other gene flow estimates between *S. droebachiensis* populations were higher with 95% confidence intervals greater than zero: across the north Atlantic from east to west ($M = 231.6$; 41.9–441.3) and across the Arctic from the northwest Atlantic to the Pacific ($M = 281.4$; 32.8–563.8). One estimate of gene flow between species (from *S. pallidus* to Pacific *S. droebachiensis*) was intermediate ($M = 134.0$) between these sets of values, with 95% confidence intervals (2.6–293.0) slightly greater than zero. This inference of marginally significant nonzero gene flow between Pacific samples of these species may be caused by the higher frequency (2/22 = 0.091) and slightly greater divergence (2/418 bp = 0.0048%) of *pallidus*-like haplotypes in Pacific *S. droebachiensis* compared to those in the northwest Atlantic (4/132 = 0.030; 1/418 bp = 0.0024%) (Table 1; Fig. 1).

Discussion

We found relatively lower levels of population differentiation—interpreted as higher rates of gene flow—between populations of two species (*A. rubens*, *S. droebachiensis*) than between species in either genus. Several other recent studies of within- and between-species genetic variation have also documented comparable patterns in animals (e.g., Alexandrino et al. 2006; Carreras-Carbonell et al. 2006; Lorenzen et al. 2006), and all of these studies suggest that hybridization has had a limited influence on within-species population genetic structure compared to effects of dispersal, genetic drift, and other within-species demographic processes. Although many plant taxa are characterized by important effects of hybridization and reticulate evolution, some recent plant studies (Drummond and Hamilton 2007) also suggest that demographic processes (especially drift and lineage sorting) are more significant than introgression as influences on local and regional genetic diversity. This is, of course, the conventional result: biological and morphological species boundaries should correspond with large population genetic differences that should be obvious from frequentist (Φ) or coalescent (M) analyses. However, this result is not trivial: numerous recent phylogeographic studies have identified unexpected strong barriers to gene flow in the ocean on spatial scales similar to or smaller than those studied here (e.g., Barber et al. 2006), in animals with long-lived planktonic larval dispersal (Swearer et al. 1999; Taylor and Hellberg 2003). Moreover, reproductive isolation among species of *Asterias*, *Strongylocentrotus*, and other broadcast-spawning marine genera depends on behavioral and molecular barriers that are porous and prone to formation of hybrids (Levitan 2002; Harper and Hart 2005). Thus, there

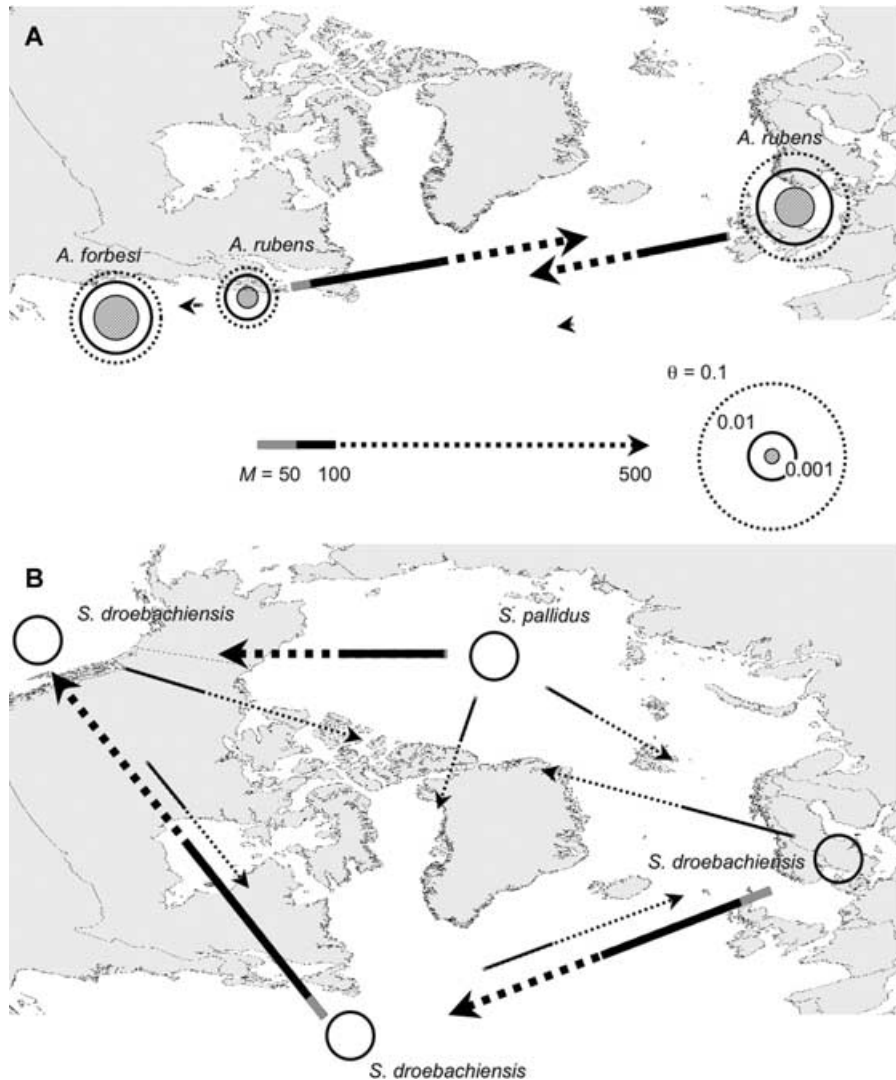


Figure 2. Summary of migration rates ($M = m/\mu$) and effective population sizes ($\theta = N_e\mu$) for (A) *Asterias* and (B) *Strongylocentrotus* species. The location of each circle on the map represents the approximate location of each population sample; the *S. pallidus* population in (B) was sampled in the northeast Pacific but is shown in the Arctic to avoid clutter and provide sufficient room to show large migration rates. The area of each circle is proportional to the point estimate for θ (black line) and the upper (broken line) or lower (shaded area) 95% confidence interval (we estimated θ only for *Asterias*). The direction of each M vector is shown by the arrow; the length of each line is proportional to the point estimate of M (black) and the upper (broken) or lower (shaded) 95% confidence interval. Migration rates for which the lower 95% confidence interval includes zero are shown as fine lines; migration rates significantly greater than zero are shown as bold lines. M and θ are drawn to the same scale in each panel (see the scale bars and circles between the panels).

might be considerable scope for interactions between heterospecific gametes to influence population genetic structure relative to rates of gene flow by larval dispersal in many marine organisms (e.g., corals; Hatta et al. 1999; Fukami et al. 2004; Vollmer and Palumbi 2007).

In spite of this potential influence, the results of this study suggest that introgression has not reduced between-species differences ($\Phi_{CT} = 0.855$) in *Asterias* relative to population genetic homogeneity ($\Phi_{SC} \sim 0$) in a species with a short amphiatlantic history (*A. rubens*), nor has introgression magnified between-population differences ($\Phi_{SC} = 0.679$) in *S. droebachiensis*

relative to spatially variable patterns of introgression between *Strongylocentrotus* species. In these two genera, larval dispersal (or other ecological factors that influence genetic differentiation) is more significant than hybridization as a force sculpturing genetic variation, even in a species (*S. droebachiensis*) in which population differentiation is strong on large spatial scales. This result is strikingly different from recent studies of threatened Caribbean corals (Vollmer and Palumbi 2007), in which introgression of *Acropora palmata* mtDNA haplotypes into *A. cervicornis* populations has blurred ($\Phi_{ST} = 0.130$) the otherwise strong evidence of population differentiation seen in analysis of *A. cervicornis*

native haplotypes ($\Phi_{ST} = 0.235$) due to restricted planula larval dispersal among *A. cervicornis* populations.

Are *S. droebachiensis* more strongly differentiated across the north Atlantic ($\Phi_{ST} = 0.701$; $\Phi_{SC} = 0.601$) than *A. rubens* ($\Phi_{ST} = 0.131$; $\Phi_{SC} = -0.007$) in spite of similar rates of trans-Atlantic gene flow ($M \sim 100$ – 200)? The standardized estimates of $\Phi'_{ST} \sim 1.0$ suggest that differentiation was about as great as possible (given within-population variation) in both species, but in the AMOVA context the population differentiation in *S. droebachiensis* (including differentiation between the Atlantic and Pacific) was similar to differentiation between *Strongylocentrotus* species (whether we included the few hybrid haplotypes), whereas population differentiation in *A. rubens* was vanishingly small compared to differentiation between *Asterias* species (Table 2). Part of this difference must certainly reflect merely the different rates of mutation of the two mtDNA markers (Hedrick 2005). However, the different Φ_{SC} patterns might also reflect the greater age and deeper gene genealogies of *S. droebachiensis* populations in the northwest Atlantic compared to the more recent establishment of *A. rubens* (Wares 2001; Addison and Hart 2005). The effective population size estimates for sea star ($\theta = 0.009$ – 0.027) and sea urchin mtDNA ($\theta = 0.001$ – 0.037 ; Addison and Hart 2005) may be sufficiently low that older sea urchin populations may have experienced considerably more drift than younger sea star populations. Alternatively, bidirectional gene flow across the north Atlantic in *A. rubens* (Fig. 2) may be more effective in limiting population differentiation compared to the inference of one-way gene flow (from east to west) between north Atlantic populations of *S. droebachiensis*.

Nonzero gene flow was inferred in both directions across the north Atlantic in *A. rubens* (Fig. 2). In contrast, west-to-east gene flow in *S. droebachiensis* was lower ($M = 95.2$; Fig. 2; see also Addison and Hart 2005) and not significantly different from zero, whereas east-to-west gene flow in this species was considerably greater ($M = 231.6$). The sea urchin pattern is the one predicted from paleontological studies. Vermeij (2005) identified 124 species with amphi-Atlantic distributions among north Atlantic mollusks, and found that about half of these examples were the product of east-to-west range expansions during or after the Pliocene (and the rest by simultaneous invasion of both sides of the Atlantic by Pacific species since the early Pliocene). Vermeij found no instances in which the fossil record suggested establishment of mollusks first in the northwest Atlantic followed by west-to-east range expansion. The *Asterias* gene flow estimates conflict with this scenario, in spite of genetic and other data that suggest a relatively recent east-to-west range expansion of this species. This conflict could be caused by constraints on the ability of coalescent simulations to distinguish recent range expansions versus recent gene flow; temporal variation in post-Pliocene direction and speed of ocean currents and larval dispersal; or qualitative

differences between the effects of older range expansions versus recent contemporary gene flow on haplotype variation within and between populations. In spite of this minor uncertainty, both the sea urchin and sea star analyses suggest that hybridization has had only a modest effect on genetic diversity and population differentiation relative to the effects of dispersal on the time scale of the trans-Arctic interchange.

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