SYMPOSIUM

It's About Time: Divergence, Demography, and the Evolution of Developmental Modes in Marine Invertebrates

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Synopsis Differences in larval developmental mode are predicted to affect ecological and evolutionary processes ranging from gene flow and population bottlenecks to rates of population recovery from anthropogenic disturbance and capacity for local adaptation. The most powerful tests of these predictions use comparisons among species to ask how phylogeographic patterns are correlated with the evolution and loss of prolonged planktonic larval development. An important and largely untested assumption of these studies is that interspecific differences in population genetic structure are mainly caused by differences in dispersal and gene flow (rather than by differences in divergence times among populations or changes in effective population sizes), and that species with similar patterns of spatial genetic variation have similar underlying temporal demographic histories. Teasing apart these temporal and spatial patterns is important for understanding the causes and consequences of evolutionary changes in larval developmental mode. New analytical methods that use the coalescent history of allelic diversity can reveal these temporal patterns, test the strength of traditional population-genetic explanations for variation in spatial structure based on differences in dispersal, and identify strongly supported alternative explanations for spatial structure based on demographic history rather than on gene flow alone. We briefly review some of these recent analytical developments, and show their potential for refining ideas about the correspondence between the evolution of larval developmental mode, population demographic history, and spatial genetic variation.

Introduction

Strathmann (1985, 1990) argued that the physical and biological chemistry of seawater has favored unique adaptations in the life histories of benthic marine animals, most notably the planktonic fertilization of small, nutrient-poor eggs that subsequently grow (as well as develop) in the plankton as specialized feeding larval forms before returning as large juveniles to the benthic habitat used by adults. Such planktotrophic larvae occur in species of most major marine animal lineages and in most marine communities, often living alongside closely related species that have evolved various combinations of internal fertilization, lecithotrophic nutrition, simplified morphogenesis, and benthic development. Subsequent efforts to understand the adaptive significance of such variation in developmental modes (Moran and Emlet 2001; Emlet and Sadro 2006; Thiyagarajan et al. 2007; Byrne et al. 2008; Marshall and Keough 2009) have arisen largely from Strathmann's fundamental question of whether or not the diversity of reproductive strategies and larval modes of marine species primarily reflect the energetic and evolutionary trade-offs between the size and number of offspring. Understanding whether the benefits of producing many small planktotrophic offspring are in fact balanced by losses to mortality during an extended larval period remains a challenging issue for the study of larval ecology and evolution, and Richard Strathmann's research, focused in large part on characterizing the adaptations of marine larvae to their planktonic environment, has been primarily responsible for creating a

comprehensive framework for understanding the trade-offs between larval transport and larval losses.

A key feature of this framework is Strathmann's successful argument that planktonic larvae are generally overdispersed relative to the distribution of suitable benthic habitat, and that individual adults gain few (if any) short-term benefits from the obligate long-distance planktonic dispersal of their offspring (Strathmann 1985). However, most larval biologists would probably agree that variation in larval developmental modes, and the expected variation in gene flow between populations that arises from the dispersal potential of different developmental modes, has important consequences over longer ecological and evolutionary timescales. For this reason, population geneticists have focused on marine species as model systems for understanding the impacts of life-history variation on realized dispersal or gene flow between populations. An important reason for the focus on life-history correlates of dispersal is the extraordinary expected variance in larval dispersal potential among species with different modes of development and potentially among conspecific larvae with the same mode of development. In species with slow-growing planktotrophic larvae, siblings are predicted to diffuse large distances from each other with a high variance in dispersion from the natal habitat, whereas offspring with benthic development are predicted to recruit to their natal habitat along with many of their siblings. Other things being equal, such differences in mode of development and larval dispersal potential are expected to strongly affect important demographic measures including functional sex ratio, effective population size, gene flow, population persistence and colonization (i.e., metapopulation dynamics), probability of extinction, and rate of speciation. Most recently, conservation biologists (Fortuna et al. 2009) have focused on life-history differences as predictors of population connectivity, metapopulation dynamics, and capacity for recovery from, or local adaptation to, disturbance by humans.

Is variation in larval developmental mode (and in gene flow by larval dispersal) the prime determinant of genetic drift and population differentiation? Marine phylogeographers have put extraordinary effort into answering this question through the analysis of the spatial distribution of genetic variation within and between populations. Because the effects of developmental mode act in the context of the overall phenotype of the organism and the historical biogeographical context of its environment, the most powerful tests of the relative effect of evolutionary changes in mode of development on population

genetic variation have used comparisons between closely related species that share otherwise similar phenotypes, or members of the same community that share a similar biogeographical context (Bohonak 1999).

Starting with the earliest comparative studies of marine population genetic structure (Berger 1973), a steady stream of comparative analyses has yielded results consistent with the predicted effects of different modes of larval development (regardless of other phenotypic or biogeographical effects), such as stronger population differentiation, smaller effective population size, or striking phylogeographic breaks in species with lower larval dispersal potential (e.g., Janson 1987; Waples 1987; McMillan et al. 1992; Duffy 1993; Hunt 1993; Shulman and Bermingham 1995; Hellberg 1996; Arndt and Smith 1998; Todd et al. 1998; Kyle and Boulding 2000; Collin 2001; Watts and Thorpe 2006; Sherman et al. 2008; for reviews see Gooch 1975; Crisp 1978; Burton and Feldman 1981; Palumbi 1994; Bohonak 1999; Kinlan and Gaines 2003). At the same time, however, there has also been a slow accumulation of a number of exceptions in which either unexpectedly strong genetic differentiation has been found over relatively small geographic scales in species with planktonic larvae (Koehn et al. 1980; Barber et al. 2000; Buonaccorsi et al. 2002; Taylor and Hellberg 2003a; Sotka et al. 2004; Marko and Barr 2007; Marko et al. 2007) or an absence of genetic subdivision over large areas has been found in species without a planktonic life-history stage (Kyle and Boulding 2000; Marko 2004; Ayre et al. 2009; for reviews see Burton and Feldman 1982; Burton 1983; Cunningham and Collins 1998). For the most part, strong differentiation in species with planktonic larvae has usually been attributed to natural selection acting directly on genetic markers (Koehn et al. 1980; Sotka et al. 2004), nearshore oceanographic processes (Gilg and Hilbish 2003; Sotka et al. 2004; Marko and Barr 2007; Banks et al. 2007), habitat specificity (Ayre et al. 2009), or larval behavior (Warner and Palumbi 2003) whereas genetic homogeneity in species lacking broadly dispersing planktonic larvae could reflect recent population expansions (Edmands 2001; Marko 2004), stabilizing selection (Karl and Avise 1992), or unexpected dispersal capability (e.g., by rafting of adults, juveniles, or encapsulated embryos attached to mobile substrates such as birds or kelps) (Highsmith 1985; Helmuth et al. 1994). Although the degree of differentiation (or absence of differentiation) in many of these cases is striking, it is important to note that many of these exceptional studies involve single species (a situation

analogous to an uncontrolled ecological experiment), and therefore lack the inferential power inherent in comparative studies. Not surprisingly, opinions differ with respect to what exactly the expected patterns of neutral genetic differentiation should be for any particular species (Collin 2003; Taylor and Hellberg 2003b; Palumbi and Warner 2003; Warner and Palumbi 2003); follow-up comparative studies involving some of these exceptional cases have been very helpful in distinguishing between contemporary and historical causes of phylogeographic patterns of differentiation (Taylor and Hellberg 2006).

One potential solution to the mixed evidence for correlated evolution of developmental mode and spatial patterns of genetic variation (e.g., Bohonak 1999 or Kinlan and Gaines 2003 compared to Ayre et al. 2009) is to refocus marine phylogeographic analyses on identifying the biogeographic and demographic processes responsible for generating different phylogeographical patterns among species, particularly among those differing in mode of larval development. For the most part, large phylogeographic breaks, or large pair-wise measures of population differentiation, are typically interpreted in terms of geographically localized limits on gene flow (Cowen and Sponaugle 2009; Pelc et al. 2009). Although reduced gene flow in itself is a valid hypothesis that can explain patterns of population genetic subdivision, genetic variation within and between populations at selectively neutral loci, of course, evolves under the combined effects of mutation, genetic drift, and gene flow acting through time.

Phylogeography has largely relied on problematic post hoc interpretations (Carstens et al. 2009) to identify spatial patterns that are consistent with hypotheses of geographical or taxonomic variation in one or more of these process-based parameters. An important recent source of insight into this problem, which promises to help overcome the limitations of post hoc interpretation, is the development of coalescent methods for the joint characterization of demographic parameters and for testing hypotheses about their magnitude and their significance for explaining patterns of spatial genetic variation. These new methods provide a more general context for marine phylogeography in which spatial patterns of genetic variation arise through a birth–death process of mutation, vicariance, genetic drift, and dispersal, all jointly modeled using the coalescent. This framework can be used to ask whether effective population sizes, rates of gene flow, and times of population divergence are predicted by variation in mode of development and larval dispersal. In particular, species with similar dispersal potential and similar phylogeographical patterns are expected to have similar underlying histories of gene flow, changes in effective population size, and population divergence time. Conversely, species with different dispersal potential and different phylogeographical patterns of spatial variation observed in contemporary populations are expected to differ mainly in the magnitude of migration and gene flow between populations, rather than in their demographic histories of population divergence and their variation in effective population size. Testing these expectations requires the joint estimation of gene flow (via high or low rates of larval dispersal) along with other parameters (population divergence time, effective population size) that might contribute substantially to the spatial pattern of differentiation in comparison to interpretations that emphasize gene flow and larval-dispersal potential. Characterizing these temporal patterns of population genetic variation, and confirming that such temporal patterns covary in a predictable way with mode of development, would greatly bolster the argument for the primacy of larval dispersal potential as the main determinant of genetic variation among populations in the oceans. Alternatively, if these predictions are often rejected, then the coevolution of population genetic variation and larval dispersal may be much weaker than has been suggested by comparative phylogeographical studies of spatial variation alone, and other phenotypic or historical effects may be much more important in shaping the magnitude of neutral genetic differentiation and its evolutionary corollaries. Here, we briefly review the development of these methods, give examples of their recent application in comparative studies of marine invertebrates, and look ahead to the prospects for fully using such methods to understand the evolution of diverse larval forms and phylogeographic patterns.

Genetic differentiation in the sea: is it always all about gene flow?

The large majority of comparative marine phylogeographic studies have characterized spatial patterns of population structure using analogs of Wright's F-statistics. Although gene flow or migration can be estimated from F_{ST} in several ways (Wright 1951; Felsenstein 1976; Slatkin 1985), this inference requires several notorious simplifying assumptions, such as constant and equal population sizes, symmetrical rates of migration, and population allele frequencies that are in a dynamic equilibrium between gene flow and genetic drift (i.e., that the F_{ST} statistic itself has reached equilibrium). Each of these

assumptions has been widely criticized for lacking biological realism in many natural situations, and violation of these assumptions can potentially severely bias estimates of migration from F_{ST} (Burton and Feldman 1982; Bossart and Prowell 1998; Cunningham and Collins 1998; Waples 1998; Beerli and Felsenstein 1999, 2001; Whitlock and McCauley 1999; Neigel 2002). The equilibrium assumption, for example, is probably wrong for many species in temperate regions that have undergone large poleward range extensions and massive demographic expansions since the end of the last glacial maximum ~10,000 years ago. The time required (in generations) for F_{ST} to reach equilibrium may be much greater than this, depending on migration rates and effective population sizes (Crow and Aoki 1984; Whitlock 1992; Whitlock and McCauley 1999). The impact of violating this assumption depends on the history of isolation between populations; estimates of migration between populations will be biased upward if populations have very recently separated but will be biased downwards if populations have newly come into contact. Comparative studies that attempt to use F_{ST}-based methods such as analysis of molecular variance (AMOVA; Excoffier et al. 2005) to account for phylogeographic differences among marine species in terms of mode of larval development and rate of gene flow are thus potentially confounded by violations of the equilibrium assumption given that the approach to equilibrium depends primarily on effective population size, rates of gene flow, and metapopulation dynamics (population divergence times), all factors expected to differ among species with different modes of development.

Although all of the variants of F_{ST} remain useful ways to summarize patterns of population differentiation (Bohonak 1999; Whitlock and McCauley 1999; Neigel 2002), estimates of migration from new analytical methods, largely those based upon neutral coalescent theory, have slowly been incorporated into marine phylogeographic studies because these newer methods have several desirable features (Kuhner 2009). Arguably the most important improvement in these methods is that they provide joint estimates of the population genetic parameters that collectively produce spatial patterns of differentiation, especially effective population size, migration, population growth rates, and divergence time. This approach is generally superior because it avoids post hoc interpretation of spatial patterns (i.e., F_{ST}) in terms of unobserved demographic processes (i.e., asymmetrical gene flow, ancient vicariance, recent range extension, population expansion, or bottlenecks), and instead directly estimates parameters

associated with those demographic processes. Like F_{ST}-based approaches, both the precision and accuracy of coalescent estimates of these population parameters are greatly improved by (or even critically dependent on) combined analysis of multiple loci in single datasets that use information from the variance in coalescent times across loci (Edwards and Beerli 2000).

For datasets with multiple loci, this coalescent approach has mainly been implemented using one of three methods implemented in user-friendly software packages running on fast publicly-accessible computer clusters: MIGRATE-N, LAMARC, and IMA (and the earlier related programs IM and MDIV). All of these methods provide maximum likelihood estimates of population genetic parameters across a group of likely gene trees simulated with Markov Chain Monte Carlo (MCMC) sampling of genealogies, and are thus dubbed "coalescent samplers" (Kuhner 2009). Using a random gene-tree topology as a starting point, MCMC methods repeatedly make small arbitrary topological changes, and assess the likelihood of the resulting genealogy at each step within a model of neutral coalescence backwards in time. Highly likely gene trees are retained and population genetic parameters are estimated across the entire sample of trees. Parameter estimation across a large sampling of trees (rather than from a single "best" tree) is important, given the high degree of uncertainty in gene-tree topologies within single species or between closely related species. MCMC sampling can be guided by either a likelihood or Bayesian approach in MIGRATE-N and LAMARC, but only a Bayesian framework is available with IMA.

The main differences among these methods (and that pertain most to our review) are the population parameters that each method estimates, and thus what each method does and does not assume about the history of the population. All three methods estimate the population genetic parameter Θ and separate migration rates ($M = N_e m$, where N_e is the effective population size and m is the probability of movement by an individual from one population to the other per generation) between populations in both directions (bidirectional migration). The parameter Θ, equivalent to $4N_e\mu$ (where μ is the mutation rate), is essentially a proxy for relative population size given that mutation rates can reasonably be assumed to be equal among populations within a single species. Both MIGRATE-N and LAMARC make these estimates for a group of n populations but IMA is limited to the analysis of a single pair of populations. MIGRATE-N and LAMARC can accommodate multiple populations because
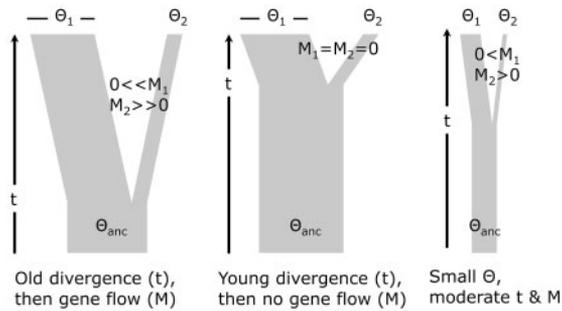
Fig. 1 The isolation-with-migration model for population histories that differ in the magnitude of gene flow, divergence time, or effective population size but have similar levels of differentiation between the population samples.

both methods assume that population dynamics have been stable for $\sim 4N_e$ generations, meaning that both effectively assume that shared polymorphisms between populations are only due to gene flow, and that gene flow and genetic drift are in equilibrium. Therefore, MIGRATE-N and LAMARC are most appropriate for cases in which populations are not expected to share ancestral polymorphisms. In contrast, IMA is ideally applied to pairs of population that have diverged recently because, in addition to M and Θ, IMA also estimates Θ for the ancestral population plus the time of divergence (t) of that ancestral population into the two sampled descendant populations; this combination of parameters allows IMA to infer whether shared alleles between populations represent either ancestral polymorphisms or recent gene flow (Hey and Nielsen 2004, 2007). In theory, IMA can therefore potentially discriminate between substantially different population histories (Fig. 1) that may show similar amounts of genetic differentiation. Because the IMA method can be applied to only two populations at a time (the isolation-with-migration model was originally developed to analyze recent speciation events), it assumes that no other populations have influenced the divergence of the sampled populations. The recently released IMA 2.0 (Hey 2010a) can accommodate multiple "populations," but because it requires an explicitly defined phylogeny for those populations, its intended use is for groups of closely related taxa whose phylogenetic relationships are known, or can be reliably inferred, from other methods (Liu and Pearl 2007; Liu 2008; Hey 2010b).

LAMARC has the option of adding a population-growth parameter (g) so that either positive or negative exponential growth can be simulated (Kuhner 2006); likewise, IM (the predecessor to IMA) has a splitting parameter that allows for growth and unequal division of the ancestral population (this

parameter is not included in IMA) (Hey 2007). LAMARC can also estimate the per-site recombination rate, which allows the user to account for intralocus recombination between alleles. Alternatively (for other methods), contiguous sequence data from individual nuclear genes must be first tested for evidence of intragenic recombination and trimmed (if necessary) to single blocks of nonrecombining sequences for use. The more recently released program MIMAR (Becquet and Przeworski 2007), which is very similar to the IMA family of programs, can accommodate intragenic recombination, which allows the use of longer nuclear sequences, and presumably leads to less uncertainty in individual gene trees.

To illustrate what we perceive to be some of the important differences among these methods, we have compared the results from different analyses using previously published data from the bay scallop, *Argopecten irradians* (Marko and Barr 2007). Bay scallops live along the southeast coast of the USA in seagrass beds found within semi-enclosed lagoonal basins or sounds that are connected to shelf waters by gaps and inlets between adjacent barrier islands. We have re-analyzed mtDNA sequence data from two adjacent basins (Bogue Sound and Back Sound) that are connected by a deep-water channel, but are potentially isolated from each other by the pattern of tidal circulation through the inlet, which creates a characteristic hydrodynamic "wall" that is expected to limit the exchange of water and veliger larvae across the inlet (Stommel and Farmer 1952; Zimmerman 1981; Luettich et al. 1998, 1999; Brown et al. 2000; Hench et al. 2002). In the original treatment of the data, migration rates were inferred from F_{ST} and with MIGRATE-N (using a maximum likelihood search), but we have repeated the MIGRATE-N analysis using Bayesian inference and have also used IMA. The estimate of gene flow ($N_e m$) between Bogue and Back Sounds from F_{ST} was 33 migrants per generation (Fig. 2), an inference that assumes symmetric or equal migration rates, equal population sizes, plus a drift-migration equilibrium. Our MIGRATE-N analysis suggested that this assumption was probably not valid: both Θ and $N_e m$ were highly asymmetrical, with much higher immigration into the much larger Back Sound population (assuming an old population divergence followed by attainment of an equilibrium between drift and gene flow). However, our IMA analysis suggested in turn that these latter assumptions are also probably not valid, and gave qualitatively different inferences about the origin of the modest spatial differentiation between Bogue and Back Sounds; IMA estimated gene flow to be zero in both directions and, most
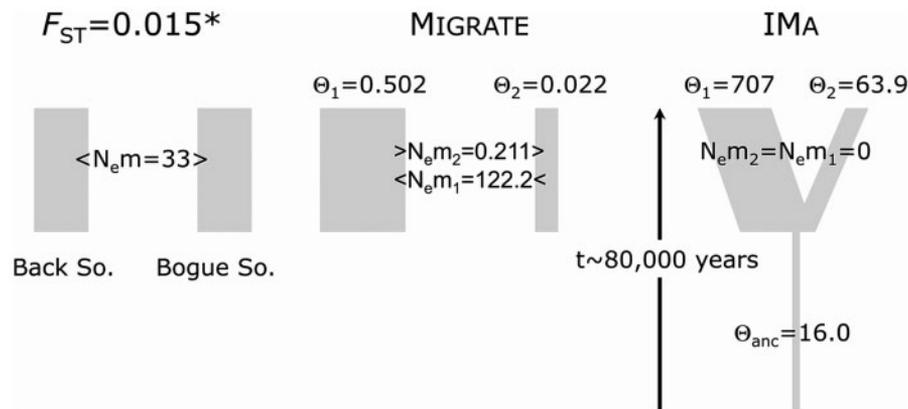
Fig. 2 Different insights into demographic processes (especially gene flow, N_em) from F_{ST} (left), MIGRATE-N (center), or IMA (right) analyses of bay scallop mtDNA variation from the Pamlico Sound region of coastal North Carolina, USA. Data from Marko and Barr (2007). The MIGRATE analysis used search methods like those described by Marko and Barr (2007) but with the faster Bayesian estimator. The IMA analyses used methods like those in Marko et al. (2010), run on the BioHPC clusters at Cornell University. We carried out a series of analyses in M mode to optimize mixing and to find a combination of search parameters and prior distributions that gave high-density coverage of the nonzero parts of the posterior distribution of demographic parameter values. The saved gene trees were then used in L mode to test nested sets of demographic models less complex than the full model including three different Θ values and two nonzero migration rates. We report parameter estimates in demographic units (population size as number of individuals, divergence time as years) for the longest of multiple runs that used the best-fit model. We used several standard biogeographical estimates of mtDNA mutation rates to convert the parameter estimates to demographic units. We used the 90% highest posterior density (HPD) to characterize the confidence interval around each parameter estimate.

surprisingly, estimated relative population size to be several orders of magnitude larger than estimates from MIGRATE-N. In short, MIGRATE-N inferred high (but asymmetrical) gene flow between small populations whereas IMA inferred the opposite pattern of no migration between very large populations that diverged from each other during the early Wisconsin glacial period (Fig. 2). Although we cannot be sure, the discrepancies between MIGRATE-N and IMA may reflect a key difference between the two methods: if the separation of two populations was sufficiently recent (Fig. 2) that they still retain many shared ancestral polymorphisms, then estimates of gene flow and population size will be biased upwards and downwards, respectively, by MIGRATE-N given that the method assumes that all shared haplotypes are due to gene flow.

Examples like the bay scallop highlight both the general advantages of the coalescent approach to understanding processes underlying spatial variation and the potential advantages of joint estimation of all three types of historical parameters (Θ, M, t). Currently the best approach to this type of problem for recent population divergences appears to us to be the IMA method because it includes all three critical population parameters. The main drawback to using IMA is its limitation to single pairs of populations: gene flow from unsampled populations may bias estimates of parameters for the sampled populations (Strasburg and Rieseberg 2010), as it will with

most other analytical approaches (Beerli 2004). Most researchers have employed a variety of strategies to overcome this limitation (depending on the spatial distribution of populations), such as conducting separate analyses for all adjacent samples or conducting all pair-wise analyses and then making qualitative inferences about the overall patterns. Other approaches to this problem may soon deliver user-friendly software that can rapidly and accurately estimate the same range of parameters for more than two populations or offer other conceptual advantages (MSBAYES: Hickerson et al. 2006; Hickerson and Meyer 2008; BEST: Liu 2008; POPABC: Lopes et al. 2009; IMA 2.0: Hey 2010a, 2010b) and thus overcome the most significant limitation of the IMA approach. However, most of these newer programs are aimed either at phylogenetic problems with well-defined populations or at species in which the genealogies of the populations are known (i.e., IMA 2.0) or migration can be assumed to be a negligible factor (i.e., MSBAYES, POPABC, BEST). Modeling population vicariance with gene flow for multiple populations is a complex problem that we can only hope to be tackled in the future.

Although the rate of application of IMA and other coalescent samplers is rapidly increasing overall, many published studies focus on single-species datasets of mtDNA sequences. So far, there are too few applications that analyze two or more sequenced loci in a comparative context, so it is not yet possible

to ask how often, or under what circumstances, the demographic history of marine invertebrate populations is consistent with predictions about patterns of spatial genetic differentiation based on similarities or differences in the mode of development and larval dispersal. Instead, we review a few such recent studies (including some of our own) with the aim of (1) highlighting the unexpected and exciting insights available from such analyses, and (2) encouraging more larval ecologists to become marine phylogeographers and analyze genetic and developmental variation within the IMA framework.

Congruent patterns of temporal demography and mode of dispersal

Because coalescent analyses could reveal different demographic histories among species with similar levels of spatial differentiation, or, alternatively, could account for differences among species in genetic diversity and differentiation, either through differences in divergence times, effective population sizes, or migration rates, the insights from coalescent analyses seem most likely to erode the strong support for the correlated evolution of larval forms and population structure evident in F_{ST}-based and AMOVA-based approaches. However, some studies find very strong similarities between patterns of spatial variation and underlying demographic histories. Many such results from comparative coalescent studies would tend to reinforce rather than erode support for dispersal as the main determinant of spatial genetic structure in marine communities. One creative example used mtDNA sequences from large samples of a snail species (*Littorina littorea*) and its host-specific trematode parasite (*Cryptocotyle lingua*). These snail populations in the northwestern Atlantic are probably descended from northeastern Atlantic ancestral populations, but the mechanism and history of their range extension to the western Atlantic have been controversial for more than 100 years: by historical human-mediated introduction since the European colonization of North America, or by an earlier nonanthropogenic mode of dispersal. Blakeslee et al. (2008) found similar spatial distributions of mtDNA variation across the North Atlantic both in host and parasite, and then used IM to test the hypothesis of ancient versus historical divergence time between North American and European populations of the snail. They reported a single-locus estimate of $t\sim500$ years, a result that is consistent with a historical anthropogenic origin (and invasive status) for *L. littorea* in North America. Blakeslee et al. (2008) reported a similar divergence time

between eastern and western trematode populations from the same locations. The results confirm the expected correspondence between similarities in dispersal potential and spatial genetic differentiation between two species that have a similar underlying demographic history. We reanalyzed the same mtDNA sequence data for both snails and trematodes in IMA: although we found population divergence times much older than any of the Holocene human colonizations of North America (Fig. 3), the trans-Atlantic divergence times were similar for the snail host and its trematode parasite. The older divergence times from IMA analyses agree with the conclusions of one of the earliest comparative population genetic studies, in which Berger (1977) interpreted fixed allozyme differences at seven of 12 loci between eastern and western Atlantic populations as indications of relatively ancient trans-Atlantic divergence in *L. littorea* (with
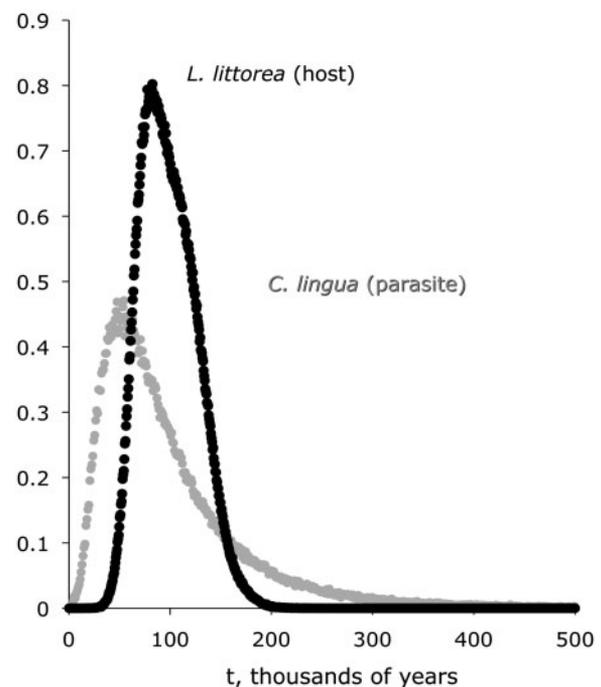

Fig. 3 Posterior probability distributions of population divergence times from IMA analysis of mtDNA of northwestern and northeastern Atlantic populations of a marine gastropod (*Littorina littorea*, black) and its obligate trematode parasite (*Cryptocotyle lingua*, gray). Data from Blakeslee et al. (2008), methods as described in Figure 2. The mode of each posterior distribution indicates the most probable population divergence time between eastern and western populations. Because we used uniform prior distributions, these most probable parameter estimates are also maximum likelihood estimates (MLE). The estimates for snails and for trematodes are considered to be not significantly different from each other because the MLE of divergence time for the snail host falls well within the confidence interval around the MLE of divergence time for the trematode parasite.

planktonic larvae) compared to much more modest allozyme divergence across the North Atlantic in two other *Littorina* species that lack planktonic larval dispersal. Later unpublished studies failed to replicate Berger's result for *L. littorea*, and suggest that Berger's observations of trans-Atlantic allozyme differentiation in that species may have been flawed (see Cunningham 2008). However, none of these studies strongly support a geologically recent introduction of North American populations of *L. littorea* associated with humans migrating from Europe. We are uncertain why the two methods (IM versus IMA) give such different absolute divergence times both for *L. littorea* and *C. lingua*; data from nuclear loci for both species might be useful in resolving the disagreement between the two results, but seem unlikely to change the conclusion that the snail and trematode had similar trans-Atlantic population histories. Such host-parasite or host-symbiont analyses seem to have great potential for testing the strength of the predicted relationship between spatial genetic differentiation and underlying demographic history in which dispersal potential is expected to be nearly identical between some strongly interacting species due to their close ecological associations.

Incongruent patterns of temporal demography and mode of dispersal

Two recent surveys of rocky-shore marine communities in southern Australia (Ayre et al. 2009) and the northeastern Pacific of North America (Marko et al. 2010) compared spatial patterns of differentiation with underlying temporal histories among large numbers of species with different larval dispersal potential. Results from both studies tend to contradict a simple correlation between mode of development and rate of gene flow as the primary determinant of spatial differentiation. Ayre et al. (2009) studied eight species using mtDNA sampled from populations within several hundred kilometers to the east and west of a significant regional biogeographic disjunction in New South Wales that is associated with a historical geological dispersal barrier (the Pleistocene land bridge between Australia and Tasmania), unsuitable adult habitat (long sandy beaches near Wilson's Promontory in Victoria), and divergent offshore currents that should tend to limit present-day gene flow across the barrier via planktonic larval dispersal. In a barnacle (*Catomerus polymerus*), a limpet (*Cellana tramoserica*), a chiton (*Plaxiphora albida*), and a sea star (*Meridiastra calcar*) Ayre et al. (2009) found no differentation among populations within each region but strong and statistically significant phylogeographic breaks between eastern and western populations on either side of the biogeographic disjunction. Notably, all four species that share this phylogeographic break have planktonic larval development. In contrast, Ayre et al. (2009) found very strong population differentiation, but no statistically significant additional regional genetic differentiation, across the same biogeographic barrier in two species with benthic development: another gastropod (*Haustrum vinosa*) and another sea star (*Parvulastra exigua*). Could gene flow actually be greater across this barrier among species with nonplanktonic larvae? Possibly, but IMA estimates of gene flow have not yet been carried out; Ayre et al. (2009) did not analyze sequence data from these species under the full isolation-with-migration model, and thus could not obtain reliable estimates of migration rates (C. Perrin, personal communication) for the four species that showed regional differentiation. No IMA analyses were carried out for the two species with benthic development that showed no additional differentiation associated with the biogeographic barrier, or for the two other species they studied (another barnacle, *Tetraclitella purpurascens*, and another gastropod, *Bembicium nanum*) that have planktonic larval dispersal and geographic ranges that span the biogeographic barrier but showed no mtDNA population differentiation. However, IMA analyses of population divergence times by Ayre et al. (2009) showed that the ages of this regional break in three of the four species were quite old (on the order of 100,000–1,000,000 years), suggesting that in three of four species the presence of the deep regional genetic break between eastern and western populations could reflect relatively ancient separation times rather than simply restricted gene flow. Whether this might also be true of the two species with benthic development (and no regional differentiation across the biogeographic barrier) is not known. Ayre et al. (2009) concluded that the most likely cause of the regional phylogeographic break seemed to be the distribution of suitable benthic habitat in the vicinity of the biogeographic barrier, and thus not simply the facilitation or restriction of planktonic larval dispersal and gene flow. Although distinguishing between these two alternative hypotheses (restricted gene flow versus very old population divergence time) will require better estimates of gene flow, presumably through the addition of more data (i.e., loci), the data and analyses from this suite of species shows no simple relationship between mode of development, genetic differentiation, and gene flow.

In the biogeographic region studied by Ayre et al. (2009), Puritz et al. (J. Puritz, C. Keever, J. Addison,
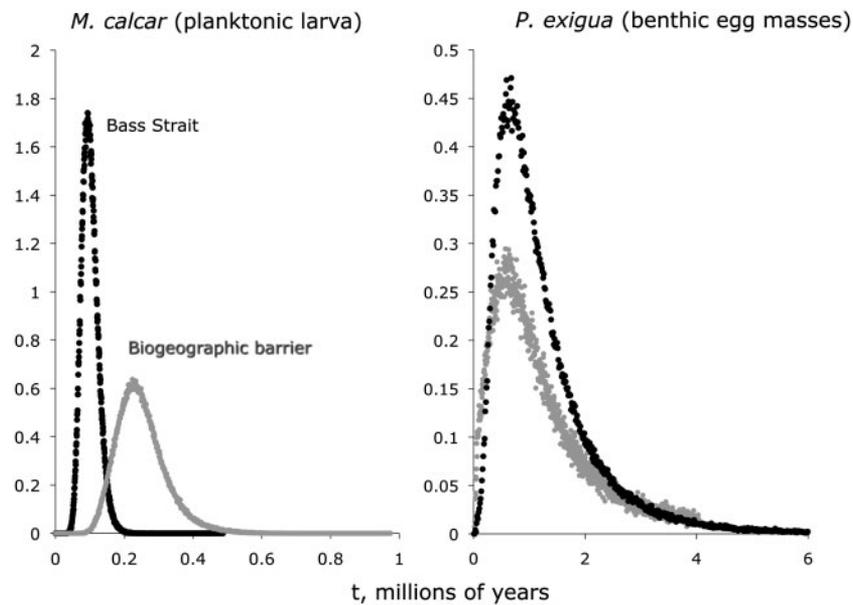
Fig. 4 Posterior probability distributions of population divergence time from IMA analysis of sea stars with (*Meridiastra calcar*) and without (*Parvulastra exigua*) planktonic larval development (J. Puritz, C. Keever, and J. Addison, unpublished data; methods as described in Fig. 2). Analyses for *M. calcar* include mtDNA only; analyses for *P. exigua* include mtDNA data plus sequences for an intron from a nuclear locus encoding glucose phosphate isomerase (as in Keever et al. 2009). For each species, two divergence times are shown: between populations from New South Wales and Tasmania (separated by Bass Strait; black); and between the same New South Wales populations and those from sites in South Australia to the west of a significant regional biogeographic barrier studied by Ayre et al. (2009) (gray).

M. Byrne, R. Toonen, R. Grosberg, M. Hart, unpublished data) have similarly tried to analyze regional variation in spatial structure and demographic history from multiple loci in the same pair of broadly sympatric sea star species with different modes of development (planktonic larvae in *M. calcar*; encapsulated development in *P. exigua*) using IMA analyses of mtDNA plus an intron from a nuclear protein-coding locus. The results of this second analysis generally argue against simple predictions about the evolution of spatial genetic structure based mainly on the mode of development and gene flow via larval dispersal. On one spatial scale, Puritz et al. estimated divergence times and other parameters for population pairs of both species from the eastern side of the biogeographic region (in New South Wales and in Tasmania, which have been linked during Pleistocene low sea level stands by the continuous coastline of the Tasmanian land bridge, and are now separated by Bass Strait). Both species show strong and statistically significant spatial differentiation in AMOVA analyses (e.g., for mtDNA variation, *M. calcar* $F_{CT} = 0.15$; *P. exigua* $F_{CT} = 0.73$) on this geographic scale, with moderate mtDNA haplotype diversity in both species, but very high intron allele diversity in *M. calcar* and very low intron allele diversity in *P. exigua* (similar to or lower than mtDNA

diversity within populations of this species). Puritz et al. found several surprising features of the demographic processes underlying this qualitatively similar pattern of spatial differentiation. First, divergence times across Bass Strait (Fig. 4) were significantly different between the two species: nearly an order of magnitude younger for *M. calcar* (93,000 years) compared to *P. exigua* (673,000 years). More recent population divergence might reflect the relative ease of colonization and population establishment or more consistent genetic connectivity across this barrier in a species with planktonic larval dispersal (*M. calcar*), or the more persistent effects of ancient extirpation with poor colonization by species without planktonic larvae (*P. exigua*). However, these two species do not differ in the rate of gene flow across Bass Strait in spite of their obvious difference in dispersal potential: in both cases, $M = 0$ after either recent (*M. calcar*) or more ancient (*P. exigua*) divergence between Australian and Tasmanian populations. This unexpected set of patterns suggests that differences in mode of development might more strongly affect metapopulation dynamics of extirpation and colonization (measured as *t*) than it would the rates of gene flow between populations after divergence (measured as *M*). Such colonization differences have been long appreciated by larval ecologists

(Johannesson 1988) but the quantitative effects of variation in colonization rate (independent of differences in gene flow after colonization) have been largely overlooked by marine phylogeographers, perhaps because the two effects could not previously be differentiated in analyses that used F-statistics interpreted mainly in terms of ongoing gene flow. Lastly, Puritz et al. found qualitatively different patterns of N_e variation between these two species: a two-fold to five-fold increase in *M. calcar* N_e (compared to ancestral N_e), and at least an order of magnitude decrease in *P. exigua* N_e (Fig. 5). We are unsure whether these two contrasting differences in t and N_e are causally linked, or independently varying, in these two species, but they strongly contradict a simple expectation of similar demographic processes underlying similar spatial patterns of genetic differentiation.

On a second geographic scale, Puritz et al. compared demographic parameters for population pairs of both species from the far ends of the eastern and western biogeographic regions documented by Ayre et al. (2009). Because these eastern and western samples were from locations in South Australia and New South Wales that are separated by an additional candidate biogeographic barrier (soft sediment shores between Wilson's Promontory and the region around Adelaide to the west in South Australia) outside of the immediate region of the phylogeographic break that was sampled on a much finer scale by Ayre et al., the results of Puritz et al. tend to complement, rather than test, the patterns reported previously. Both species show very strong spatial differentiation on this geographic scale (for mtDNA variation, *M. calcar* $F_{CT} = 0.58$; *P. exigua* $F_{CT} = 0.72$), with reciprocally monophyletic allele or haplotype genealogies at one or both loci. In this comparison, east–west population divergence times were generally similar to those estimated by Ayre et al. (2009) but significantly younger in *M. calcar* (223,000 years) than in *P. exigua* (>600,000 years; Fig. 4), and older than the *M. calcar* divergence times across Bass Strait. Like the Bass Strait comparison, migration rates were zero for both species and the two species differed in the pattern of evolution of population size (expanding in *M. calcar*,
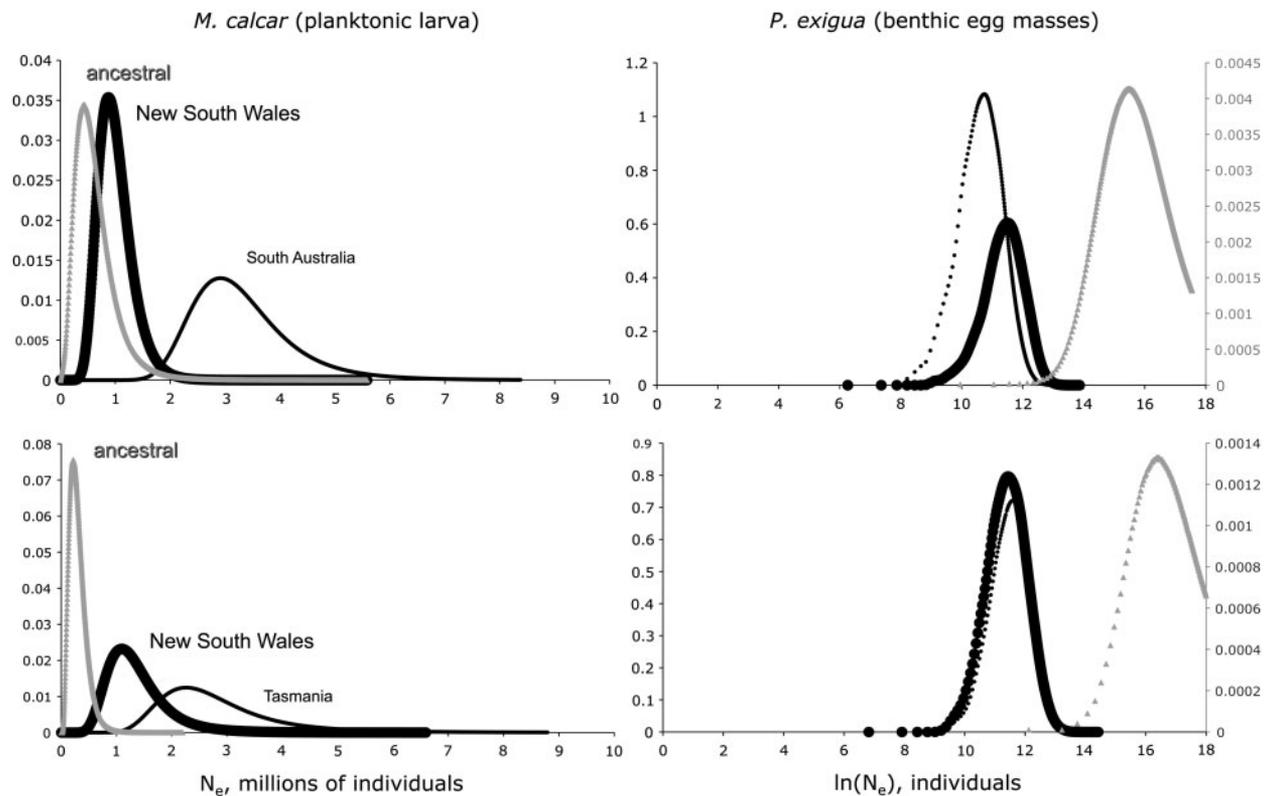

Fig. 5 Posterior probability distributions of effective population sizes (N_e) of sea stars as in Fig. 4. Each panel shows estimated ancestral N_e (gray symbols and text), plus the estimate for extant populations from New South Wales (black, large symbols and text) and from either South Australia (above; small symbols and text) or from Tasmania (below). For *P. exigua* (right), N_e estimates were log-transformed in order to show both small (extant) and large (ancestral) N_e values on the same scale. Note the second dependent variable scale (gray) for lower probability values of ancestral N_e estimates in *P. exigua*.

shrinking in *P. exigua,* relative to the ancestral population; Fig. 5). Because all estimates of migration rates on these spatial scales were zero, the spatial differentiation and contrasting histories of population growth are not likely to be explained by differences in larval dispersal. One candidate explanation is based on differences in mating systems: *P. exigua* individuals are small sex-changing hermaphrodites with fecundities on the order of 10^2–10^3 per egg mass, whereas *M. calcar* individuals are large gonochoric broadcast spawners with clutch sizes several orders of magnitude larger than in *P. exigua*. Small N_e in *P. exigua* may reflect population bottlenecks exacerbated by the evolution of very small clutch size. IMA analyses of variation in both species on smaller spatial scales (within each of the three regions we considered in this comparison) will be useful in estimating the possible difference between species in local migration rates within biogeographical regions (as suggested by Ayre et al. 2009). However, none of these comparisons so far suggest a primary role for gene flow via larval dispersal (or differences between species in mode of development) for generating large-scale phylogeographic structure or determining species differences in these spatial patterns.

In the northeastern Pacific, Marko et al. (2010) combined mtDNA with some nuclear intron or other noncoding nuclear DNA sequences for a similar taxonomically broad sample of co-occurring species from a temperate rocky-shore community. This study did not include a single putative regional biogeographic barrier (although some strong phylogeographic breaks occur among some of the species surveyed), but rather focused on inter-specific patterns of spatial genetic diversity caused by late Pleistocene glaciations, especially the last glacial maximum (LGM) ~20,000 years ago. Among the 14 species studied, 12 showed statistically significant evidence of past population expansions as measured by standard summary statistics that assume equilibrium rates of mutation, gene flow, and genetic drift (i.e., Tajima's D, Fu's F_s, and Ramos-Osins and Rozas' R2; Tajima 1989; Ramos-Onzins and Rozas 1992). Eleven species showed no strong population differentiation, together suggesting a shared demographic history of recent postglacial expansion of the population across the entire community. However, when analyzed with IMA, some species showed consistently ancient inter-population divergence times that were much older than the LGM (*Balanus glandula, Patiria miniata,* and *Xiphister atropurpureus*) whereas others displayed younger population divergence times (e.g., *Nucella lamellosa,*

X. mucosus) consistent with post-LGM recolonization. Old population divergences were found in species with (*B. glandula, P. miniata*) and without (*N. lamellosa, X. atropurpureus*) long-lived planktonic larvae, and IMA estimates of migration rates were not consistently correlated with either the mode of reproduction or the age of the most significant population differentiation. Notably, Marko et al. (2010) found very old (*B. glandula*) or very young (*X. mucosus*) multilocus population divergence times in species without significant mtDNA population differentiation. Population separation times inferred with IMA were consistent with demographic reconstructions of changes in past population size (from Bayesian skyline plots); many species exhibited evidence of large demographic expansions in the past, but the timing of these expansions varied dramatically among species in an unpredictable manner with respect to developmental mode. Marko et al. (2010) concluded that there might be more evidence for long-term population persistence (population-pairs with old divergence times) among species with planktonic larval dispersal than among species with benthic embryos and larvae, but found no statistically significant association between population history and mode of development.

In the same biogeographic region studied by Marko et al. (2010), we have tried to carefully compare regional variation in spatial structure and demographic history in two broadly sympatric species with different modes of development (*N. lamellosa, P. miniata*) using IMA analyses of a large sample of mtDNA plus six anonymous noncoding nuclear DNA sequence markers for each species (T. McGovern, C. Keever, C. Saski, M. Hart, P. Marko, unpublished results). This detailed comparison has revealed clear examples of three types of incongruence between spatial population structure and underlying demographic history that tend to reject the simple predicted effects of mode of development and larval dispersal on rates of gene flow and the evolution of population differentiation. The first example demonstrates how unexpected patterns of differentiation with respect to larval dispersal potential can be more readily explained once the patterns are considered in the temporal context provided by IMA. Across Queen Charlotte Sound in British Columbia (between the northern end of Vancouver Island and the Haida Gwaii/Alexander archipelagos of northern BC and southeastern Alaska), *P. minata* shows a large and highly statistically significant mtDNA population genetic break (F_{CT} ~0.4) but *N. lamellosa* shows no significant genetic differentiation across the same expanse. Given their different modes of development, this spatial pattern of

differentiation appears to completely contradict the expectation that the greater potential for gene flow by larval dispersal in *P. miniata* should erase the signature of older vicariant events and that the genetic evidence of older vicariant events might be more easily preserved in *N. lamellosa*, which lacks planktonic larvae (Pelc et al. 2009). However, IMA estimates of the age of this divergence between northern and southern populations on either side of Queen Charlotte Sound (Fig. 6) are about an order of magnitude older in the sea stars (~280,000 years) than in the snails (~15,000 years, consistent with colonization and population divergence at the end of the last glaciation), and imply that population structure on this geographic scale is a consequence of different responses to vicariant events of very different ages (possibly including environmental effects of the same age as the most recent glaciation, plus one or more much older processes) rather than differences in dispersal ability. The multilocus estimate of gene flow across Queen Charlotte Sound from IMA was significantly greater than zero

for *P. miniata*, but not substantially lower than comparisons between populations in other parts of the species range. This consistency across the species range indicates that the phylogeographic break reflects a relatively long history of population separation, rather than a regional restriction on gene flow. For *N. lamellosa*, even though no significant mtDNA structure was found across Queen Charlotte Sound, IMA inferred zero gene flow from the multilocus data, similar to estimates of gene flow between most other pairs of population.

The second example from this detailed comparison involves similar demographic histories underlying a different spatial pattern of differentiation. In *N. lamellosa* the most significant regional mtDNA phylogeographic break (F_{CT} ~0.07) occurs between British Columbian populations in northern and southern Vancouver Island (~500 km to the south of the large break in *P. miniata* at Queen Charlotte Sound). On this spatial scale, populations of *P. miniata* show no significant differentiation in
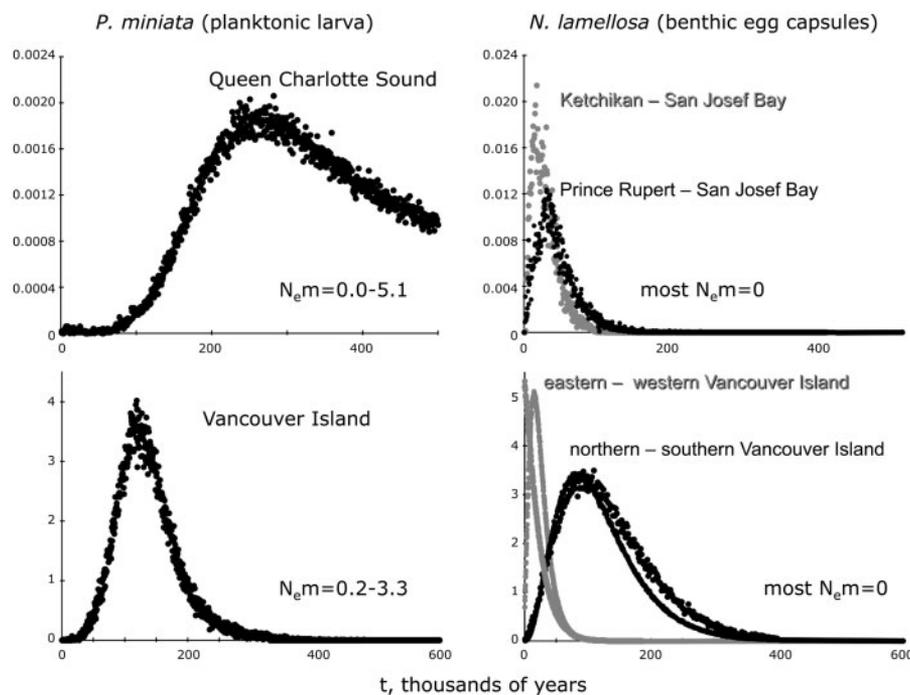

Fig. 6 Posterior probability distributions of population divergence times from IMA analysis of mtDNA and six anonymous nuclear loci of a northeast Pacific whelk (*Nucella lamellosa*) and a sympatric sea star (*Patiria miniata*). Data from Marko et al. (2010); methods as in Fig. 2 and Marko et al. (2010). Population sampling for both species included locations north and south of a significant regional phylogeographic break at Queen Charlotte Sound, British Columbia, Canada (in the sea stars), and north or south of a phylogeographic break on Vancouver Island (in snails). Note the slightly different time scales for the two comparisons. For snails (upper right), two posterior distributions are shown for comparisons between a population south of Queen Charlotte Sound on Vancouver Island (San Josef Bay) and one of two populations from Ketchikan, Alaska (gray), or from Prince Rupert, British Columbia (black), to the north of Queen Charlotte Sound. Snail populations were sampled on both the eastern and western sides of Vancouver Island, and all four posterior distributions are shown (lower right) for divergence times between eastern and western populations at the same latitude (gray), or between northern and southern populations along the wave-exposed western coast or along the wave-protected eastern coast (black) of Vancouver Island.

mtDNA. However, this qualitative difference in the spatial pattern of mtDNA differentiation hides a statistically indistinguishable history of population separation of ~112,000 years in the sea stars and 98,000–106,000 years in snails (for north–south divergence times between snail populations on the exposed western or protected eastern sides of Vancouver Island, respectively) (Fig. 6). Gene flow at this scale in both species appears to be low, but the effective population sizes of the sea stars dwarf those of the snails, and this difference may have been important in slowing the evolution of spatial differentiation between sea star populations as measured by AMOVA analyses. Given their different modes of development, this temporally coincident vicariance is difficult to explain in terms of limitation of dispersal (for the snails) and high rates of gene flow via planktonic larvae (for the sea stars), and thus probably requires an explanation based on the environmental effects (such as Pleistocene ice) on adult population persistence in both species.

In both of these examples, IMA estimated scaled migration rates ($N_e m$) that were similar for pairs of populations separated by a phylogeographic break and for pairs on the same side of such a break. Overall, migration rates were lower (and more often estimated to be zero) between snail populations than between sea star populations, but in both species the migration rate across a significant phylogeographic break (Queen Charlotte Sound for sea stars, Vancouver Island for snails; Fig. 6) was not significantly lower than migration rates between populations on one or the other side of the break. In both cases, this result argues strongly against the primary significance of gene flow via larval dispersal (or philopatric recruitment via encapsulated development) in the evolution of regional population genetic structure.

Obstacles to full exploitation of new analytical methods

Hare (2001) reviewed the prospects for use of nuclear gene trees in phylogeography at about the same time as coalescent MCMC methods were first made widely available. Hare noted the liabilities associated with reliance on a single gene tree (from mtDNA or other organellar genomes) for inferences about population histories, and the critical need for multiple loci in demographic inference. His review identified three main obstacles at that time to the use of nuclear DNA sequences in phylogeography: lack of standardized methods for PCR amplification of nuclear genes from under-studied genomes; expected

high rates of recombination between alleles; and unknown but possibly low rates of polymorphism. Progress in the short time since that review has largely resolved these issues through the (1) development of relatively accessible methods for individual researchers to clone and sequence anonymous nuclear loci, and through the development of partial or complete genome sequences from EST and genomic libraries for hundreds of animal, plant, and microbial genomes; (2) development of methods for quantifying site-specific recombination rates in sequence alignments, incorporating recombination into isolation-with-migration models, and identifying blocks of nucleotide sites unaffected by real or apparent recombination; and (3) demonstration of remarkably high rates of polymorphism, with deep intraspecific coalescent times, at many nuclear loci across a wide variety of organisms.

Recent developments have also resolved a significant issue associated with coalescent population models: the impact of demographic or spatial structure within the two population samples in IMA analyses. A recent simulation study (Strasburg and Rieseberg 2010) suggests that the IM model is not much affected by population structure within samples, and the development of IMA2 may further resolve such issues in favor of splitting some differentiated samples for analyses of more than two populations. The most significant remaining obstacle to successful application of the IMA method across species and loci may be the fit of individual sequence alignments to the DNA sequence mutation model implemented in current versions of the software. Only two (out of many possible and more realistic) mutation models for DNA sequences are implemented in IMA and IMA2. Strasburg and Rieseberg (2010) showed that a poor fit between the mutation model and the real pattern of nucleotide substitution can be a significant source of error in IMA analyses. Microsatellites, which often contain many "imperfect" repeats, may be particularly problematic in this regard, given that IMA only allows the use of the stepwise mutational model (Kimura and Ohta 1978).

Since Hare's (2001) review, the progressive development of more complex and realistic coalescent population models has put increasing pressure on larval ecologists (working as phylogeographers) to keep pace with the data requirements of the models by adding nucleotides (for greater resolution of genealogies), loci (for capturing more of the population history sampled among individuals), individuals (for capturing more of the coalescent history of each locus), populations (for comprehensive

understanding of population history across each species' range), and species (for comparative analyses within communities or within higher taxa). In spite of a decade of advice from reviews, models, and simulation studies to "add loci", a glance at the recent literature shows that many larval ecologists have found it difficult to satisfy all five of these imperatives, and many have found it necessary instead to trade-off some against others, most often by relying on single-locus mtDNA analyses that use highly reliable PCR methods for previously well-characterized mitochondrial genes, and avoid the labor, expense, and errors associated with cloning PCR products from heterozygotes at nuclear loci. It seems likely that many of these cases stem from lack of knowledge of the nuclear genome of the study organism, or the perceived difficulty of developing PCR primers for polymorphic nuclear gene sequences.

The effects of dependence on mtDNA alone (and the need for multiple loci in order to take full advantage of the insights from IMA and other coalescent MCMC methods) are apparent in the results from some recent comparative phylogeographical studies whose design and research questions were directly relevant to the goals of our review but which yielded inconclusive results from IM or IMA analysis. Teske et al. (2007) reported variation in patterns of mtDNA spatial differentiation (F_{ST}) in southern Africa among five sympatric rocky-shore species that was correlated with the mode of larval development in a predictable way (higher for species without planktonic larvae). However, IM estimates of population parameters from those mtDNA sequence alignments were ambiguous in the sense that the posterior distributions of most estimated migration rates and population divergence times had weakly defined lower bounds (including zero) and upper bounds (the posterior probabilities for the highest parameter values failed to decline to zero within the range of the prior distribution). Similarly, Crandall et al. (2008) followed a heroic sampling design that included 1182 individuals and 133 populations from 97 localities spanning the 7000 km east–west range of two closely related *Nerita* gastropods. Crandall et al. found striking differences between the large-scale phylogeographic structures in mtDNA sequence alignments from these two species (which have similar high-dispersal modes of larval development), but were unable to use IM to compare the population demographic histories underlying those two different patterns of spatial variation because one of the sequence alignments stubbornly resisted all efforts to converge on a reliable IM result.

Instead, Crandall et al. used alternative methods (Bayesian skyline plots) to infer differences among species and lineages in the history of variation in effective population size but without jointly estimating variation in migration rates or population divergence times (as in IM). In an analogous study (of ecotypes within a snail species complex, rather than among species with different distributions or modes of development), Quesada et al. (2007) found that mtDNA sequences alone could clearly identify four geographically distinct parallel divergences between the two ecotypes, but three of those four population divergence times could not be estimated with any precision in IM (with poorly defined posterior distributions as described above). In all three of these cases, the inability to resolve the population demographic histories that underlie the observed patterns of mtDNA spatial differentiation does not invalidate the characterization of those spatial patterns, but it does prevent us from discovering whether the spatial differentiation is a direct reflection of differences in rates of gene flow, or a more complex outcome of different population histories, divergence times, and patterns of change in population size. It seems likely that in all three of these cases (and others we have not reviewed that did not focus on marine invertebrate populations), mtDNA variation alone contained too little coalescent information for the joint estimation of many demographic parameters, and that greater sampling of loci (with the same or even less intensive sampling of nucleotides, individuals, and populations) could have sidestepped these limitations on the coalescent information content from mtDNA alone.

An important but underappreciated consideration in the development of new nuclear sequence alignments for coalescent population genetic analyses is the potential bias in marker selection associated with a historic preference for highly variable markers. In the past, a standard procedural step in the process of development of genetic markers used by many population geneticists was to "screen loci for polymorphism" by focusing on loci with high levels of allelic variation in test samples and discarding those with low levels of polymorphism. Although loci with low levels of polymorphisms are not useful for studies of individuality (e.g., kinship and parentage) or for some methods that attempt to infer recent dispersal events using multilocus linkage disequilibrium (e.g., STRUCTURE, BAYESASS), loci with low levels of nucleotide and haplotype diversity can provide important information about variance in the coalescent process. Because the relative mutation rates of loci are estimated separately in IMA (i.e., each locus

receives its own scalar of mutation rate), the effect of discarding less variable loci depends on the prior distribution of mutation-rate scalars with and without such loci included. If the prior distributions with, and without, less variable loci were substantially different, then their exclusion could bias the results (Hey 2010a) unless some other information (such as very low relative rates of nonsynonymous substitutions in coding sequences) can be used to argue for a nonneutral basis for the low polymorphism.

The need for multiple loci in coalescent demographic analyses, and the perils of reliance on mtDNA alone in animal phylogeography, is an increasingly difficulty problem for larval ecologists to avoid. In the IMA2 documentation, Hey suggested as a rule of thumb that we should double the number of loci for each additional population in order to avoid the crippling limitations on the quality of estimation of parameters as the number of parameters increases approximately exponentially with the number of populations. Whether larval ecologists will have to meet this or some less onerous standard in order to achieve reliable and precise coalescent MCMC results in future comparative analyses is an open question, but there seems little doubt that the future of comparative phylogeography will be built mainly on coalescent hypothesis-tests using multiple nuclear-sequence alignments (Hurt et al. 2009).

The new marine phylogeography

Are marine invertebrate larvae entirely responsible for determining population genetic structure in marine communities? Although relevant evidence has been presented for >30 years, we suggest that the jury should continue deliberations. On the one hand, post hoc interpretations based on dispersal capability for results from F_{ST} and AMOVA analyses often suggest a strong correlation. On the other hand, coalescent methods will tend to give evidence that often contradicts AMOVA-type results by accounting for some large or small proportion of population differentiation in terms of variation in divergence times and changes in population sizes rather than strictly in terms of gene flow. Reaching a reliable verdict will depend on reanalysis of previously published (mainly mtDNA) sequence data, and the accumulation of new (multilocus) sequence alignments analyzed using improved coalescent samplers.

In this short review, we advocated comparative tests of the correlation between modes of development, spatial population genetic structure, and rates of gene flow (jointly estimated with other population demographic parameters). However, additional insight could come from better within-species hypotheses of the spatial distribution of gene flow and population differentiation. One hypothesis that might bear repeated testing involves regional biogeographic patterns like those studied by Ayre et al. (2009) and Marko et al. (2010): does IMA analysis of pairs of populations reveal higher rates of gene flow (N_em) between pairs on the same side of such a phylogeographic break, and lower gene flow between pairs of populations separated from each other by the biogeographic "barrier"? The expectation is intuitively obvious, but our observations of spatially uniform gene flow around a phylogeographic break in P. miniata suggests that vicariance and not barriers to gene flow may account for this phylogeographic structure. Many such analyses might reveal whether this pattern is an anomaly or a typical explanation for unexpected population differentiation in those marine invertebrates with apparently high potential for larval dispersal. For example, Ayre et al. (2009) sampled on both sides of a distinctive biogeographic barrier, and found patterns of spatial variation on either side that were consistent with high rates of gene flow within each region for species with planktonic larvae, but did not test the hypothesis of larval dispersal by estimating migration rates and other demographic parameters for those pairs of populations within each region (or compare such results between species with and without planktonic larvae). We look forward to many such analyses as the product of increasingly powerful analytical methods and increasingly inexpensive DNA sequencing services for compiling multilocus sequence alignments for comparative phylogeography.

We foresee a surprising but very happy convergence of interests and research tools not previously shared in common by phylogeographers and comparative developmental biologists (including larval ecologists), both intent on understanding the evolution of marine animal development. Phylogeographers have a critical need for datasets composed of sequence alignments from multiple nuclear loci for coalescent MCMC demographic analyses, but often lack easy access to preliminary genomic data for the design of PCR and sequencing strategies other than those for mtDNA. In contrast, comparative developmental biologists require (among other things) genomic resources for understanding the evolution of gene expression networks underlying differences in developmental programs that have led to the evolution and loss of dispersing planktonic larval forms. An important driver of the explosive growth of

comparative genomics has been the development of second-generation sequencing technology. In large part at the urging of developmental geneticists, this high-throughput sequencing capacity has led to the sequencing of hundreds of complete eukaryotic genomes. In addition to providing information for analysis of gene expression patterns (for comparative developmental biology), these new complete genome sequences can provide initial insight into genomes of nonmodel organisms that present interesting phylogeographic problems, and can be used for the design of sampling strategies for phylogeographic studies based on PCR amplification of known target loci and high-throughput sequencing of PCR amplicons. In addition, these complete genome sequences can also serve as reference sequences for the computational assembly of whole genome sequence data from individual organisms in phylogeographic studies. These two complementary approaches to using second-generation sequencing in phylogeographic studies have different design requirements and might have different goals, but both approaches seem to hold considerable promise for fully exploiting the new analytical methods and for resolving some of the most important problems in larval ecology: how far do larvae go, how often, and for how long have they done so?

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