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ORIGINAL PAPER

Retrospective coalescent methods and the reconstruction of metapopulation histories in the sea

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Abstract Phylogeographic analyses are a key interface between ecological and evolutionary ways of knowing because such analyses integrate the cumulative effects of demographic (ecological) processes over geological (evolutionary) time scales. Newly developed coalescent methods allow evolutionary ecologists to overcome some limitations associated with inferring population history from classic methods such as Wright's F_{ST}. Here we briefly contrast classic and coalescent methods for looking backward in time through a population genetic lens, focusing on the key advantages of the isolation-with-migration (IM) class of coalescent methods for distinguishing ancient connectedness from actual recurrent contemporary gene flow as causes of genetic similarity or differentiation among populations. Making this critical distinction can lead to the discovery of otherwise obscured histories underlying conventional patterns of spatial variation. We illustrate the importance of these insights using analyses of Pacific fishes, snails, and sea stars in which population sizes and divergence times are more important than rates of contemporary gene flow as determinants of population genetic differentiation. We then extend the IM method to genetic data from two model metapopulation species (California abalone, Australian damselfish). The analyses show the potential use of non-equilibrium IM methods for differentiating among metapopulation models that make different predictions about population parameters and have different implications for the design of marine protected areas and other conservation goals. At face value, the results largely rule out classic metapopulation dynamics (dominated by extinction and colonization rather than connectivity via ongoing recurrent gene flow) but, at the same time, do not strongly support a modern marine metapopulation dynamic (ecologically significant connectivity between demes). However, the results also highlight the need for much more data (i.e., loci) sampled on different spatial scales in order to determine whether metapopulation dynamics might exist

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on smaller scales than are typically sampled by most phylogeographers and landscape geneticists.

Keywords Connectivity · Extinction · F_{ST} · Island model · Gene flow · Marine ecology · Mitochondrial DNA · Population genetics · Recolonization

Introduction

For most of its history, population genetics has been conducted within a prospective framework established in the early to mid-twentieth century, growing from relatively simple single-locus algebraic models by Fisher (1930), Wright (1931), and Haldane (1932) that consisted of equations that could be solved to determine the rate at which evolutionary change would occur given particular values of important parameters, such as the strength of natural selection, population size, and gene flow (Provine 1971; Ewens 1990; Mayr and Provine 1998; Wakeley 2008). Compared to traditionally retrospective disciplines focused on the direct observation of historical data (i.e., fossils) as a source of information to reconstruct the past, classical population genetics has had little to contribute to historical ecology, a field exemplified by the many influential paleobiological and historically-oriented ecological studies that came to define a large part of the research career of Jeremy Jackson. Despite the prospective framework of classical population genetics, population geneticists have long considered the idea that different demographic histories will leave behind distinctive signatures on genetic diversity and, more recently, that the structure of gene trees—the coalescent—could be used to reconstruct population history (Cannings and Thompson 1981; Felsenstein 1982; Kingman 1982; Tavare 1984; Waterson 1985; Slatkin 1985; Avise 1989; Griffiths and Tavare 1993). As a result, population genetics has undergone a slow but profound transformation in perspective brought on by the development and availability of coalescent methods (e.g., Kuhner et al. 1995; Beerli and Felsenstein 1999, 2001; Bahlo and Griffiths 2000; Hickerson et al. 2010), stimulated in part by the rapid increase in the amount of DNA sequence data available in the 1990s that turned the focus of population geneticists from the analysis of allele or haplotype frequencies to the analysis of gene trees, which are readily constructed from DNA sequences. In sharp contrast to classical population genetic approaches emphasizing evolutionary forces currently acting on genetic variation within and between living populations, coalescent methods are retrospective methods that look backward in time to make inferences about evolutionary forces that *have* acted on molecular variation in the past (Ewens 1990). Because the demographic forces that shape patterns of neutral genetic variation within species over time are of great interest to ecologists, coalescent population genetic methods potentially provide a way for ecologists to travel back in time to sample and reconstruct population histories of genetic effective population size or of gene flow in a way that can potentially complement other historical data (such as fossils) or ecological data (from census measures of population size or direct observations of dispersal), or provide novel insights into the demographic pasts of taxa that lack any trace of a history in the fossil record.

The development of a retrospective framework for population genetics has changed how population geneticists and phylogeographers need to think about spatial patterns of genetic differentiation, particularly with respect to understanding patterns of gene flow among populations. In his influential reviews on gene flow in natural populations, Slatkin (1985, 1987) highlighted the important difference between recurrent contemporary and historical

gene flow or connectedness in an explanation for unexpectedly high multilocus genetic similarity across populations of the checkerspot butterfly, *Euphydryas editha* (Ehrlich et al. 1975; McKechnie et al. 1975), proposing that either a recent population expansion or frequent extinction and recolonization might explain very high levels of apparent recurrent gene flow estimated with a classical inferential approach. In both of Slatkin's hypotheses, processes that introduce new alleles to populations (gene flow and mutation) and those that cause the loss of alleles within populations (genetic drift) are far from equilibrium, such that classical inferences that assume a drift-migration-mutation equilibrium lead to gross overestimates of recurrent migration between populations. Although the potential impact of demographic expansion on spatial patterns of genetic diversity has been well appreciated by phylogeographers (e.g., Ovenden and White 1990; Ibrahim et al. 1996; Avise and Walker 1998; Marko 1998; Edmands 2001), range expansions are just one of several evolutionarily unstable non-equilibrium situations that ecologists think quite common in nature, including extinction and recolonization, population growth and decline, persistence in multiple refugia (e.g., at high and low latitudes during Pleistocene glaciations), and secondary contact between previously allopatric (and genetically differentiated) populations. In these demographically complex situations, only analytical methods that can simultaneously estimate each of the relevant and interacting population genetic factors (effective population size, isolation time, mutation, and gene flow) can potentially make accurate inferences specifically about recurrent gene flow. Although by no means ignored (see Kuhner 2009), the most appropriate methods for making gene flow inferences in non-equilibrium situations are new enough that recent reviews have often not acknowledged the potential application of these methods to non-equilibrium situations (e.g., Broquet and Petit 2009; Holsinger and Weir 2009; Anderson et al. 2010; Lowe and Allendorf 2010).

In this contribution, we provide a brief overview of coalescent population genetic methods with a focus on the non-equilibrium isolation-with-migration (IM) class of model, highlighting a handful of recent studies that illustrate how the use of this non-equilibrium method can lead to novel insights into the demographic histories of marine species that cannot be reliably and robustly inferred from classical approaches, post-hoc interpretation of patterns, or coalescent approaches that require an equilibrium assumption. Our first goal is to highlight the critical problems associated with gene flow inferences from genetic data caused by violations of assumption of population genetic equilibrium (between mutation, genetic drift, and gene flow), and the associated conclusion that different population histories and different rates or directions of gene flow may often underlie very similar patterns of genetic differentiation. We conclude that population genetic measures of differentiation can be very poor proxies for connectivity and gene flow. We then extend these ideas by applying the IM model to genetic data gathered from two model marine metapopulation species. Wakeley (2004) noted the parallels between metapopulation models in ecology and the hierarchical structure of within- and between-population variation in population genetic analyses; the idea that many marine species exist as metapopulations has gained great popularity in marine ecology (Kritzer and Sale 2006), but the growth of marine metapopulation theory has been severely limited by the fact that the most important emergent property of marine metapopulations—interpopulation connectivity—is typically poorly quantified in marine species (Gerber et al. 2003; Sale et al. 2006). Even though genetics has contributed greatly to understanding patterns of spatial subdivision in the sea (e.g., Barber et al. 2000; Taylor and Hellberg 2003), most genetic estimates of metapopulation connectivity that use classic methods and inference (based on subdivision or differentiation) are hopelessly confounded by an unknown history of population size change, vicariance, extinction, and recolonization: populations may share alleles or haplotypes due

to both historical processes and contemporary connectivity (recurrent gene flow), and classic methods cannot distinguish between these historical and ongoing causes of differentiation or similarity among populations. For these reasons, coalescent IM methods that can distinguish these processes from each other seem likely to improve understanding of the history of life in the ocean, over both ecological and evolutionary timescales. We develop a series of analyses from two well-known metapopulation case studies (in abalone and in coral reef fish). We conclude that existing metapopulation genetic datasets (based on mtDNA) may often have too little information in them to adequately fit the IM model or to test specific metapopulation models, and that more data from faster-evolving regions of the nuclear genome will be needed for such IM studies in the future.

Classical population genetics, genetic differentiation, and gene flow in marine species

Nineteenth-century biogeographers recognized the important role of physical isolation as a factor promoting evolutionary divergence (Darwin 1859; Wagner 1868; Wallace 1869; Jordan 1908), leading to the concept that gene flow plays a fundamentally important role as a homogenizing evolutionary force (Mayr 1942; Slatkin 1985). As a consequence, evolutionary ecologists have been justifiably preoccupied with the direct and indirect measurement of gene flow as a means to characterize the degree of connectivity among natural populations for the purpose of understanding the extent to which local populations are independent both evolutionarily and demographically (Lowe and Allendorf 2010). This sharp focus on gene flow has provided insight into the potential for population divergence and speciation, which also has important implications for the preservation, protection, and management of threatened populations, species, and ecosystems (Larson et al. 1984; Slatkin 1985, 1987; Avise 2004; Palumbi 1994, 2003; Roderick 1996; Koenig et al. 1996; Bossart and Prowell 1998; Waples 1998; Bohonak 1999; Hare 2001; Mora and Sale 2002; Kinlan and Gaines 2003; Lowe and Allendorf 2010). Given the high density of water, rapid speed of ocean currents, and high fecundity of marine species (combined with the fact that most marine organisms spend significant time in a pelagic larval stage), marine biogeographers have been particularly passionate about understanding gene flow, creating a massive literature that includes >3 review papers per decade focused at least in part on the relationship between gene flow and genetic differentiation in the sea (e.g., Gooch 1975; Crisp 1978; Burton and Feldman 1981; Palumbi 1992, 1994; Bohonak 1999; Waples 1998; Grosberg and Cunningham 2001; Hellberg et al. 2002; Hellberg 2006, 2009; Hart and Marko 2010).

Most of these review articles on marine gene flow unavoidably establish the fact that the vast majority of studies that have sought to understand rates and patterns of gene flow do so via the estimation and interpretation of Wright's *F*-statistics and their many analogs (i.e., F_{ST}, G_{ST}, Φ_{ST}, R_{ST}). In addition to being mathematically tractable for theorists (see Holsinger and Weir 2009), F_{ST} has also been a popular measure of differentiation among empiricists because rates of gene flow (i.e., migration) may be inferred from values of F_{ST} if several simplifying assumptions hold: given sufficient time such that the appearance of new alleles within populations from gene flow and mutation is in an evolutionary equilibrium with the loss of alleles via genetic drift, the extent of neutral genetic differentiation among two or more populations will reflect the average rate of gene flow provided each population is the same size and that rates of gene flow are equal among all populations. Collectively, these assumptions are known as Wright's Island Model, which predicts $F_{ST} \approx 1/(4N_em + 1)$, where N_e is the effective population size and m is the per generation

proportion of individuals in each population that are immigrants, such that the product of N_e and m is equivalent to the number of migrants entering each population per generation (referred to hereafter as "Nm").

Calculation of Nm from F_{ST} (and the idea that populations can be adequately characterized with just one parameter) has been frequently and duly criticized because many of the island model assumptions are highly unrealistic in many situations (Slatkin 1985, 1987; Slatkin and Barton 1989; Bossart and Prowell 1998; Beerli and Felsenstein 1999; Whitlock and McCauley 1999; Mallet 2001; Neigel 2002). As Slatkin (1985, 1987) and others have pointed out, the equilibrium assumption is probably violated for many species that have undergone large population expansions following the end of the last Pleistocene glacial (e.g., Hellberg 1994; Marko 1998; Edmands 2001; Hickerson and Cunningham 2005; but see Wares 2010). Because the time required for F_{ST} to reach equilibrium depends on N_e and rates of gene flow (Crow and Aoki 1984; Whitlock 1992; Whitlock and McCauley 1999; Kuhner 2009), F_{ST} will only be near an equilibrium state in species with relatively long histories of population stability. For example, Hellberg (1994) interpreted lower genetic diversity in high latitude populations of the solitary cup coral *Balanophyllia elegans* as evidence of a recent poleward range expansion, and estimated that a drift-mutation-migration equilibrium would not be reached for at least 40,000 generations, roughly half the time of the last Pleistocene glacial and more than twice the length of the current interglacial. Because other species with much larger effective population sizes can retain ancestral alleles for even longer periods (Hurt et al. 2009; Marko and Moran 2009), the retention of ancestral alleles is surely a potential confounding factor for gene flow estimates based on differentiation between populations that have only separated on timescales of hundreds to thousands of years. Species that exist as metapopulations, with frequent extinction and recolonization, may rarely, if ever, reach a drift-mutation-migration equilibrium (Whitlock and McCauley 1999). The empirical consequences of violating the equilibrium assumption depend in part on the history of population separation. Gene flow will be systematically underestimated between populations that have only recently resumed genetic exchange because insufficient time has elapsed for gene flow to erode the accumulation of neutral genetic differences. In contrast, gene flow will be overestimated between recently separated populations if they share many alleles due to common ancestry ("ancestral polymorphisms") rather than due to gene flow that occurred after their separation.

Coalescent population genetics

Given these and other important problems with estimation of F_{ST} (e.g., Crow and Aoki 1984; Weir and Cockerham 1984; Neigel 1997; Waples 1998; Hedrick 1999), the intellectual stigma associated with the calculation of Nm from F_{ST} is so great that population geneticists do not present such estimates without considerable qualification. However, equating spatial F_{ST} variation with gene flow variation has proven a very hard habit to break for most population geneticists: despite near elimination of Nm estimates based on F_{ST} from the empirical literature, many population geneticists and phylogeographers (including the authors of this paper) continue to interpret patterns of genetic differentiation largely in terms of gene flow (Cowen and Sponaugle 2009; Pelc et al. 2009). This routine bias towards one explanatory factor as a post-hoc explanation for patterns of spatial differentiation ignores the otherwise well-known fact that F_{ST} variation is shaped by a suite of evolutionary forces interacting with each other over time. In the absence of methods that

could jointly estimate the magnitude of all of these interacting forces, population geneticists working on marine organisms (with vast ranges and easily dispersed larval propagules that face few obvious obstacles to gene flow) could perhaps be forgiven for overlooking other post-hoc explanations for differentiation in favor of explanations based on gene flow. More recently, however, population genetics has slowly shifted towards the application of new retrospective coalescent approaches that jointly estimate population genetic factors responsible for creating spatial patterns of diversity sampled from contemporary populations.

"The coalescent" is the stochastic mutation-extinction process that generates a gene genealogy, which is characterized by the phylogenetic relationships among gene copies (i.e., branching order) and the times to common ancestry (i.e., branch lengths) within the genealogy. Initially developed in the early 1980s (Kingman 1982; Hudson 1983; Tajima 1983), basic coalescent theory has been extended to far more complex situations, such as population growth and decline (Griffiths and Tavare 1993; Kuhner et al. 1998), population separation and gene flow (Beerli and Felsenstein 1999; Nielsen and Wakeley 2001), as well as natural selection (Krone and Neuhauser 1997) and recombination (Griffiths and Marjoram 1996). Because these models were only incorporated in pioneering computer programs such as GENETREE (Bahlo and Griffiths 2000) and MIGRATE-N (Beerli and Felsenstein 1999, 2001) at the turn of the last century, their use has only become common in the last few years (see Kuhner 2009). Even though the theory is mathematical and the application of the theory is highly statistical, the basic idea of the coalescent is readily understood by most biologists familiar with phylogenetic trees and the expected impacts of different histories on the overall shape and structure of trees. By the time coalescent approaches were first made available, phylogeographers were particularly familiar with the idea that different population histories could be distinguished on the basis of obvious visual structural differences among gene trees from different populations and species (e.g., Ovenden and White 1990; Templeton et al. 1995; Hewitt 1999). For example, going from the present to the past, a rapid increase in the rate of coalescence (i.e., a decrease in the length of internal branches) within a genealogy is consistent with a reduction in population size, or, alternatively, when viewed from the past moving forward to the present, a population expansion. Although the development of formal methods that could estimate genetic parameters from gene tree shapes has somewhat blurred the boundary between phylogeography and population genetics (Edwards and Bensch 2009), not all phylogeographers and population geneticists have embraced coalescent approaches that focus on the estimation of population genetic parameters over post-hoc interpretation of phylogeographic patterns (e.g., Zink and Barrowclough 2008). Although we argue from some convincing examples that these methods can provide deeper and more statistically robust insights into long-standing questions about the demographic histories of species, many empiricists still favor post-hoc interpretation of genetic data over model-based inferences and hypothesis testing (e.g., Horne et al. 2008; Chabot and Allen 2009; Reece et al. 2010).

An important distinction between classical population genetics and coalescent theory is that coalescent methods emphasize the calculation of likelihoods for features of the population that can often be successfully characterized from relatively small samples in comparison to classical population genetics theory that emphasizes estimates of allele frequencies and often requires large samples (especially for rare alleles) (Wakeley 2008). This has obvious practical advantages in that extensive sampling of variation at a locus is not necessary for coalescent methods given that any sample of alleles from a population has a coalescent structure that was shaped (at least in part) by the demographic history of the population, and will have important implications for analyses of large genomic data

sets (Wang and Hey 2010). As with all population genetic inferences, however, there is a rub that must always give empiricists pause. First, even for a simple demographic history such as a constant population size, there exists a disconcertingly high interlocus variability in the coalescent for neutral loci. Therefore, the coalescent must be empirically "replicated" across multiple unlinked loci (usually from the same sample of organisms) so that the variability in the coalescent process—whether caused by stochastic (drift and migration) or deterministic (selection) evolutionary forces—can be considered in parameter estimation. Although the impact of genetic drift will be greater on haploid and maternally-inherited markers such that patterns indicative of isolation may be more evident in animal mtDNA, the rapid divergence of mtDNA via drift also causes populations to rapidly lose information about ancestral population size; thus empiricists must always be wary of mtDNA-only inferences from sharply divergent populations. A second problem with the implementation of coalescent methods is that, because many alleles or haplotypes within a sample often differ by a small number of mutations, intraspecific genealogies can rarely be recovered with much certainty. For that reason, all of the coalescent methods commonly used today simulate population genetic parameters across a large sample of highly likely but slightly different genealogies for each locus considered, to take into account the unavoidable uncertainty in gene tree reconstruction. Hence, coalescent methods have been referred to as "genealogy samplers" (Kuhner 2009) because parameters are inferred across a large sample of highly likely gene trees rather than a single, best tree. Lastly, both the demographic and mutation models that characterize each particular coalescent method must match the history of populations from which the genetic data were gathered, otherwise the methods are likely to lead to biased results (e.g., Beerli 2004; Kuhner and Smith 2007; Strasburg and Rieseberg 2009).

All coalescent methods represent an improvement over Wright's Island Model because they lack one or more of its unrealistic assumptions. Several different classes of coalescent methods have been developed recently, including some that fit a fully specified likelihood function to a particular (relatively simplified) population model, as well as approximate Bayesian computational (ABC) methods that add model flexibility (and realism) at the expense of not fitting a fully specified likelihood model (reviewed by Hickerson et al. 2010). Among the full-likelihood approaches, such as MIGRATE-N (Beerli and Felsenstein 1999, 2001), LAMARC (Kuhner 2006), GENETREE (Griffths and Tavare 1993), and IMa (Nielsen and Wakeley 2001; Hey and Nielsen 2004, 2007), only the IM class of methods (IMa and its predecessors, MDIV and IM; also see MIMAR, Becquet and Przeworski 2007) lacks the assumption that allele sharing is exclusively due to gene flow. Therefore, MIGRATE-N, LAMARC, and GENETREE can only be used for populations that have been stable and exchanging alleles for a relatively long time: a good rule of thumb is $\sim 4N_e$ generations (Kuhner 2009), the so-called "time to monophyly" or the time required for alleles to coalesce backward in time to a single common ancestor. Therefore, only the IM approach can potentially distinguish ancestral polymorphism from recurrent contemporary gene flow that occurred after population separation. Because IMa is a non-equilibrium method in which pairwise population divergence time is the focal model parameter, the major limitation of IMa is that single analyses are limited to two populations at a time; the recently released IMa2 can analyze multiple populations, but (for computational purposes) requires knowledge of the hierarchical splitting history for all of the populations and (in order to estimate very large numbers of parameters for >2 populations) requires data from large numbers of unlinked loci. Researchers generally compensate for these IMa constraints by carrying out multiple analyses of either all pairwise or geographically adjacent samples followed by qualitative inferences about the overall patterns.

Like all model-based methods in population genetics, the implementation of the IM model makes a series of assumptions about some aspects of the population history in order to infer other features of the demography (Beaumont et al. 2010). We argue that the IM model assumptions are considerably more realistic than those of the island model and other equilibrium models, but some of those assumptions are not easily tested. One indication of important violations of the IM model assumptions might come from iterated analysis of different population pairs that show significant differences in the posterior distribution of N_e for a focal population when analyzed in combination with a series of other populations. Users of these methods must be aware of the potential bias caused by the two-population limitation, because both historic and recurrent gene flow from a third population can alter IMa parameter estimates for the two focal populations (Strasburg and Rieseberg 2009).

IM estimates of gene flow (Nm) in marine species

Our own studies of the population structure of the dogwhelk gastropod *Nucella lamellosa* and the bat star *Patiria miniata* along the coasts of British Columbia and southeastern Alaska illustrate the potential errors inherent in equating the absence of differentiation with high rates of gene flow, and how patterns of population differentiation are often shaped largely by historic rather than contemporary or recurrent gene flow (Marko 2004; Keever et al. 2009). The bat star, which has a highly dispersive planktonic larval stage, shows a very large and highly statistically significant population genetic break across Queen Charlotte Sound (QCS), between the northern end of Vancouver Island and the Haida Gwaii/Alexander Archipelagos of northern BC and southeastern Alaska (Fig. 1a). The authors speculated that this strong phylogeographic break might be caused by limited larval dispersal and low gene flow across QCS due to the divergence of a major nearshore ocean current at the same latitude (Keever et al. 2009). The snail, however, which completely lacks a planktonic larval stage, shows no significant genetic differentiation across the same deep water expanse (Fig. 2a). Is the absence of a genetic break across QCS in *N. lamellosa* explained by greater rates of contemporary gene flow in this poorly dispersing species? Probably not: a subsequent study (McGovern et al. 2010) re-visited this and other disjunctions in the two species by combining data from six anonymous nuclear loci with existing mtDNA sequences from each species and re-analyzed with IMa. The multilocus estimate of population divergence time was very old for the bat star ($\sim$282 kyr) but significantly more recent for the snail (15 kyr); the results also suggested that relatively small effective population sizes in the north may have been important in driving the QCS divergence in the bat star. The IMa estimates of gene flow across QCS (since the time of population separation) are in fact greater for the bat star, consistent with differences in the two species dispersal potential. Taken together, the results from IMa show that the absence of a genetic break in the poorly dispersing snail is best explained by a recent population separation across QCS, probably a consequence of a recent colonization across Queen Charlotte Sound and thus greater allele sharing due to historical, rather than contemporary, gene flow. The more surprising result from these analyses was that the multilocus estimates of gene flow in the bat star were no lower across QCS than between any other adjacent populations elsewhere in the species' range, emphasizing that the unusually large break across QCS in the bat star is not a consequence of restricted present-day gene flow, and requires no explanation based on present-day physical oceanography, but instead can be explained mainly by a relatively ancient population separation (whose cause is unknown but might have been based on Pleistocene climate and ocean current fluctuations). An

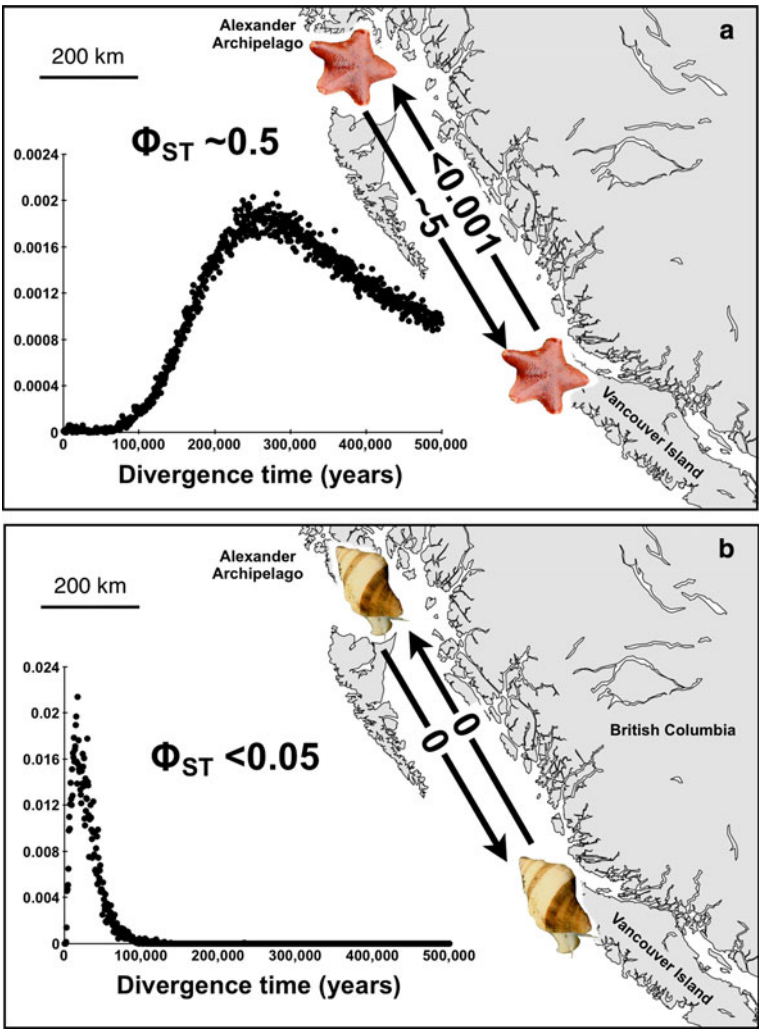

Fig. 1 Genetic differentiation and multilocus (mtDNA, 6 anonymous nuclear loci) posterior distributions for population divergence times across Queen Charlotte Sound, British Columbia from IMa for populations of **a** *Patiria miniata* and **b** *Nucella lamellosa*. Values on *arrows* are jointly estimated (*modes*) rates of gene flow (Nm). See McGovern et al. (2010)

important result from this study was that IMa consistently emphasized isolation times and population size variation, rather than gene flow, as explanatory factors for spatial patterns of genetic differentiation.

Although the difference in population separation time was not altogether unexpected given the differences in dispersal potential between the two species (and thus the improbability that recurrent gene flow has been greater in the poorly dispersing snail), the study of snails and sea stars from the northeastern Pacific clearly illustrates the potential pitfalls of post-hoc interpretations of patterns of population differentiation, and shows that non-equilibrium approaches may be necessary to distinguish recurrent contemporary from historic gene flow. Very few other comparative studies have used these methods to

disentangle the relative importance of gene flow, isolation time, and population size as explanatory factors for patterns of population differentiation in the sea, particularly across multiple independent comparisons. One recent study by Lessios and Robertson (2006), however, compiled mtDNA sequence data from central and eastern Pacific populations from 20 teleost fish species whose ranges span the entire Pacific basin. The species were selected based on morphological criteria such that each was regarded to be the same species across the entire Pacific basin so that rates and patterns of intraspecific gene flow could be compared. Unlike the study of bat stars and snails, the dispersal potential for all of the species considered by Lessios and Robertson was thought to be similar given that all possess a pelagic larval stage, so differences in levels of genetic differentiation cannot be easily attributed to differences in larval dispersal potential. Among species, the mtDNA sequences showed highly variable patterns of genetic differentiation across the Pacific, with anywhere from zero to more than 40% of the variation being attributable to differences between central and eastern Pacific populations. Because many eastern and central populations shared mtDNA haplotype lineages, the authors used the IM model to jointly estimate gene flow, effective population size, and population divergence time.

Overall, the IM analyses showed that in most species, recurrent gene flow was highly asymmetrical, with greater migration from the eastern Pacific to the central Pacific than in the opposite direction (Lessios and Robertson 2006). Not surprisingly, the results also provided examples where the expected qualitative relationship between genetic differentiation and gene flow is readily apparent. For example, some species with highly divergent trans-Pacific populations have exchanged no migrants since the time of population separation (e.g., *Doryrhamphus excisus*, $\Phi_{CT} = 0.489$, $Nm = 0$ in both directions) whereas some completely undifferentiated population pairs have been exchanging migrants at very high rates (e.g., *Mulloidichthyes vanicolensis*, $\Phi_{CT} = 0$, $Nm > 700$ from eastern to central Pacific) in at least one direction across the Pacific. However, other completely undifferentiated population pairs have unexpectedly low rates of gene flow (e.g., *Scarus rubroviolaceus*, $\Phi_{CT} = -0.108$, $Nm < 0.01$ in both directions); high genetic similarity across the Pacific in these cases is instead explained by relatively recent population separations ($\sim$30 kyr). Conversely, some highly divergent populations show high rates of gene flow (*Arothron meleagris*, $\Phi_{CT} = 0.346$, $Nm > 17$ from eastern to central Pacific) in at least one direction across the Pacific since their initial separation $\sim$68 kyr ago. Collectively, these examples illustrate that the expected relationship between gene flow and genetic differentiation may be present in some species, but may be entirely absent in others. More surprisingly, the results reveal that large differences in recurrent gene flow may underlie similar levels of variation as characterized by *F*-statistics, such as with *A. meleagris* and *D. excisus*, which show similar amounts of differentiation but markedly different rates of trans-Pacific gene flow. Across all of the comparisons in which the analyses converged on clear results, divergence time explains approximately four times as much variation in genetic differentiation (Φ_{CT}) as does gene flow and only the relationship between genetic differentiation and isolation time was statistically significant (Fig. 2).

Although we wish to highlight the perils of framing intraspecific patterns of genetic differentiation in terms of gene flow as a prelude to applying the non-equilibrium IM approach to metapopulations, some estimates of gene flow from equilibrium and non-equilibrium methods often show remarkably similar results, which may be evidence of a drift-mutation-migration equilibrium. For example, a MIGRATE-N analysis (which assumes equilibrium) of mtDNA sequences from Pacific lingcod (Marko et al. 2007) showed an asymmetrical pattern of gene flow (Nm) between adjacent populations in the Puget Sound region of the northeast Pacific, although migration rates (expressed here as the

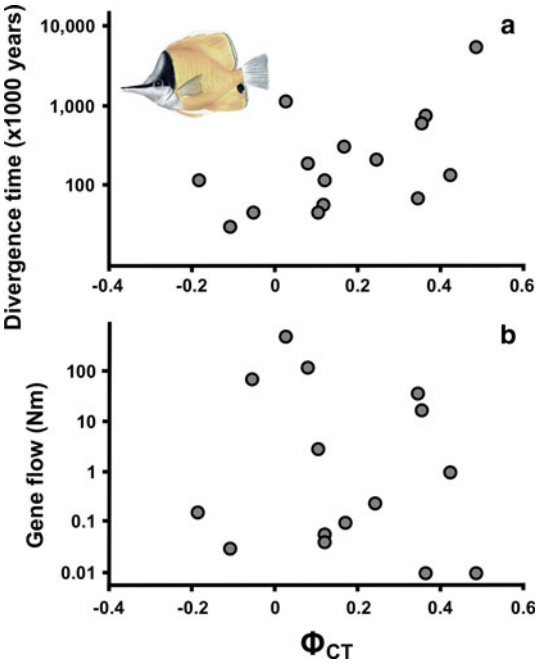

Fig. 2 Relationship between regional mtDNA genetic differentiation and pairwise IM estimates of **a** population divergence time ($R^2 = 0.228$, one-tailed $P = 0.033$) and **b** gene flow (Nm) ($R^2 = 0.057$, one-tailed $P = 0.195$) between central and eastern Pacific populations of 15 reef fish species. Parameter estimates from Lessios and Robertson (2006). The correlations were not biased by inclusion of three cases in which the measure of regional trans-Pacific population differentiation (Φ_{CT}) was <0: both of these correlations were actually strengthened by adjusting these three cases of negative Φ_{CT} values to zero

parameter m due to uncertainty in some estimates of Θ) are all generally small in all directions (Fig. 3a) indicating that migrants make up a very small fraction of the population (<1%) each generation. A re-analysis with IMa (which does not assume a drift-migration equilibrium) returns qualitatively similar results (Fig. 3b).

Non-equilibrium genetics and metapopulation dynamics

Non-equilibrium coalescent methods seem likely to have a large positive effect on ecology by allowing ecologists to use genetic approaches to look backwards in coalescent time in order to estimate population parameters from sequence data for organisms of particular conservation interest that exist in fragmented habitats and temporally unstable environments. The capabilities of IM methods to distinguish historical connectivity from recurrent gene flow suggests one especially rich area of application of these methods might be the improved understanding of metapopulation dynamics in threatened or endangered species, where the design of conservation or recovery plans might critically depend on a clear understanding of the historical role of recurrent gene flow versus other evolutionary processes in producing population differentiation and metapopulation structure (Kritzer and Sale 2006). In marine ecology, metapopulation theory has largely replaced the dynamic equilibrium theory of island biogeography (MacArthur and Wilson 1967) as a conceptual framework for conservation (Hanski and Simberloff 1997), probably because of the perception that metapopulation theory provides a non-equilibrium framework (Hanski and Simberloff 1997; Hanski 2001) and because many species living in patchy or fragmented seascapes usually lack large "mainland" populations that largely replenish smaller patches through re-colonization, as envisioned in the island theory (Sale et al. 2006).

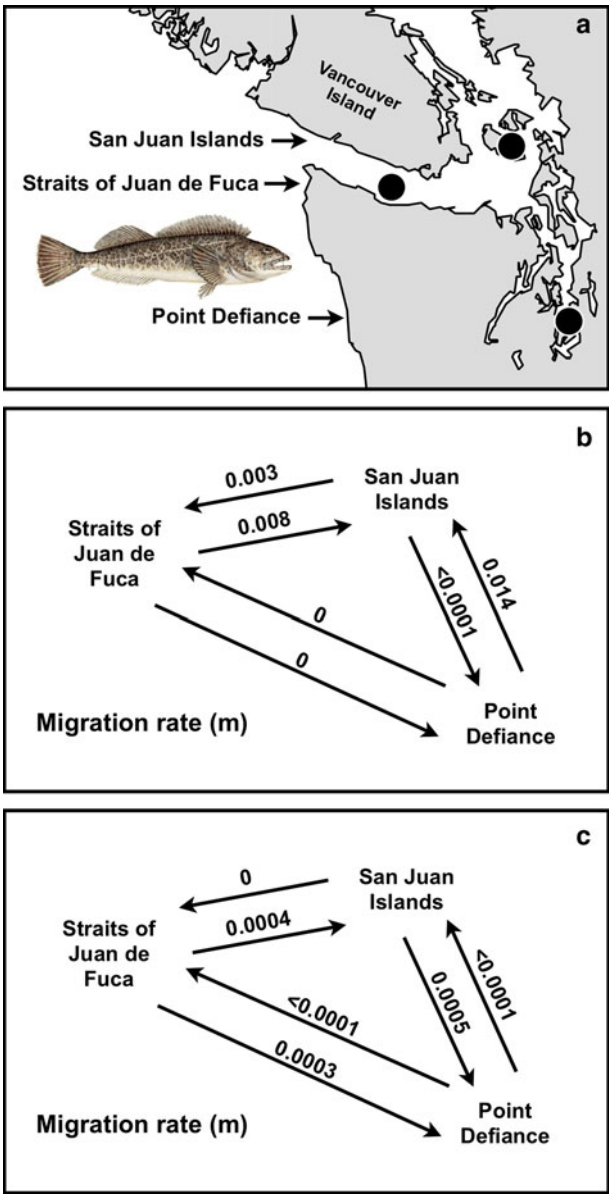

Fig. 3 Migration rates (m, per generation) between populations of *Ophiodon elongatus* estimated from mtDNA sequences using **a** MIGRATE (Marko et al. 2007) and **b** IMa. To convert estimates of m/μ from both methods, we assumed a substitution rate equivalent to a 2% per million years divergence rate and a generation time (average age of breeders) of 10 years. We make the more direct comparison between estimates of m rather than Nm (number of migrants per generation) because values of Nm depend on estimates of population size (i.e., $\Theta = 4\ N\mu$), which had large credibility intervals (Drawing by D. Smith)

Many different definitions of metapopulations have been proposed, but most are variants on the original concept introduced by Levins (1969), a theoretical framework for the population dynamics of species inhabiting patchy habitats. What we call a classic "Levins

metapopulation" (LM) is a group of demes (in terminology suggested by Wakeley 2004) separated from each other in space but linked in time by a dynamic pattern of local extinction and recolonization (Levins 1969). Although proponents of the Levins model do not contend that dispersal between existing demes is nonexistent, the Levins model focuses on re-colonization as a source of connectivity by incorporation of the simplifying assumption that the local size of each deme is either zero or the carrying capacity, eliminating dispersal between existing demes from the model. In contrast, a "Kritzer-Sale metapopulation" (KSM), used by many marine ecologists, focuses on recurrent connectivity between existing demes (via larval dispersal) in spatially discrete locations, placing greater emphasis on the coupling of recruitment dynamics acting over different spatial scales, in which local recruitment within patches is modulated by immigration from other patches such that individual demes are not in total isolation and extinction probabilities for local demes are lower overall (Kritzer and Sale 2004, 2006; Sale et al. 2006). In the language of the IM model, classic LMs consist of demes that exchange no migrants once established or separated, have relatively recent but variable isolation times, and retain low genetic diversity given that intra-deme diversity is determined entirely by rare colonization events (historic gene flow) rather than recurrent contemporary migration. Local demes within KSMs have older and less variable separation times and remain connected by demographically significant recurrent migration after colonization, resulting in the maintenance of greater intra-deme diversity. Although larval dispersal and inter-deme connectivity are fundamentally important factors influencing (and defining) metapopulation structure, the nature of inter-deme connectivity—whether dominated by extinction-recolonization processes, recurrent gene flow, or some combination of both—is typically poorly characterized for most marine species studied within a metapopulation framework (Kritzer and Sale 2006).

The IM models and analytical methods described in the examples above could be used to test the predictions of the classic LM and the KSM models against population parameters (especially t and Nm) estimated from genetic data in order to ask, for example, how often or under what circumstances marine metapopulations conform to the classic LM model or some version of the KSM model that also includes variation in gene flow and in effective population sizes. Surprisingly, it seems that this empirical approach using IM models has not yet been used. Although population geneticists have long recognized the potential impacts of extinction and recolonization on patterns of differentiation within metapopulations (e.g., Wade and McCauley 1988; Giles and Goudet 1997; Pannell and Charlesworth 1999; Pannell 2003; Wakeley 2004), only a handful of the hundreds of papers that cite the original applications of the IM (Hey and Nielsen 2004) and IMa (Hey and Nielsen 2007) methods include the keyword "metapopulation" in a searchable field of the ISI Web of Knowledge database (searched 20 July 2010), and none have used IM or IMa to test different metapopulation model predictions. Even the most recent analyses of marine population genetic structure that explicitly invoke metapopulation dynamics and use coalescent demographic models to analyze sequence data have used methods like MIGRATE-N that assume dispersal as the cause of allele sharing between demes, neglecting ancestral polymorphisms and divergence times (e.g., Bay et al. 2008).

To illustrate some of the potential insights from IM analyses of metapopulations, we reanalyzed data from one of two marine invertebrate case studies (abalone and sea urchins) developed in detail as metapopulation exemplars by Morgan and Shepherd (2006). We focused on the California red abalone, *Haliotis rufescens*, for which there are suitable mtDNA (and other) population genetic data (Gruenthal et al. 2007). *Haliotis* species form patchily distributed demes on rocky reefs in the northeast Pacific that are separated from

each other by habitat unsuitable for the benthic adults. Because adults move at most a few tens of meters during their lifetimes, demes are most likely established (and linked to each other after colonization) by the dispersal of larvae during a brief period of nonfeeding planktonic development. All of these species have been targeted for commercial or recreational or traditional food fisheries, many have experienced severe population declines, and two have made the IUCN Red List of endangered (*H. kamtschakana*) or critically endangered (*H. cracherodii*) species (IUCN 2010). The design of marine reserves or management plans for abalone recovery could depend in part on understanding whether abalone form classic LMs in which demes are not connected to each other by ongoing larval dispersal (a prediction consistent with strong population genetic differentiation in some *Haliotis* species) such that population structure reflects only the history of local extinction and recolonization of demes, or KSMs with ongoing gene flow in some directions between some demes leading to greater genetic mixing of demes and relatively higher local diversity (a prediction consistent with genetic homogeneity in other *Haliotis* species; Morgan and Shepherd 2006).

We used IMa (Hey and Nielsen 2007) to analyze mtDNA sequences collected by Gruenthal et al. (2007) from three pairs of adjacent demes including, from north to south: mainland demes from 7 House Cove (7HC, near the putative biogeographic break at Cape Mendocino), Horseshoe Cove (HSC, just north of San Francisco Bay), and Monterey (MY, the most genetically distinctive deme sampled by Gruenthal et al. 2007); and an offshore location on San Miguel Island (SMI, the southernmost deme in the study, and south of the putative biogeographic break at Point Conception). The *Haliotis* mutation rate for mitochondrial protein-coding genes is unknown, so we used the same rate (7.6×10^{-9} substitutions per site per year) used in our earlier analyses of the same gene in *Nucella* and we assumed a generation time (the average age of breeders, for calculation of N_e from Θ) of 10 years for large, long-lived, and slow-growing abalone (IUCN 2010). We used an analytical strategy similar to that suggested in the IMa documentation and used by us in other studies. First we ran a series of analyses in "M mode" (Markov-Chain Monte Carlo or MCMC mode) in which we varied the prior distributions, number of search chains, and heating scheme in order to find parameters for the Metropolis-coupled MCMC search of likely gene trees that gave evidence of good mixing (large effective sample sizes, similar demographic parameter estimates from the first and second halves of each search, and similar results from replicate searches under identical conditions from different random number seeds) and that densely sampled the non-zero part of the posterior distribution of the six demographic parameter values: divergence time (t), three population size parameters ($\Theta = 4N_e\mu$, where μ is the locus specific mutation rate) for each deme and for the ancestral deme; and two migration rates (m/μ). The M mode analyses invoke the MCMC search, generate posterior distributions of model parameter values, and save a sample of gene trees on which the parameter value posteriors are based; for each population or deme pair, we then analyzed the saved gene trees from the best M mode result in "L mode" (Load Trees mode) to carry out likelihood ratio tests that compare a set of nested models with progressively fewer parameters against the full six-parameter model. These L mode tests allow the user to ask whether population sizes have changed from the ancestral population size, whether population sizes of the sampled populations differ, whether the two migration rates differ from each other, and whether either or both of those migration rates are not significantly different from zero. In some cases where the L mode results suggested a less parameter-rich model was not a significantly poorer fit to the data, we reran the M mode analyses using a simplified model (e.g., one or the other migration rate set with an extremely low prior distribution to approximate $m \sim 0$) and estimated the

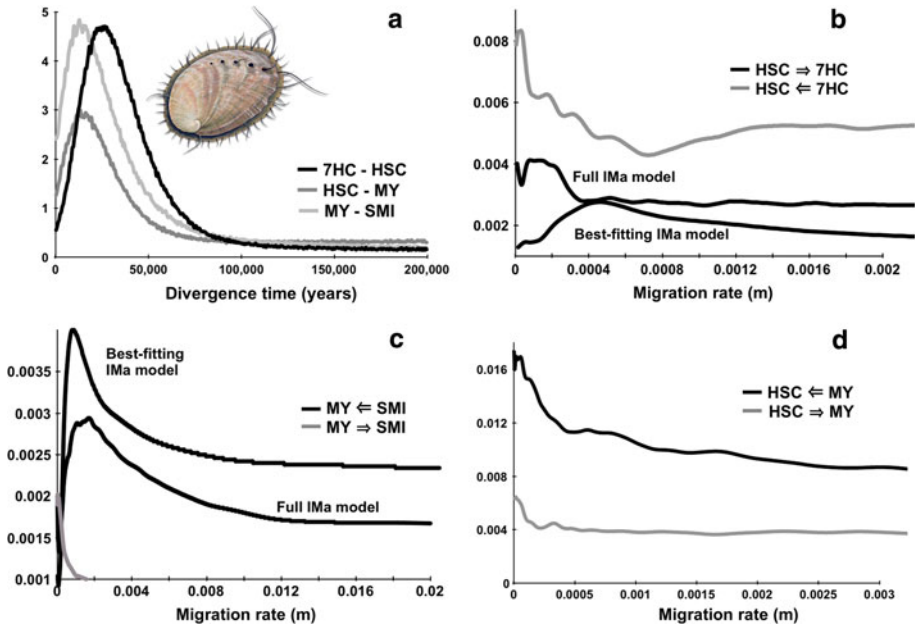

Fig. 4 Pairwise IM estimates of divergence time and migration (m, per generation) among populations of *Haliotis rufescens* in California. 7HC, 7 House Cove. HSC, Horseshoe Cove. SMI, San Miguel Island. Data from Gruenthal et al. (2007) (Drawing by E. Damstra)

remaining parameter values under that better-fitting model. We primarily used the L mode results to identify migration rates for each pair of demes that were not significantly different from each other or from zero.

Our results tended to reject the classic LM model for *H. rufescens* in favor of KSM dynamics with spatial variation in gene flow but not in the history of extinction-recolonization. First, population divergence times all had sharp modes in the late Pleistocene (12,000–27,000 years) with broadly overlapping posterior distributions (Fig. 4a) that suggested these demes were probably all established at about the same time. This result suggests that, at least on this geographic scale, abalone metapopulations are not mainly structured by regional patterns of stochastic local extinction and recolonization (Morgan and Shepherd 2006). This conclusion is fairly robust with respect to conceivable error in our assumed mutation rates: a five to tenfold larger mutation rate would still result in modal divergence times exceeding several thousand years. Instead, population pairs appear to differ mainly in the magnitude and direction of gene flow. Although the posteriors for migration were fairly flat in some comparisons, L mode testing rejected models in which migration rates in both directions were zero: for the northern (7HC-HSC, Fig. 4b) and southern (MY-SMI, Fig. 4c) pairs of demes, L mode testing could not reject models with northern gene flow and subsequent M mode analyses using the best-fitting models showed that south-to-north gene flow (into 7HC, into MY) was low but significantly greater than zero. For the central pair of demes (HSC-MY, Fig. 4d), gene flow was significantly greater than zero in both directions, but the shapes of the migration rate posterior distributions were poorly resolved with long right-hand tails of non-zero probability for very high parameter values. This and other features of these results highlight the limited information

content from single loci in coalescent demographic analysis using IMa (and other models or methods). Effective population sizes (i.e., Θ) could not be precisely estimated in nearly all comparisons, and tended to have posterior distributions with a high mode but with very broad non-zero posterior probabilities for very large population sizes (such a result is typical of mtDNA sequence data with relatively few coalescent events). For this reason, we were not able to express gene flow as the population migration rate $M = 2Nm$. Such results do not give us much confidence in a quantitative comparison between demes and strongly suggest that more data (i.e., more loci) are needed in order to fully characterize metapopulation structure in these demes under the IM model. However, our preliminary results based on mtDNA alone do not appear to favor a metapopulation structure defined by relatively recent variation in the timing of local extinction and colonization (Fig. 4a).

We have also re-analyzed mtDNA sequence data from a second metapopulation case study species, *Acanthochromis polyacanthus* (spiny chromis), a common western Pacific damselfish found throughout Australia's Great Barrier Reef (GBR). Reef fish are often considered as model metapopulations by many marine ecologists (Figueira 2002; James et al. 2002; Kritzer and Sale 2006; Lipcius et al. 2008), and a recent genetic analysis (Bay et al. 2008) of mtDNA control region (or D-loop) sequences from *A. polyacanthus* concluded that patterns of genetic diversity among reefs on several spatial scales (several to 100s of km) were broadly consistent with metapopulation dynamics. At the largest spatial scale sampled (populations from northern, central, or southern regions of the GBR separated by up to 1,200 km), Bay et al. found very strong genetic differences ($\Phi_{ST} = 0.81$) among regions (partly concordant with color-morph differences) plus evidence of isolation by distance, indicating that each region is effectively a closed system with respect to gene flow. Within regions, there was consistently significant but variable spatial genetic structure among many reefs, no evidence of isolation by distance, and evidence of recent population expansions of different ages. However, despite the overall pattern of significant genetic differentiation, statistically significant (and often asymmetric) gene flow ($Nm > 1$) was inferred with MIGRATE-N among 20–40% of reefs on the smallest spatial scales sampled. Overall, Bay et al. concluded that the combination of results was consistent with a non-equilibrium metapopulation structure among reefs, but did not investigate whether patterns of asymmetric gene flow from MIGRATE-N were actually caused by recurrent contemporary migration among established demes (KSM dynamics) or a history of extinction and recolonization among demes (LM dynamics).

To separate historic from contemporary gene flow in *A. polyacanthus*, we re-analyzed a portion of the sequence data with IMa. We focused on the central GBR region because of the apparent pattern of one-way gene flow among reefs inferred by MIGRATE-N: Myrmidon Reef (MYR) → Trunk Reef (TRU) → Pith Reef (PIT) → Britomart Reef (BRI); we attempted similar analyses of the northern region studied by Bay et al. but these results were complicated by the presence of two highly divergent haplotype clades in the northern region that could represent cryptic species. We used the same analytical procedures as with the abalone data, using both the M and L modes within the program IMa for parameter estimation and hypothesis tests, respectively. We employed a mutation rate of 7.8×10^{-8} substitutions per site per year based on D-loop sequence divergences between a geminate species pair of *Chromis* separated by the Isthmus of Panama at least 3.1 million years ago (Domingues et al. 2005). This geminate-based rate is quite fast (equivalent to a divergence rate of $\sim 16\%$ per million years), and could reflect a divergence time that substantially predates the final closure of the Central American Seaway (see Marko 2002), although the results and conclusions from our analysis appear to be conservative with respect to the possibility that this is an overestimate of the actual rate. We also assumed a generation time

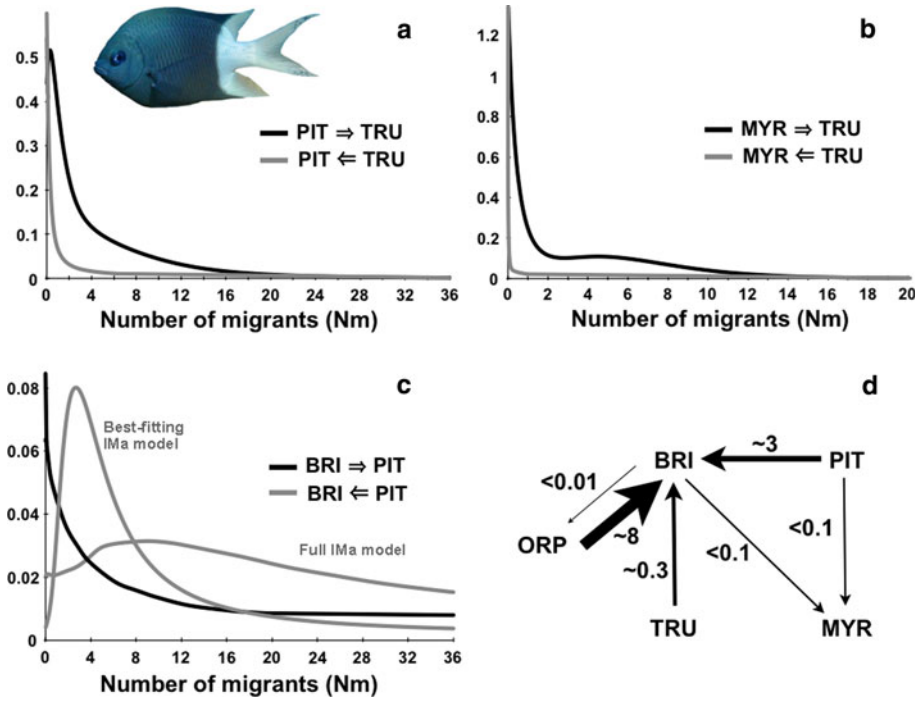

Fig. 5 Estimates of gene flow (Nm) from pairwise IMa analyses of central Great Barrier Reef populations of *Acanthochromis polyacanthus*. Data from Bay et al. (2008) (Image courtesy of L. Bay)

of 2 years based on the average age of breeding adults (Kavanagh 2000) for the purpose of estimating effective population size.

For two (MYR-TRU and TRU-PIT) of the three pairwise IMa comparisons among central GBR reef demes of *A. polyacanthus* for which strong asymmetrical gene flow was inferred with MIGRATE-N, M mode analyses in IMa revealed that estimates of gene flow in both directions were zero (Fig. 5a, b) and L mode testing was unable to reject simpler demographic models in which gene flow was zero in both directions in both cases. Therefore, re-analysis with IMa suggests that MIGRATE-N probably over-estimates gene flow between some demes that share haplotypes from an earlier period of greater connectivity, potentially from their initial colonization or separation. In the third comparison with strong asymmetric gene flow inferred with MIGRATE-N (PIT-BRI), the posterior probability distributions for migration parameters from IMa were consistent with the MIGRATE-N results in that the posterior distribution for gene flow from PIT to BRI had a non-zero peak (Fig. 5c); although L mode model testing for this last comparison rejected all models in which gene flow was zero in both directions, model testing was unable to reject a model in which gene flow was asymmetric, from PIT to BRI. Re-running the analysis in M mode under the best-fitting model yielded a migration posterior with a stronger and smaller mode (Nm $\sim$ 3). Other pairwise comparisons from IMa among these four demes returned some gene flow results consistent with MIGRATE-N, such as asymmetric gene flow between MYR and PIT, but also some results that differed substantially from MIGRATE-N estimates, such as no gene flow between BRI and MYR, significant asymmetric gene flow between TRU-BRI, and very high gene flow from a fifth

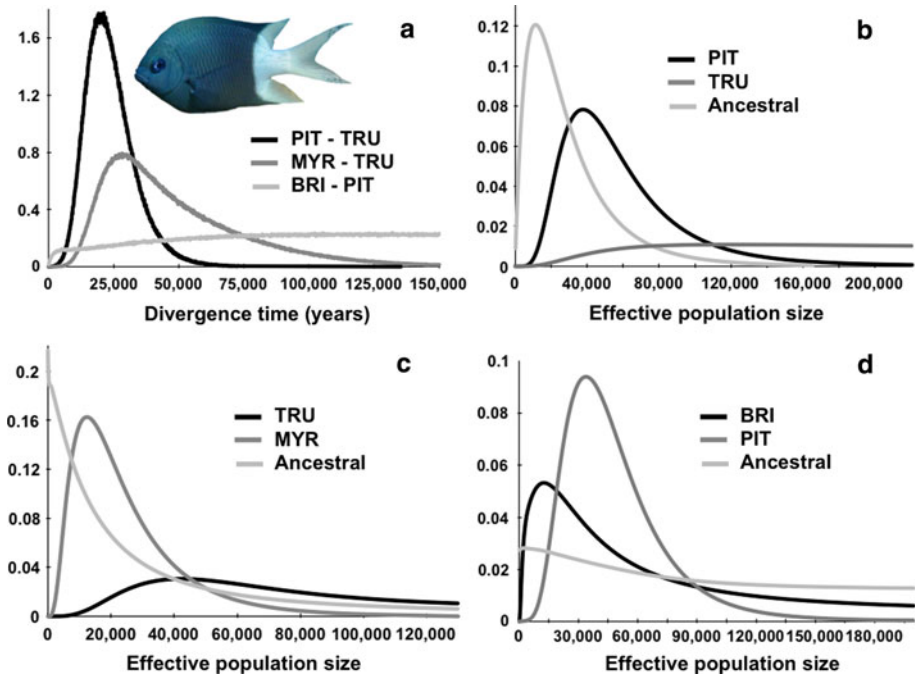

Fig. 6 Estimates of divergence time and effective population size from pairwise IMa analyses of central Great Barrier Reef populations of *Acanthochromis polyacanthus*. Data from Bay et al. (2008)

population (ORP, Fig. 5d). Overall, the results from the IMa analyses were only very broadly qualitatively similar to those from MIGRATE-N (spatially variable asymmetric gene flow), but differed with respect to which demes have exchanged migrants since separation. The final analyses in M mode, in which we set the prior for gene flow to zero in either both directions (TRU-PIT, MYR-TRU, and BRI-MYR) or only one (PIT-BRI, MYR-PIT, TRU-BRI) direction between demes (depending on L model model testing), showed that, despite our use of a very fast mutation rate, deme separation times were similar but also quite old (14,300–17,500 years ago) among IMa comparisons for which the posterior had a single peak (Fig. 5); several comparisons resulted in inconclusive posterior distributions with long right-hand tails of nonzero probabilities that were consistent with a broad range including much older divergence times. Effective population sizes for most comparisons were also surprisingly large (most $N_e > 10{,}000$, Fig. 6) with single and well-defined posterior peaks, despite the dependence of this parameter estimate on our assumed generation time of 2 years: a doubling of generation time only reduces these N_e estimates by half.

Are jointly inferred patterns of gene flow, isolation time, and effective population size consistent with KSM or LM dynamics in *A. polyacanthus*? Old separation times, large effective population sizes, and consistent nonzero gene flow among some reefs are patterns all broadly consistent with KSM dynamics. Even if we assume a tripling of the mutation rate (equivalent to a 50% per million years divergence rate), population separation times would be on the order of several thousand years, still too old to be considered consistent with LM dynamics over temporal scales relevant to issues of interest to ecologists and

managers. The unexpectedly old divergence times among reefs sampled by Bay et al. within the central GBR region are probably instead consistent with a history in which present-day reefs were colonized from further down the slope as sea-level rose following the last glacial maximum. However, a true KSM metapopulation consists of demes that are connected by demographically significant dispersal (Sale et al. 2006). Relative to the large estimates of effective population size (10^3–10^4 individuals) and the usual (albeit coarse) definition of 'significant' demographic connectivity (populations consisting of 10% migrants; see Hastings 1993; Waples and Gaggiotti 2006; but see Lowe and Allendorf 2010), the rates of migration that we estimated (Nm = 0–2.6 migrants per generation) are probably too low to be considered demographically significant, even if nothing is known about local population growth rates (see Lowe and Allendorf 2010). Thus, from this perspective, such low migration rates are not entirely consistent with KSM dynamics in which local retention of larvae on reefs and recruitment from exogenous sources are both important to local demography. Although there are several important caveats to consider with demographic parameter estimation from genetic data, such as the fact that single locus estimates of population genetic parameters must always be considered with caution and that estimates of effective population size depend fundamentally on assumptions about mutation rate and generation time, the spatial scale of the sampling by Bay et al. may simply be too large with respect to the spatial scale of coral reef metapopulation dynamics. Taken at face value, the analyses indicate that there is probably too little migration (whether inferred from IMa or MIGRATE-N) among reefs that were colonized too long ago to be considered a metapopulation at this spatial scale, and that more intensive sampling of patch reefs on smaller scales may be more helpful for characterizing coral reef fish metapopulations and distinguishing between patterns consistent with KSM or LM dynamics over smaller distances and between smaller populations.

Conclusions

The new direction provided by retrospectively oriented coalescent methods has revolutionized population genetics by providing a statistical framework for reconstructing demographic histories from genetic data. One of the reasons coalescent theory has been so influential is that nearly any demographic history can be modeled and applied to genetic data to infer population history (Wakeley 2008). As we have noted, the utility and wide applicability of the basic approach also represents one of the important limitations of each individual coalescent method: the underlying demographic model that characterizes any particular analysis must match the history of the populations from which samples are taken. The rapid growth and proliferation of situation-specific analytical methods available for use (Kuhner 2009) reflects this basic fact about coalescent methods. For population geneticists and phylogeographers, data analysis (like genetic marker choice) involves finding the right tool for the right job.

Although some studies have shown that gene flow inferences from F_{ST} can be accurate (e.g., Tatarenkov et al. 2010), the ongoing practice of explaining patterns of population differentiation largely in terms of gene flow runs counter to the modern perceptions of most ecologists that many populations, both on land and in the sea, often exhibit characteristics consistent with metapopulation structure, in which individual populations or demes will share alleles or haplotypes due to both historical connectivity (i.e., colonization and range expansion) and recurrent contemporary migration. Although gene flow estimates from F_{ST} may be biased for a variety of reasons, we have focused on examples where non-

equilibrium IM methods show strongly (in contrast to inferences based on F_{ST}) that variation in isolation time and effective population size can be more important factors explaining spatial patterns of population differentiation than gene flow. Thus, we urge population geneticists to be skeptical not only of explicit quantitative inferences of gene flow based on differentiation but also of qualitative inferences of connectivity that implicitly rely on equilibrium assumptions, and to compare results from different methods that differ in assumptions that are most relevant to the ecological and evolutionary circumstances of the particular species or populations under study.

Overall, our attempts to use IM methods to distinguish between different metapopulation structures appear to provide little support for classic "Levins" dynamics in two model marine metapopulation species (given relatively ancient population isolation times). Yet, at the same time, the results from these analyses (especially the low rates of immigration into relatively large populations) do not provide unequivocal support for the modern marine metapopulation or "Kritzer-Sale" paradigm, the dominant framework for studying marine ecology. In addition to several important assumptions about mutation rates and generation times, the most obvious caveat for this initial conclusion is that either type of metapopulation structure may exist on smaller spatial scales, and that further discrimination of metapopulation models will require greater sampling of smaller reefs and demes separated by smaller distances not typically sampled by marine population geneticists and phylogeographers. A common misconception about coalescent methods is that because they return information about population history over "evolutionary" timescales they have little relevance to ecological questions, which are better answered with other types of analyses based on allele frequency data. Given our emphasis on the intertwined relationships among gene flow, population size, and isolation time, our review did not focus on methods based on genotypic clustering or assignment tests (e.g., Pritchard et al. 2000) that can provide estimates of migration rates in recent generations and can be compared to estimates of gene flow from coalescent methods (e.g., Yao et al. 2007). Coalescent methods provide time-averaged estimates of demographic parameters only over the temporal scale of the coalescent process for a specific genetic marker, and our analyses of abalone and damselfish data suggest that mtDNA data may often contain too little information for successful application of such methods. Like other potential objections to reliance on mtDNA data alone for phylogeography (e.g., inadvertent sampling of nuclear pseudogenes, selection on mtDNA coding sequence variation), the inability of mtDNA polymorphisms to resolve ecological-scale coalescent histories argues strongly for the use of other (nuclear) loci, especially those with faster mutation rates. We emphasize that this is an argument against particular genetic markers and not an argument against coalescent demographic analyses of those genetic markers: just as there are no limits on phylogenetic reconstruction that restrict its use to ancient species diversifications, there is no such analogous limitation on coalescent population genetic methods per se. Moreover, we think the frequently stated opinion that population genetics only reveals processes over "evolutionary" timescales is somewhat unfortunate given that the evolutionary structure of the coalescent is ultimately created by ecological and demographic processes acting over time. Therefore, we think that IM methods have the potential to improve the understanding of average rates of connectivity, but future applications will require more intensive sampling of demes, larger samples of loci, and inclusion of markers that coalesce over a range of time periods, including more quickly evolving sequences (e.g., anonymous non-coding nuclear loci) whose alleles potentially coalesce between populations on spatial and temporal scales closer to those that interest ecologists and resource managers.

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