Genetic Approaches to Measuring Connectivity

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INFERRING GENE FLOW AND MIGRATION FROM SPATIAL VARIATION IN ALLELE AND GENOTYPE FREQUENCIES

Understanding the connectivity of marine populations is vital for conservation and fisheries management, particularly for the strategic design of reserve systems. A recent proliferation of molecular and statistical tools allows increasingly sophisticated integration of genetic and geographic data (e.g., Manel et al., 2003). Such advances have fueled considerable hope that the challenging problem of tracking movement of individuals within the vast ocean will soon be solved. Here, we focus on some of the inherent limitations of genetic approaches to inferring connectivity, particularly in marine environments. More optimistically, we also point to a number of situations where genetic approaches have been particularly successful in the past, as well as newer integrative approaches that deserve further attention.

Basic Population Genetic Concepts

Populations evolve by changes in the frequencies of alleles, the alternative forms of genes that constitute heritable diversity among conspecific individuals. Allelic-frequency change results from evolutionary forces: *mutation* of genes; *random genetic drift* caused by stochastic fluctuations in finite populations; *gene flow*, resulting from migration among populations; and *selection* within and among populations. Over sufficiently long periods of time and in the absence of environmental change, the genetic composition of a population arrives at an equilibrium often determined by a balance among opposing forces. Random genetic drift and diversifying selection cause populations to diverge, for example, while migration acts to homogenize populations and to maintain the genetic cohesion of a biological species (Mayr, 1963). The theoretical effect of migration or gene flow on allele-frequency differences among populations is the obvious conceptual link between the topic of marine connectivity and population genetics. Note, however, that "migration" in population genetics means not only dispersal from one location to another—accomplished in the sea by planktonic larvae—but also the survival and reproduction of migrants, so that they contribute to the local gene pool.

Direct and Indirect Methods for Measuring Gene Flow

Gene flow among populations can be estimated by a variety of direct and indirect methods. Indirect methods estimate gene flow from genetic differences among populations on the assumption that those populations have reached equilibrium (Slatkin, 1993). Direct methods, on the other hand, focus on assignment of individuals to populations of origin (e.g., Manel et al., 2005) or to specific parents (Jones et al., 2005) and, in this way, are conceptually similar to evidence obtained from physical (e.g., Block et al., 1998) or chemical/environmental tags (Thorrold et al., this issue). Indirect methods rely heavily on theoretical models of population structure, such as Sewall Wright's Island Model (Wright, 1931). Differentiation among subpopulations has traditionally been measured by Wright's F_{ST}—a standardized variance in allele frequencies among populations—although a variety of conceptually similar metrics are now used (G_{ST}, ϕ_{ST}, AMOVA, private alleles, etc.). For loci with two alleles, F_{ST} ranges from 0, when populations have identical allele frequencies at all loci, to 1, when populations share no alleles. In the Island Model at equilibrium, the absolute number of migrants exchanged each generation can be estimated as $Nm = (1–F_{ST})/(4F_{ST})$, where N is the effective size of the local population and m is the proportion of migrants entering that population each generation.

While many have estimated Nm using this equation, Whitlock and McCauley (1999) point out that, in practice, such estimates are only "likely to be correct within a few orders of magnitude," owing to unmet assumptions of the Island Model (aka, the "Fantasy Island" model) and a variety of technical issues. For example, it may take tens to hundreds of thousands of generations for large marine populations to reach equilibrium, resulting in retention of historical patterns of gene exchange by contemporary populations (Benzie, 1999). Moreover, owing to the reciprocal relationship between F_{ST} and Nm (Figure 1), small errors in measuring the low F_{ST} values that characterize many marine populations (often < 0.05) make moderate gene flow indistinguishable from random mating (Palumbi, 2003). New

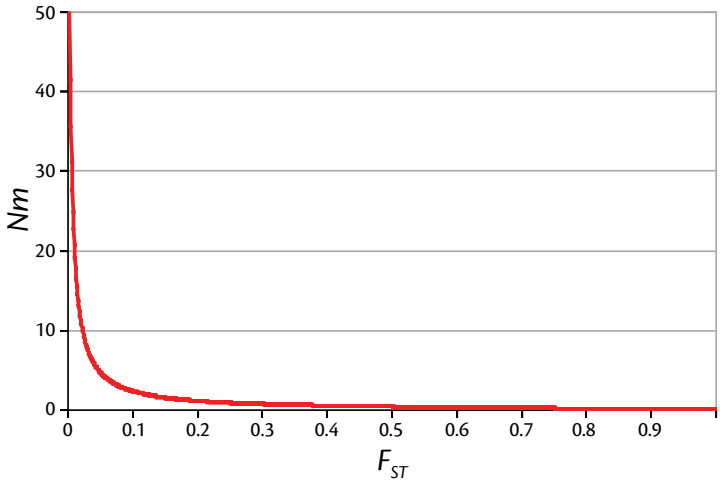

Figure 1. The difficulty of accurately estimating migration in marine species with high gene flow is illustrated by the reciprocal relationship between F_{ST} (the standardized allele-frequency variance) and Nm (the absolute number of migrants exchanged each generation) in the Island Model.

coalescent approaches overcome some of these hurdles (Beerli and Felsenstein, 2001; Nielsen and Wakeley, 2001) but are not without their own shortcomings, such as sensitivity to unsampled populations (Abdo et al., 2004; Slatkin, 2005). F_{ST} may be overestimated (and migration thus underestimated) in hierarchical population models diverging by random genetic drift, owing to correlation of allele frequencies among populations or regions (Song et al., 2006). Finally, G_{ST}, a commonly used estimate of divergence at loci with multiple alleles, is sensitive to intrapopulation variation at highly variable markers, such as microsatellite loci; as a result, it does not range from 0 to 1, as F_{ST} does, and must be standardized for comparisons across markers and species (Hedrick, 2005a).

Direct estimates of connectivity via assignment or parentage tests take advantage of variable molecular markers to calculate the probability that a given individual originated from a particular source population or set of parents. For example, an individual can be assigned to a source based on the expected frequency of its multilocus genotype in various putative sources (Paetkau et al., 1995). Assumptions of this original approach are that all potential source populations are defined in advance, sampled randomly, and do not depart from Hardy Weinberg or linkage equilibria. More recently, maximum likelihood and Bayesian approaches have been developed, which in some cases involve fewer assumptions and provide higher accuracy (Manel et al., 2005), as well as the ability to infer migration rates among populations (Wilson and Rannala, 2003). However, these methods are most effective when $Nm < 5$ (Waples and Gaggiotti, 2006) and genetic structure is high (Cornuet et al., 1999), suggesting they may be most useful in determining patterns only when connectivity is low.

As mentioned above, population assignment and parentage tests are conceptually similar to studies using environmental signatures (Swearer et al., 1999; Thorrold et al., 2001) or chemical tags (Jones et al., 1999; Thorrold et al., this issue) of fish otoliths (ear bones). Although the integration of otolith microgeochemisty and microsatellite-based paternity testing has demonstrated pronounced larval retention (Jones et al., 2005), only rarely have otolith studies examined the origin of larvae that are not self-recruiting (Thorrold et al., 2001), specifically addressing connectivity.

Different Time Scales and Dynamics of Genetic and Demographic Equilibria

The direct and indirect approaches described above estimate connectivity on opposite ends of a temporal spectrum. Indirect genetic methods estimate connectivity over evolutionary time scales but are incapable of estimating contemporary demographic exchange. Direct methods excel at estimating connectivity or retention over a single or a few generations but are snapshots unlikely to document stochastic (e.g., hurricanes) or recurrent (e.g., ENSO, decadal-scale regime shifts) environmental impacts on connectivity and demography. In between these extremes lies a range of time scales of biological importance that escape present analytical methods. For example, the Banggai cardinalfish, a mouth brooder, lacks a dispersive stage, and shows pronounced genetic structure over its geographic range; yet, many populations separated by over 50 km of open ocean are genetically similar (Bernardi and Vagelli, 2004). This pattern has two potential explanations. First, population similarity may reflect historical rather than contemporary gene flow, perhaps during periods of lowered sea levels when habitat was more continuous. Alternatively, local retention may be punctuated with rare events that connect otherwise isolated populations on intermediate time scales. Indirect methods cannot distinguish exchange of 1000 individuals every 100 generations from exchange of 10 individuals every generation ($Nm = 10$ in both cases). While intermediate time-scale events can profoundly affect the dynamics of marine populations (see Glynn, 1988, for review), at present neither indirect nor direct measures of migration are capable of elucidating the temporal scale and magnitude of realized connectivity in such systems.

Structure of Marine Populations

Estimating connectivity depends on the extent of spatial genetic variation. Estimates of connectivity are generally

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effective when genetic subdivision is high (i.e., in the absence of significant connectivity) and the literature is somewhat biased toward positive examples, which are nevertheless instructive, especially when combined with predictions of physical advective models (Galindo et al., 2006; Treml, 2006) (Figure 2). Still, a major challenge is to develop methods for measuring connectivity in the more difficult, high-gene-flow and/or non-equilibrium situations common to many exploited marine species.

The majority of marine species with planktonic larvae, especially planktotrophic (feeding) larvae, lie toward the open end of the connectivity continuum (Waples, 1998). Intermediate connectivity patterns include stepping-stone gene flow driven by restricted dispersal, geographic clines driven by natural selection or secondary introgression of previously separated populations, and chaotic patchiness driven by temporal genetic variation among cohorts. Hellberg et al. (2002) grouped genetic structure of marine populations into six patterns, ranging from completely closed (high F_{ST}) to completely open (low F_{ST}). However, care must be exercised in generalizing about F_{ST} and related measures of genetic divergence, as different markers can yield different F_{ST} values for the same populations. In the case of the eastern oyster, for example, F_{ST} across populations from the Gulf of Mexico and the Atlantic Ocean are approximately 1.0 for the mitochondrial genome (Reeb and Avise, 1990), 0.04 for 20 allozymes (Buroker et al., 1983), 0.26 for 4 single-copy nuclear DNA (scnDNA) markers (Karl and Avise, 1992), but 0.05 for another 6 scnDNA markers (McDonald et al., 1996). Levels of F_{ST} discussed in the next section are broad, heuristic categories corresponding to the very weak, moderate, or very strong genetic structures likely to be encountered in the sea.

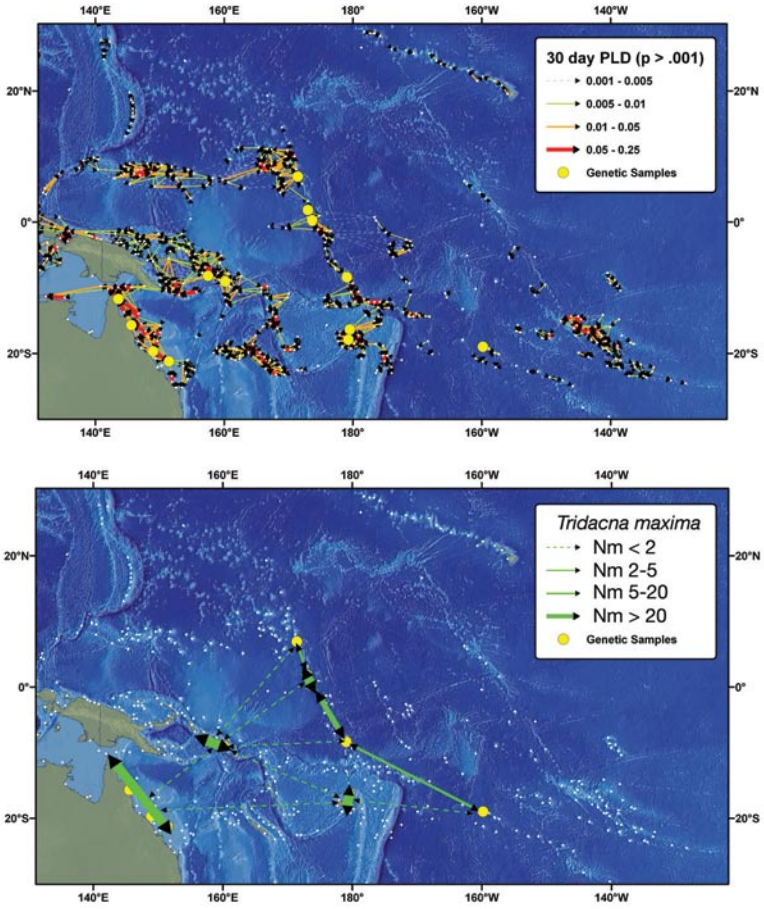

Figure 2. An example of congruence between predicted patterns of larval dispersal and inferred patterns of genetic exchange for western Pacific populations of the giant clam *Tridacna maxima*. The upper panel depicts among-site dispersal probabilities obtained through a two-dimensional Eulerian advection-diffusion model (Treml, 2006) that incorporated geography, hydrodynamics, spawning location, pelagic larval duration, larval behavior, and mortality. The model produces a connectivity matrix that can be compared to a matrix of estimated gene flow among sampled populations (lower panel—arrow width is proportional to level of migration, *Nm*, among populations of *Tridacna maxima*, after Benzie and Williams, 1997). Although the predicted connectivity and gene flow matrices are significantly correlated ($r = 0.567$), past variation in current patterns, population demography, and reproductive success may account for particular discrepancies between modeled and observed genetic connectivity.

INFERENCES OF MIGRATION RATE FROM GENETIC STUDIES

Absence of Connectivity, When $F_{ST} \approx 1$

When F_{ST} approaches 1 and *Nm* approaches 0, an absence of connectivity is relatively easy to infer. One marine example of extremely low effective migration is the tidepool copepod *Tigriopus californicus*. Despite seemingly

high dispersal potential, neighboring populations routinely show fixed differences (e.g., Burton and Feldman, 1981; Burton and Lee, 1994) and overall population subdivision is extremely high (F_{ST} = 0.80 for microsatellites [Edmands and Harrison, 2003] and 0.98 for mitochondrial DNA [Edmands, 2001]). Importantly, this subdivision appears to be temporally stable as evidenced by repeated sampling over 18 years, the equivalent of approximately 200 copepod generations (Burton, 1997). In other marine examples, minimal connectivity is associated with particular geographic barriers. An extreme case is the mantis shrimp *Haptosquilla pulchella* in the Indo-West Pacific (Barber et al., 2000, 2002). Despite a four- to six-week planktonic larval duration (inferred from closely related species) and strong regional currents, this species exhibits sharp genetic breaks (ϕ_{ST} = 0.87) among regions that may have been isolated ocean basins during periods of lowered sea level. In the copepod and mantis shrimp examples, we can be confident that migration is too low to maintain demographic connectivity.

Assignment Tests, When $F_{ST} \geq 0.1$

Direct genetic estimation of connectivity is a relatively recent application of assignment tests (Manel et al., 2005); as such, examples utilizing this approach are comparatively rare. Assignment tests rely on highly polymorphic markers and are most effective for moderate to highly structured populations (e.g., microsatellite $F_{ST} \geq 0.1$; Cornuet et al., 1999). Galindo et al. (2006) used assignment tests to demonstrate concordance between regional population differentiation in Caribbean *Acropora cervicornis* and predications based on a physical advective model. Baums et al. (2005) demonstrated high self recruitment in *Acropora palmata* and determined the relative connectivity among these populations, despite F_{ST} values < 0.1. In Northwest Australia, Underwood et al. (2007) assigned putative source populations to individual coral colonies that had recruited and grown since a catastrophic beaching event. Hare et al. (2006) used assignment tests to identify larvae of an artificially selected oyster strain to determine the spatial scale of connectivity in the Chesapeake Bay. Combined, these studies demonstrate the utility and versatility of assignment tests, although for many marine species genetic subdivision is too low for accurate assignment.

Isolation by Distance and Genetic Clines

For species characterized by stepping-stone gene flow, mean dispersal can be estimated from the slope of the increase in genetic distance with geographic distance, a relationship that might be expected to be less prone to artifact than calculation of a single F_{ST} value. In this way, mean larval dispersal distances for a variety of fish and invertebrates with pelagically dispersing larvae have been estimated as between 25 and 150 km (reviewed in Palumbi, 2003). The precision of such estimates is limited, however, by untested assumptions of migration-drift equilibrium and constant dispersal rates across the entire geographic range. The regression approach, moreover, is appropriate for only a limited range of distances and is compromised by even moderate mutation or immigration (Hardy and Vekemans, 1999).

For species exhibiting genetic clines or gradients, mean larval dispersal can be inferred from cline width and selection coefficients (estimated by experimentation or from levels of linkage disequilibrium), assuming equilibrium between migration and selection. While many marine species exhibit clines, appropriate data to estimate dispersal is available in only a few cases (reviewed in Sotka and Palumbi, 2006). Average larval dispersal in these cases ranges from 0.189 km per generation in the damselfish *Acanthochromis polyacanthus*, which lacks pelagic larvae (Planes and Doherty, 1997), to ~ 70 km per generation in the acorn barnacle *Balanus glandula*, which has a minimum planktonic larval duration of two weeks (Sotka et al., 2004).

Connectivity, When $F_{ST} \approx 0$

Information for judging the degree of connectivity among populations is lacking when $F_{ST} \approx 0$. The temptation might be to conclude that there is high connectivity, but such a conclusion would not be warranted from genetic data alone, because there is no way of knowing whether the sampled populations have reached equilibrium. If a barrier to dispersal suddenly divides a single population, the two completely isolated daughter populations will diverge over time by random genetic drift and diversifying selection. However, the rate of divergence can be slow, depending on the effective sizes of the two populations. Lack of allele-frequency variance could simply reflect insufficient time since separation to establish the drift-migration equilibrium.

Still, careful multidisciplinary analysis of cases in which there is weak but temporally consistent differentiation can yield insights into larval dispersal and connectivity. For example, Knutsen et al. (2003) studied differentiation of Atlantic cod populations in six samples collected along a 300-km region of coastline lacking any obvious physical barriers. They detected weak but consistent and highly significant differentiation (F_{ST} = 0.0023) that exhibited no geographical or isolation-by-distance pattern. Stenseth et al. (2006), using a combination of long-term ecological monitoring data and oceanographic modeling, subsequently supported the hypothesis that these low levels of differentiation are attributable to intermittent passive transport of eggs or larvae from an offshore cod stock. Similarly, Selkoe et al. (2006) found that a combination of kin aggregation and changes in larval delivery from differentiated source populations explains genetic patchiness among cohorts of kelp bass recruits (*Paralabrax clathratus*).

TEMPORAL VARIATION IN ALLELE AND GENOTYPE FREQUENCIES

Temporal genetic change, resulting from random genetic drift, fluctuating delivery of larvae from genetically different source populations, or selection, has until very recently been regarded as unimportant in abundant marine organisms. Yet, the temporal dimension of the population genetic structure of marine species may ultimately prove more important than the spatial dimension, which remains the focus of most genetic analyses (Hellberg et al., 2002; Palumbi, 2003). Temporal genetic change within adult populations or between adults and recruits has been confirmed in several studies of marine fish and invertebrates (Koehn et al., 1980; Hedgecock, 1994; Edmands et al., 1996; Moberg and Burton, 2000; Hauser et al., 2002; Planes and Lenfant, 2002; Turner et al., 2002; Árnason, 2004; Knutsen et al., 2003; Hoarau et al., 2005; Maes et al., 2006; Selkoe et al., 2006) (Figure 3). Temporal stability, on the other hand, has been documented in only a few populations (e.g., Burton, 1997). Many more temporal studies will be required to determine the importance of temporal genetic variation in marine metapopulation dynamics, connectivity, and genetic structure.

Sweepstakes Reproductive Success (SRS)

The enormous fecundity (10^6–10^8 eggs per female per season) and high larval mortality of most marine animals make them fundamentally different from better-studied mammalian, fruit fly, or nematode model species (10^1–10^3 eggs

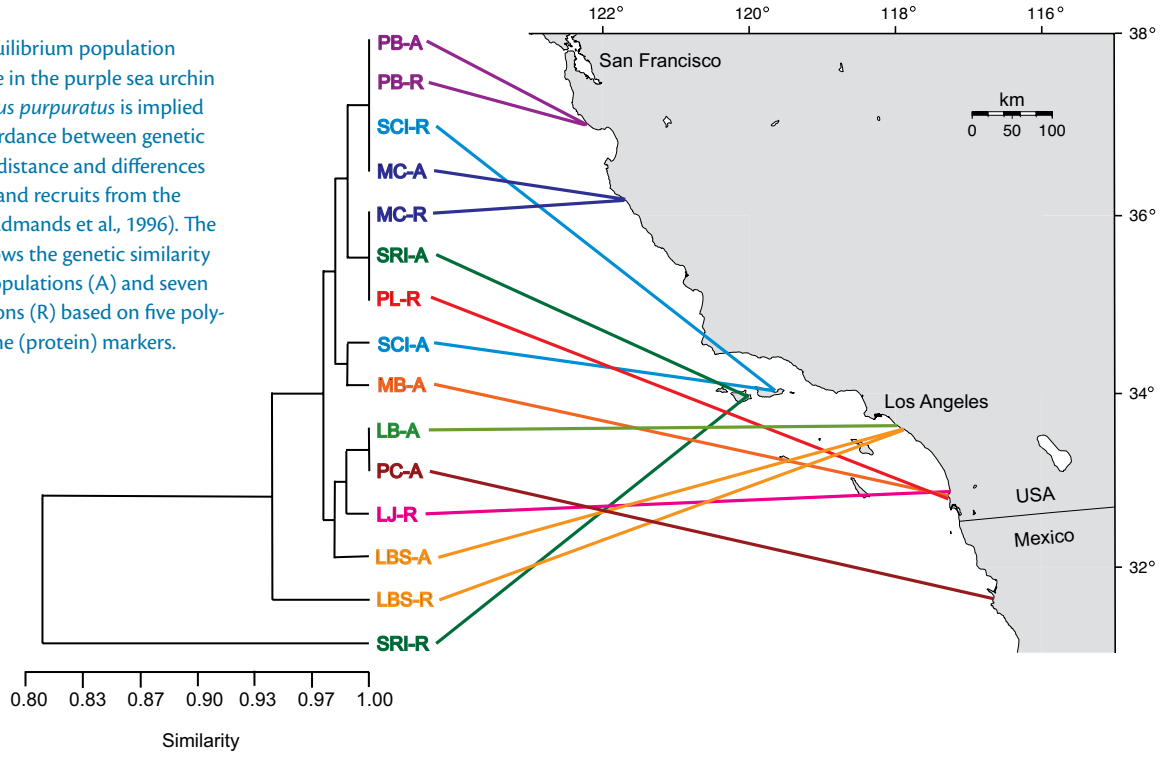

Figure 3. Nonequilibrium population genetic structure in the purple sea urchin *Strongylocentrotus purpuratus* is implied by lack of concordance between genetic and geographic distance and differences between adults and recruits from the same location (Edmands et al., 1996). The dendrogram shows the genetic similarity of eight adult populations (A) and seven recruit populations (R) based on five polymorphic allozyme (protein) markers.

per female per lifetime). High fecundity creates the potential for sweepstakes reproductive success (SRS), in which a relatively small proportion of adults may account for the bulk of reproduction and recruitment, owing to chance matching of reproductive activity with temporally and spatially varying conditions conducive to fertilization, larval development, and settlement (Hedgecock, 1986, 1994; Beckenbach, 1994; Li and Hedgecock, 1998; Planes and Lenfant, 2002; Turner et al., 2002; Waples, 2002). With SRS, the effective size of a natural marine population (N_e) would be determined by the winners of the sweepstakes reproductive lottery (Hedrick, 2005b), comprising a small fraction of the actual population number (N). The ratio of N_e/N in marine populations might be 0.001 or less, much less than ratios of 0.1–0.7 measured for terrestrial organisms (Frankham, 1995; Nunney, 1996). Two important aspects of SRS must be emphasized. First, the severity and frequency of SRS depend on environmental conditions that vary on time scales yet to be determined. Second, the statistical power to detect a sweepstakes reproductive event depends on its severity and the method used to detect it.

With respect to connectivity, SRS complicates the indirect inference of migration rate from F_{ST}. Measurable genetic variance among adult populations could result from transitory effects of variance in reproductive success rather than from the equilibrium between genetic drift and migration (cf. Whitlock and McCauley, 1999). The spatial distribution of reproductive success, moreover, is critical information for location-dependent management. Turner et al. (2002) show by simulation that variance in reproductive success among different local populations may be a more potent way of reducing N_e/N ratios than variance among individual reproductive contributions. This hypothesis, which bears directly on the identification of reproductive sources and sinks, merits further consideration and testing.

Cohort Analyses (Allele Rarefaction, H Excess, LD, Relatedness)

Production of a cohort of offspring by small numbers of adults yields several telltale genetic signals. First, allelic diversity of a cohort is reduced relative to that of the adult population when the number of successful parents is restricted (Allendorf, 1986; Hedgecock and Sly, 1990). Second, proportions of heterozygotes in the cohort sample may exceed those expected based on allele frequencies (Pudovkin et al., 1996; Luikart and Cornuet, 1999). Third, significant levels of gametic phase or linkage disequilibrium (LD), the nonrandom association of alleles at different genes, may also distinguish cohorts produced by a small number of parents, owing to the limited number of chromosomes and genetic combinations present. Fourth, a cohort produced by a finite number of parents may comprise full and half siblings, which can be detected by log likelihood ratio tests of relatedness. Finally, temporal variance between the adult population and a cohort of offspring may be significant (e.g., Pujolar et al., 2006). All of these telltale genetic signals of a restricted number of parents were found in a recent study of flat oyster recruits (Hedgecock et al., 2007) (see Figure 4), implying that the cohort came from only ~ 10 parents, an astonishingly small number for a broadcast spawning marine invertebrate. The frequency of such extreme sweepstakes reproductive events is unknown; the power to detect less-extreme sweepstakes events is limited (Flowers et al., 2002). For example, had the effective number of parents for the cohort of flat oysters been on the order of 10^2 to 10^3—still a significant reduction compared to adult abundance—none of these characteristic genetic signals would likely have been detected with just four microsatellite markers.

INTEGRATED APPROACHES NEEDED

Knowledge about the connectivity of marine populations is likely to be gained only through integrated, multidisciplinary efforts in which genetic methods can and should play an important part. Although the "big" picture afforded by indirect estimates of gene flow should always be considered, direct genetic methods are likely to take precedence in future studies focusing on ecological time scales and processes. Cohort analysis appears to be a particularly fruitful approach for interdisciplinary studies. If sweepstakes reproductive events or delivery from slightly divergent adult populations imparts a characteristic genetic signal to a cohort of larvae or juveniles, as illustrated by some of the case studies cited above, then it might be possible to track that cohort in the water column or to identify the spatial extent of its recruitment into juvenile habitat (its dispersal kernel). However, a multidisciplinary team of physical oceanographers, larval ecologists, modelers, and geneti-

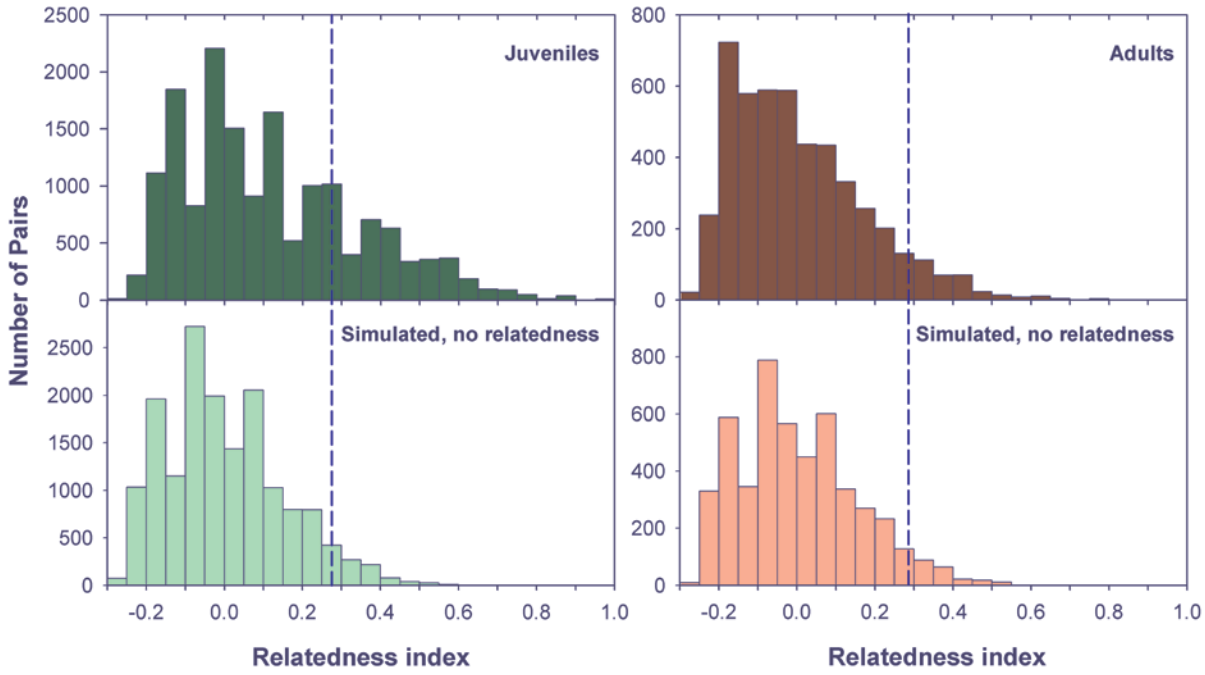

Figure 4. Evidence that a relatively small number of parents contributed to a cohort of European flat oysters (after Hedgecock et al., 2007), which could potentially impact inference of genetic connectivity. The upper panels show distributions of a relatedness index (calculated from allele sharing at four microsatellite DNA markers) for all pairwise comparisons of individuals in adult and juvenile samples. The relatedness index should average zero in randomly mating populations, though variation about the mean is expected and is evaluated by simulating random production of the same numbers of pairs as obtained in the actual samples (16,110 for the juveniles, 4,848 for the adults; lower panels). Vertical dashed lines mark the upper 5% thresholds in the simulated distributions. The juveniles but not the adults show levels of relatedness above the threshold, suggesting the presence of both full siblings (0.5 expected relatedness) and half siblings (0.25 expected relatedness) in this cohort.

cists, using adaptive sampling strategies, large sample sizes, and high-throughput genotyping methods, will be needed to accomplish this. Such studies will benefit from direct genetic methods for determining the provenance or relatedness of recruits. For species in which all or most potential adults in a local population can be genotyped, parental assignment of recruits directly measures local retention (e.g., Jones et al., 2005).

Estimates of connectivity also depend on the assumption that natural mortality is constant for all larvae. Experimental studies of oysters have uncovered a large load of highly deleterious recessive mutations in natural populations (Bierne et al., 1998; Launey and Hedgecock, 2001). These laboratory results explain previously observed correlations between marker heterozygosity and individual size in natural mollusc populations and suggest that much of the variance in larval fitness may be attributable to selection against harmful mutations. A high frequency of harmful mutations may be a byproduct of high fecundity, which is common for marine species with planktonically dispersing larvae. Genomic understanding of such endogenous sources of variation in fitness should be incorporated into individual-based models of marine population dynamics, just as the vertical migratory behavior of larvae has been incorporated into physical oceanographic models (Cowen et al., 2006). Oceanography needs to adopt and promote model organisms that are amenable to experimental culture and crosses in order to bring the power of genomic and postgenomic technologies to bear on the basis for variation among individual larvae and their performance in the plankton.

CONCLUSION

Genetic methods and approaches can be used to detect migration among conspecific populations and can thus play an important role in studies of connectivity of marine populations. Nevertheless, the limitations and assumptions of these methods need to be appreciated. Genetic

differences among geographic populations cannot simply be interpreted as evidence of low connectivity, nor can genetic homogeneity be taken as evidence of high connectivity. Ancillary information is required to support inferences about connectivity from spatial genetic data. Temporal as well as spatial genetic variability needs to be assessed, and one cannot assume that marine populations are at equilibrium for demographic and evolutionary processes. Genetic methods, which do not assume or require equilibrium, particularly when applied in the context of cohort studies and supported by rigorous physical and biological oceanographic analyses, offer the best hope for resolving connectivity among genetically similar, continuous, or contiguous marine populations.

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