BULLETIN OF MARINE SCIENCE, 70(1) SUPPL.: 273–290, 2002

GENETIC ASSESSMENT OF CONNECTIVITY AMONG MARINE POPULATIONS

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ABSTRACT

Geographical surveys of genetic variation provide an indirect means of tracing movements made between marine populations by larvae and other propagules. Genetic markers can provide strong evidence that populations are closed (self-recruiting) because genetic differentiation is highly sensitive to migration. However, inferences based on genetic data must necessarily be based on models that make assumptions concerning inheritance, selective neutrality of markers, and equilibrium between genetic drift, migration, and mutation. We briefly introduce the types of genetic markers that can be used to infer demographic connections between populations and the forces causing evolutionary changes in these markers, and then we outline six patterns revealed by geographic surveys of genetic markers in marine species. Four of these patterns represent the possible combinations of high or low migration rates and large or small effective population sizes; two others are due to history and natural selection. Future genetic surveys should include more detailed spatial and temporal sampling and employ analyses of DNA sequence data that can reveal the signatures of natural selection and historical changes.

Given the vast size of the ocean and the small size of most marine propagules, determining whether propagules settle away from their natal site or close to their parents can be a daunting task. Successful migrants should leave a genetic trail of their movements, offering an indirect means of estimating population connectivity. Genes are also recombined and passed through multiple generations, however, so the genotype of a larva cannot indicate its origin in the same direct way as a physical tag (Hedgecock, 1994a). Instead, the geographic distribution of genetic markers must be interpreted using population genetic models (Neigel, 1997; Waples, 1998). Clear interpretation of population genetic data, then, requires understanding and acknowledging the powers and pitfalls of these models.

Here, we summarize how geographic patterns of genetic variation can be used to estimate the degree to which marine populations are closed or open. Our intended audience is the marine biologist who is new to genetic approaches to population biology; more detailed reviews can be found in Palumbi (1994) and Grosberg and Cunningham (2001). We begin by briefly categorizing the types of genetic markers that can be employed as population markers, the forces affecting their evolution, and the general models of population genetic structure that provide the framework for the interpretation of data. Next, we outline six patterns seen repeatedly in geographic surveys of genetic variation among marine populations, along with examples and possible underlying mechanisms for each. Finally, we discuss instances where patterns appear to conflict and point to future research that might both resolve these conflicts, and provide greater insights into the degree of connection between marine populations.

Types of Genetic Markers

To be potentially useful for inferring whether or not populations are closed, a genetic marker must vary intraspecifically and have a known means of inheritance. Finding that two distant populations share a single fixed allele provides no information about movement between populations, but a shared frequency of, say, 0.5 suggests that some force of genetic homogenization, perhaps gene flow, acts on the marker. Well-developed theoretical backgrounds facilitate the interpretation of both codominant nuclear markers and maternally transmitted mitochondrial DNA; dominant markers (such as RAPDs) cannot be simply interpreted in a population context (see Sunnucks, 1999). In addition, safeguards such as Hardy-Weinberg equilibrium cannot be employed to flag dominant markers behaving in non-Mendelian fashion. While dominant markers may prove valuable in some instances (e.g., identification of clones, paternity exclusion), we will not consider them here. Other factors worth considering when choosing a genetic marker include: the expense and time required to survey a sufficient number of populations and individuals to resolve the question of interest, the quantity and quality of sample tissue needed to obtain individual genotypes, and the likelihood that a chosen marker might be subject to strong evolutionary forces other than genetic drift and gene flow (i.e., selection).

Allozymes.—Allozymes are products of alternative alleles of a particular enzyme locus that have different electrophoretic mobilities. To score this variation, protein homogenates are separated by an electric field within a support matrix (starch, acrylamide, or cellulose acetate). After separation, the locations of different allozymes are revealed biochemically by using the catalytic activity of the enzyme to produce visible bands.

With a few notable exceptions (mainly esterases and peptidases), the vast majority of allozymes exhibit codominant Mendelian inheritance. Thus, resulting genotype frequency data fit easily into classical population genetic models. Screening for variation is a trial-and-error process but, aside from costs associated with disposal of toxic stains, it is relatively inexpensive.

Allozymes reveal a limited proportion of the total variation in the DNA sequence at the encoding locus: only amino acid-changing substitutions that alter charge density are detected. These amino acid alterations might also be subject to natural selection, as suggested by some detailed studies of particular allozymes (see Geographic Clines below). Because the visualization of allozyme bands requires active enzyme, samples must be kept alive or frozen before scoring.

Mitochondrial DNA.—Mitochondria carry their own DNA (mtDNA). In animals, offspring usually receive mtDNA only from their mother (although some bivalves are exceptional; see Hoeh et al., 1991) and the mitochondrial genome does not usually recombine. MtDNA thus provides a clonally inherited marker that traces maternal lineage. Distinct mtDNA sequences are referred to as haplotypes rather than alleles, because mtDNA is inherited as a single non-recombining unit.

Variation in mtDNA was first surveyed using restriction analysis of centrifugally isolated whole mtDNA. Over the last decade, phylogenetically conserved primers have been developed to amplify several regions of the mitochondrial genome using the polymerase chain reaction (PCR, see Palumbi, 1996). Variation within this amplified fragment can then be assayed by digestion with restriction enzymes or by direct DNA sequencing. PCR-based markers offer one practical advantage over allozymes: DNA (unlike most

enzymes) is stable at ambient temperatures once tissue has been dehydrated with salts or alcohol.

Although the frequencies of mtDNA haplotypes fit classical population genetic models developed for haploid systems, they are more profitably analyzed using methods adapted from phylogenetic systematics. These genealogical models may offer important insights into population history that frequency-based models cannot (Avise, 2000).

MICROSATELLITES.—Microsatellites are tandem repeats of 2–10 base pair nucleotide motifs. Microsatellite loci are often highly variable with respect to the number of these small repeats, so allelic variation can be surveyed as size polymorphisms, which can be scored relatively cheaply and quickly. Microsatellite loci combine the advantages of high polymorphism and multiple independently segregating loci, however they are not without drawbacks. Aside from complications with interpreting allele size, some trinucleotide repeats are coding (and thus subject to selection at the protein level) and, as with any nuclear gene, duplications can confuse the assumption of codominant Mendelian inheritance.

NUCLEAR SEQUENCES.—DNA sequences from genes encoded in the nucleus potentially offer the benefits of both allozymes and mtDNA. Like allozymes, codominant Mendelian inheritance can generally be assumed and as such the data fit well into classical population genetic models. Like mtDNA, nuclear sequences allow relationships between alleles to be inferred, gene regions can be amplified using the PCR, and restriction analysis can be used to assay some variation. However, because PCR amplifies both alleles of heterozygous individuals, diploid genes generally cannot be sequenced directly as PCR products. For example, if two alleles differed by a single insertion/deletion, all sites after such a mutation would be overlaid but offset, resulting in unreadable sequence. Even if all variation consisted of single-site polymorphisms, substitutions could not be assigned to one allele or the other. Many nuclear genes are duplicated, so alleles from different loci might be simultaneously amplified and later confounded. Much non-coding DNA likewise occurs at high copy number. Finally, nuclear genes can recombine, potentially fusing sequences with different histories and complicating genealogical approaches to estimating gene flow.

FORCES ACTING ON GENETIC MARKERS

GENETIC DRIFT.—In a randomly mating population of infinite size, the frequencies of genes unaffected by selection would not change over time. However, in a finite population, allele frequencies will vary due to chance sampling events. The intensity of genetic drift varies inversely with the number of breeding individuals in a population. As a result, mtDNA markers (passed on as single copies only by females) drift more rapidly than nuclear markers (Birky et al., 1989). The time required for all but one allele to be lost from a population as a result of drift is likewise inversely related to the effective population size.

The high population densities and large geographic ranges of many marine species would seem to suggest that drift is inconsequential in the sea. However, the effects of drift hinge not on the total number of individuals in the population (the census size), but on the effective population size, roughly, the number of individuals that actually contribute genetically to the next generation. Thanks to the vagaries of spawning success and pelagic larval survival, effective population size may be only a small fraction of census

population size in many free-spawning marine organisms (see Chaotic Genetic Patchiness below). Furthermore, the harmonic mean of the effective population size averaged over generations determines the degree of drift in populations of variable size, so even a few bouts of depressed population size could dramatically lower effective population size.

GENE FLOW.—In theory, gene flow is the *number* of migrants between populations each generation; exactly the number that interests ecologists studying connectivity in marine populations. Standard population genetic models do not estimate migration rates directly, but rather as $N_e m$, the product of the *proportion* of individuals migrating each generation (m) and the effective population size (N_e). Only those migrants that successfully breed after migration contribute to gene flow; those selected against or failing by chance to reproduce are invisible to inferences made with genetic techniques.

MUTATION.—The rate of mutation (μ) may have a substantial impact on estimators of population structure and levels of gene flow. At one extreme, low mutation rates may limit the variation of markers. At the other, high mutation rates may cause alleles to mutate before ever leaving the population where they originated, a real possibility for some microsatellite markers (Neigel, 1997; Hedrick, 1999). Generally, high mutation rates (μ approaching m) will lead to overestimates of m in frequency-based methods (Slatkin, 1995; Neigel, 1997), leading to the conclusion that connectivity is greater than is actually the case. This is because estimates of genetic subdivision are based on the average difference between individuals in different populations divided by the average difference between individuals within the same populations. At high mutation rates, the differences in alleles (or nucleotides) begin to saturate, and this more strongly underestimates the number of mutational differences between individuals in different populations compared to the differences between individuals in the same population. The result is a bias that makes different populations look more similar. If the between-population differences were not normalized by within-population differences, this problem would not occur, and higher mutation rates would lead to more (absolute, not relative) differences between populations.

SELECTION.—The unfortunate truth is that most patterns of geographic genetic variation could be explained by some scenario involving selection acting either directly on the markers in question or on genetically linked sites. Selection for different alleles in different populations could increase apparent differentiation. Selective sweeps (where one globally favored variant takes over an entire range) or stabilizing selection (where the same selected genotypes are favored over a wide range) could enforce homogeneity, misleadingly suggesting high levels of connectivity. These sobering truths should trigger caution, but need not result in despair. A growing battery of tests can be employed to detect selection (Skibinski, 2000). Simulations show that under certain conditions selection has a limited effect on estimates of gene flow (Slatkin and Barton, 1989). Selection should act independently on unlinked loci, so utilizing a broad arsenal of markers is the best way to confront difficulties posed by this force.

HISTORY.—As with selection, ignoring the vestiges of history when interpreting genetic data can be misleading. The estimation of many population genetic statistics (such as F_{ST}, see Neigel, 1997) rests on the assumption of an equilibrium between processes, some of which (such as genetic drift) may proceed very slowly in large populations. A common scenario where history could bias inference about population connectedness involves recolonization after local population extinction. A recolonized population will, for a time,

be genetically similar to the population that served as the source of recolonizing propagules. The time required to return to equilibrium levels of differentiation following such a demographic perturbation is inversely proportional to the migration rate between populations. As a result, historical changes tend to exert a one-way bias on estimates of population connectedness: species with limited dispersal potential will carry the mark of history for a longer period of time than broad dispersers. Consequently, genetic approaches will tend to overestimate gene flow between recolonized populations and their sources. More generally, nonequilibrium between gene flow and drift can result in disagreement between patterns of connectedness inferred indirectly from genetic markers and patterns predicted by present-day current patterns (Benzie and Williams, 1997) or based on direct ecological observations of settlement (Gaines and Bertness, 1992; Brown et al., 2001).

Models of Population Structure

Geography.—Most applications of genetic data to population questions have used Sewall Wright's island model to relate the geography of gene frequency variation to levels of gene flow (Neigel, 1997). In the island model, all populations are linked by equal levels of gene flow, with a proportion of migrants (m) every generation. Differences between populations are all assumed to reflect the same parameter and thus are pooled as replicates to provide a single estimate with low variance. Island models may be appropriate in two-population cases, or in describing equally-spaced oceanic islands, but probably do not describe most real population structures (especially those along coastlines) very well.

The most commonly considered alternative to the island model is the stepping stone model (Slatkin, 1993), in which only adjacent populations exchange migrants. In such circumstances, there is a distinct geography, and populations that are closer are linked by larger amounts of genetic exchange. This seems to reflect the organization of many coastal marine species more accurately, in which dispersal between localities is often related to geographic distance.

Identity.—Ultimately, conclusions about levels of connectedness between populations are based on the genetic similarity of those populations. Different types of genetic data allow similarity to be assessed and measured in different ways. In frequency-based models, levels of gene flow are estimated as an inverse function of F_{ST}, which summarizes departures of heterozygosity from expectations for freely interbreeding populations (Neigel, 1997). There can be no degrees of similarity between variants (e.g., similarity of sequences), only degrees of similarity between frequencies of these variants in populations. These models have been applied most often to allozyme data.

While the frequencies of different genetic types can also figure into sequence-based models, these data (usually mtDNA sequences) can also provide information about the genealogical relationships of alleles. These models are not so heavily dependent on assumptions of equilibrium as frequency-based models, and as a result are far better for teasing out the effects of history from ongoing gene flow (Wakeley, 1996; Nielsen and Slatkin, 2000).

Models for analyzing microsatellites tend to straddle frequency- and sequence-based approaches. Frequency-based analyses can be applied, but similar-sized alleles are probably closely related due to the stepwise mutations that produce most new microsatellites. F_{ST} estimates that take this mutation process into account have been developed (Goldstein et al., 1995; Slatkin, 1995).

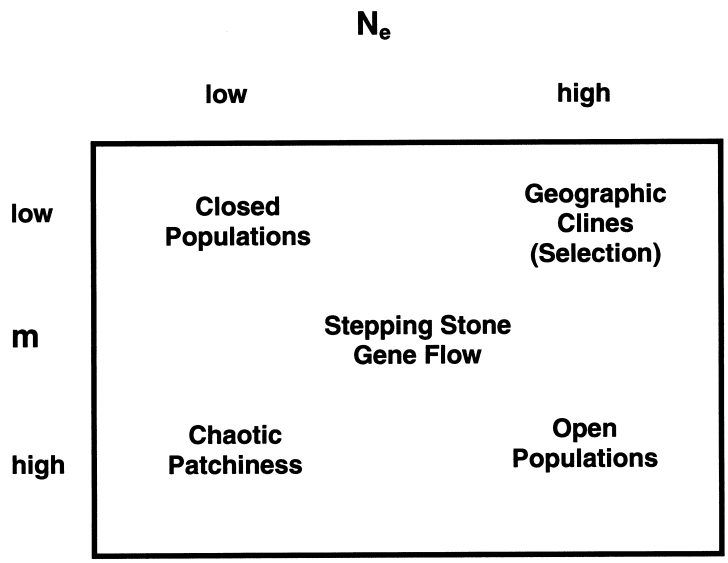

Figure 1 Combinations of migration rate (m) and effective population size (Ne) most favorable to five described patterns. Historical effects must also be considered, especially for low migration rates; see text.

Six Patterns of Genetic Differentiation

Different patterns of geographic genetic differentiation evolve based on the magnitude of the migration rate (m) and the effective population size (N_e) (Fig. 1). Populations can be completely closed (all recruits from within) when $N_e m$ is small, or completely open (all recruits from other populations) when $N_e m$ is large. Between these two extremes of dispersal, populations may show gradually reduced genetic similarity with increasing geographical isolation owing to restricted dispersal (stepping stone gene flow). More detailed temporal sampling may reveal that apparently open populations actually consist of mixed cohorts recruited from a relatively small number of breeding adults. In rare cases, selection on the markers themselves (especially allozymes) may override the forces of ongoing gene flow and drift. Finally, historical effects must always be taken into account, especially when m is small and N_e is large.

Closed Populations.—The primary genetic signature of a *closed population* is its persistent genetic differentiation from other conspecific populations. The extent of this differentiation will depend on the combined action of mutation, drift, migration, and selection and their interplay with time and population size. When gene flow between two populations ceases, drift and selection will initially play the greatest role in differentiation by acting on extant genetic variation. In time, mutations will result in the appearance of 'private' alleles, i.e., genetic variants that only occur in the population in which they originated. In the absence of gene flow, such alleles may reach high frequency (via drift or selection) in one population without ever appearing in others (Slatkin, 1985). Phylogenetic relationships among haplotypes can also provide signals of population closure. In the absence of gene flow, new alleles arise from those already extant within the population, producing a pattern where the most similar haplotypes are distributed within (rather than between) populations. Given sufficient time and continued self-recruitment, a pat-

tern of reciprocal monophyly, whereby all alleles in each population are more closely related to each other than to those from any foreign population, will eventually result (Cunningham and Collins, 1998). Finally, although persistent genetic differentiation may suggest closure, it is not sufficient to demonstrate closure. Selection may favor different genotypes in different geographic locales, so population differentiation at some loci may persist in the face of significant gene flow (see the *Lap* example from *Mytilus edulis* in Geographic Clines, below).

One marine species characterized by closed populations is the tidepool copepod *Tigriopus californicus*. Sharp genetic differentiation is common between neighboring *T. californicus* populations despite its free-swimming life style within high intertidal splash pools (Burton and Feldman, 1981; Burton and Lee, 1994). Allelic frequency differences among populations have been shown to be stable over a period of 18 yrs, corresponding to approximately 200 generations (Burton, 1997). Such differentiation of local populations is probably not uncommon among taxa that lack pelagic development (e.g., the amphipod *Excirolana braziliensis*: Lessios et al., 1994; gastropods with crawl-away larvae: Hoskin, 1997).

Organisms exhibiting strong natal philopatry may also have closed populations. Tagged female green turtles (*Chelonia mydas*) show strong nest site fidelity despite broad foraging ranges. The genetic consequences of this fidelity are evident in fixed or nearly fixed mtDNA haplotype differences between most rookeries (Bowen et al., 1992). Nesbo et al. (2000) found genetic differences between different eastern Atlantic spawning stocks of Atlantic mackerel (*Scomber scombrus*), even after trans-Atlantic samples of non-spawning adults had failed to reveal differentiation.

Closure may also be more common than we currently assume in species possessing pelagic larval stages. Jiang et al. (1995) found fixed differences in mitochondrial DNA haplotypes between neighboring populations (35 km apart) of Taiwanese abalone (*Haliotis diversicolor*), a species with a 4–10 d pelagic larval life. Near-reciprocal monophyly of mtDNA haplotypes has also been reported for populations of fish with moderate (2–3 wks) pelagic larval periods separated by only 100–200 km (Riginos and Nachman, 2001).

Analyses of temporal stability of allelic frequencies can provide the heightened sensitivity needed to detect more subtle genetic signatures. For example, although Lewis and Thorpe (1994) found that allozyme differentiation among populations of the queen scallop, *Aequipecten opercularis*, was not pronounced, similar allelic frequency differences were apparent across all four year classes studied. Such temporal stability of subtle differentiation would not be expected under a regime of extensive dispersal; Lewis and Thorpe (1994) suggest that the populations were self-recruiting despite a pelagic larval duration of up to several weeks. Such temporal analyses have been underutilized in studies of the genetic structure of marine organisms.

Abrupt Genetic Change at a Geographical Barrier.—A *phylogeographic break* can be defined as the coincidence of a genealogical divide and a geographic barrier (category I of Avise et al., 1987). Such a pattern should result when a barrier sunders a species' range and subsequent gene flow has been limited for a sufficient time for alleles in each population to drift to fixation of alternate alleles or reciprocal monophyly. MtDNA sequences can define this pattern more clearly than nuclear markers because their smaller effective population size means divided populations will drift to reciprocal monophyly more quickly (Birky et al., 1989).

The best-studied marine phylogeographic break occurs at Cape Canaveral on the eastern coast of Florida. Several marine invertebrates and teleost fish show concordant change in mtDNA RFLPs at this break, most notably the oyster *Crassostrea virginica* (Reeb and Avise, 1990). This latter result is quite surprising, given the oyster's pelagic larvae and an allozyme survey (Buroker, 1983) that did not show abrupt change at Cape Canaveral. Similar concordance between a classically recognized biogeographic boundary and intraspecific genealogy also appears in the Indo-Australian archipelago (Lavery et al., 1995; Williams and Benzie, 1998).

The persistence of strong phylogeographic breaks in species with pelagic larvae implies that little gene flow is realized across biogeographic barriers; thus populations to either side may be closed with respect to one another. However, inferring that populations separated by similar distances elsewhere in a species' range are likewise closed would be an unwarranted extrapolation, as biogeographic boundaries are by definition atypical. Given that the time to achieve reciprocal monophyly is long when the effective population size is large, populations to either side of the barrier may even be cryptically reproductively isolated species.

Not all large scale surveys of genetic variation in marine populations employ mtDNA sequences, and only a subset of mtDNA surveys reveal reciprocally monophyletic populations. We can define a geographic genetic break as any abrupt change in genetic similarity that coincides with a past or present biogeographical barrier. Such breaks can be detected as changes in not only genealogical history as inferred by mtDNA sequences, but also by changes in gene frequencies or genetic diversity between populations. This expanded definition allows non-genealogical data to point to possible phylogeographic breaks (see Ayre et al., 1991; Billingham and Ayre, 1996 for possible allozyme examples in eastern Australia).

More broadly, this definition embraces a variety of patterns of population relationships that result from the interplay of divergence due to past barriers and similarity owing to subsequent recolonization and gene flow. One such pattern results when a barrier marked the endpoint of a species' range in the past. As conditions changed, colonizing propagules could pass this endpoint and establish populations in previously vacant regions. If only a limited number of propagules initially crossed the former barrier, and subsequent gene flow has been limited, such recolonized populations should exhibit a genetic signature of population expansion in the form of low heterozygosity and a high degree of relatedness both to other recolonized populations and the propagule source population. The gastropod *Acanthinucella spirata* provides an example: subdivided and variable populations from southern California contrast markedly with populations from north of the present-day biogeographic barrier at Point Conception, which are nearly fixed for a single mtDNA haplotype (Hellberg et al., 2001).

Such patterns of past range expansion can also provide information on the magnitude and direction of gene flow since the barrier's demise. For example, populations in the Black Sea have been repeatedly isolated from the greater Mediterranean Sea due to Pleistocene climatic changes. However, genetic mixture between populations of anchovy (*Engraulis encrasicolus*) during times of connection appears to be highly unidirectional; the 'Black Sea' clade occurs at frequencies near 40% throughout much of the Mediterranean, but the ancestral 'Mediterranean' clade is nearly completely absent from the Black Sea (Magoulas et al., 1996). This implies that Black Sea populations may serve as sources

for some Mediterranean populations (especially nearby Aegean populations), but must be closed to recruitment from other populations.

In short, abrupt genetic changes may provide insights into recruitment patterns under special circumstances, but usually owe more to past extinctions and recolonizations than to ongoing patterns of gene flow (Cunningham and Collins, 1998). Even such historical interpretations should be made only with some independent corroboration (e.g., concordant patterns between species or markers, fossil evidence) since even randomly generated lineages may exhibit apparent phylogeographic breaks (Neigel and Avise, 1986).

GEOGRAPHIC CLINES.—A cline may be defined as a consistent and progressive change in gene frequencies along a geographic axis. In contrast, phylogeographic breaks are abrupt rather than progressive, and the patterns caused by spatially restricted gene flow (including isolation by distance) are characterized by a random pattern of allele frequency changes. Clines may be generated either when selection acts in opposite directions at the ends of a geographic range (primary differentiation), or by secondary intergradation between genetically divergent populations (Endler, 1977).

Regardless of their cause, the presence of clines in marine species suggests that gene flow is not always the most powerful force acting on allele frequencies. Primary clinal variation demonstrates that selection may be strong enough to overcome even high rates of migration. In the blue mussel, *Mytilus edulis*, an 'oceanic' allele of the leucine aminopeptidase (*Lap*) locus confers high enzymatic activity in high salinity environments (Koehn and Siebenaller, 1981), but can lead to a lethal depletion of nitrogen reserves during the stressful autumn season (Hilbish and Koehn, 1985). As a result, the oceanic allele is disadvantageous in low salinity environments, and frequencies of this allele decline from the mouth of Long Island Sound to its head. The oceanic allele is common among newly recruited individuals (Koehn et al. 1980, Hilbish, 1985); a progressive reduction in frequency within each cohort establishes the low frequencies characteristic of adult populations in low salinity environments each generation. This form of habitat-associated selection may increase the closure of a marine population if it favors recruits that have returned to their parental population.

The recognition that history has played an important role in establishing the clines has helped to change the view that in marine systems gene flow is strong enough to homogenize allele frequency distributions (Hilbish, 1996) quickly. Concordance of clines at multiple allozyme loci and in mtDNA haplotypes in the killifish, *Fundulus heteroclitus* suggests that these clines initially formed as the result of a secondary contact between two previously isolated subspecies of *F. heteroclitus* (Ropson et al., 1990; Gonzalez-Villaseñor and Powers, 1990). Even in species with a long planktonic phase, a history of isolation and secondary contact can create highly complex patterns that are surprisingly resistant to gene flow (Karl and Avise, 1992).

STEPPING STONE GENE FLOW.—When migration occurs solely between neighboring populations (so that distant populations are linked via intermediate 'stepping stones'), genetic differentiation between populations increases with increased geographic distance. This pattern has also been termed isolation-by-distance, although Wright originally coined the expression to describe a process (not a pattern) of change in small, continuous (not discrete) populations (Wright, 1943). Under stepping stone gene flow, pairwise gene flow estimates are high for close populations, but lower for more distant populations. The exact relationship between genetic distance and geographic distance depends on the configuration of the stepping stones, the mutation rate, the migration rate among adjacent

populations, and the time populations have had to come into equilibrium between drift and migration (Slatkin, 1993; Hutchison and Templeton, 1999).

Studies of fish and invertebrates from the Atlantic (Pogson et al., 2001), the Mediterranean (Borsa et al., 1997), deep sea (Vrijenhoek, 1997), and the tropical Pacific (Palumbi et al., 1997) all show isolation by distance. Spatial autocorrelation analysis in red drum (*Sciaenops ocellatus*) and black drum (*Pogonias cromis*) from the Gulf of Mexico suggests a strong isolation by distance effect in these commercially important fish, despite very low overall genetic differentiation among localities (Gold et al., 1994).

Analysis of patterns of change in gene flow with distance from one set of populations can also potentially help interpret dispersal range in another set of populations from that same species. The crown-of-thorns starfish shows a marked pattern of isolation by distance across the entire Pacific (Benzie and Stoddart, 1992). Within the Great Barrier Reef, F_{ST} values are about 2% for non-outbreaking populations (Benzie and Stoddart, 1992) but only about 0.7% for populations in the middle of population explosions that sweep the reef from north to south (Benzie, 1992). The genetic isolation by distance and higher overall F_{ST} of non-outbreaking populations suggests that these populations show limited larval exchange in most years. Only during outbreaks is there significant population connectivity.

Simulations that estimate genetic structure under a variety of different dispersal schemes along a coastal stepping stone show that the signal of stepping stone gene flow is fairly robust, and occurs in populations with a wide variety of dispersal schemes and oceanographic settings (Palumbi, 2002). The simulations suggest that small overall genetic differentiation (e.g., F_{ST} on the order of 1% or so) can be distinguished from measurement noise by examining the relationship between F_{ST} and distance. Accurate interpretation of such slight genetic differentiation could be a powerful tool in understanding realized genetic exchange among populations.

An important assumption of the simulation models is that species are distributed continuously and that the relationship between larval dispersal and distance is constant over the entire range. In fact, populations often occupy discontinuous habitat, such as rocky outcrops interspersed by sand, or island archipelagoes separated by open ocean. Comparisons between geographically disjunct populations frequently generate much of the signal in isolation-by-distance comparisons (e.g., crown-of-thorns starfish, Benzie and Stoddart, 1992). Ideally, short-range larval dispersal should be measured through genetic isolation by distance of relatively nearby populations (see Hellberg, 1994; Johnson and Black, 1998), and be compared with estimates based on genetic analyses across larger distances.

Chaotic Genetic Patchiness.—For several species with pelagic larvae (limpets: Johnson and Black, 1982, 1984; echinoids: Watts et al., 1990; Edmands et al., 1996; Moberg and Burton, 2000; barnacles: Hedgecock, 1986, 1994b), adult populations show low levels of genetic subdivision, yet repeated sampling of recruits from the same locale over time reveals that different cohorts are genetically differentiated. This pattern is termed *chaotic* (or *fluctuating*) *genetic patchiness*. A role for the pelagic larvae must be invoked to explain this pattern (Hedgecock et al., 1982; Hedgecock, 1986; Johnson and Black, 1984; Kordos and Burton, 1993). These studies suggest that as much genetic variation can be observed among recruits at a single place as can be observed among adult populations on spatial scales of 100s to 1000s of kms (Hedgecock, 1994c). Thus, the population genetics of marine planktonic larvae or new recruits are spatially and temporally much more dy-

namic than expected, given that their adult populations are large and apparently well connected by larval dispersal.

There are three potential explanations for the dynamic population genetics of larvae or recruits. First, larvae may be coming from different source populations at different times, depending on the direction of currents (Kordos and Burton, 1993). Genetically differentiated sources are found where formerly isolated (and thus differentiated) populations presently make contact (Hare and Avise, 1996), but most marine populations with pelagic larvae are genetically similar over broad regions (Hedgecock, 1994b; Edmands et al., 1996; Moberg and Burton, 2000). A single hidden source population would also not be consistent with heterogeneity among settling cohorts or with consistently reduced genetic diversity within pulses of settlers relative to adult populations.

A second possibility is that selection on early life stages may winnow the genetic diversity of larvae and recruits. Selection on pelagic larvae was invoked to explain chaotic patchiness in a well-studied limpet (Johnson and Black, 1984) and deficiencies of heterozygotes in young bivalves (Singh and Green, 1984). However, the conditions under which polymorphism is maintained at loci experiencing large reversals or fluctuations in fitness are limited (Zouros and Foltz, 1984). Differential survival of genotypes after settlement and before sampling could also explain differences between recruits and adults (Koehn et al., 1980). Such selection would not, however, explain chaotic temporal variation in adult marine populations. Future comparisons of settlers and larvae should shed light on the selection hypothesis as well; selection would be supported if larval samples were consistently more genetically diverse than samples of settlers.

A final explanation begins with the observation that free-spawning marine invertebrates may produce millions of gametes. The chances that any of these are fertilized and survive their pelagic dispersal are very small. This combination of high reproductive output and high rates of failure during early life history stages mean that only a few adults may be responsible for much of the recruitment during any particular season. Such 'sweepstakes' reproductive success may limit the diversity of recruits (Hedgecock et al., 1982; Hedgecock, 1986, 1994c) and reduce the observed genetically effective population size (Palumbi and Wilson, 1990). Although marine species have exquisite adaptations for reproducing when and where recruitment is broadly predictable (Parrish et al., 1981; Morgan, 1995), they may be unable to respond evolutionarily to fine-grained oceanographic variation affecting reproductive success.

Broad-Scale Homogeneity.—Some marine species with planktotrophic (feeding) larvae show high genetic similarity over broad geographic scales that is apparently due to ongoing gene flow. Winans' (1980) study of geographical variation in the milkfish *Chanos chanos* provides one of the earliest examples of this pattern. Analysis of numerous variable allozyme loci from juvenile milkfish collected from locations between the Philippine Archipelago to the Hawaiian Islands revealed only low levels of differentiation (F_{ST} = 0.039). Most of this owes to the Hawaiian populations; setting these aside leaves no significant variation in allelic frequencies over a range spanning more than 12,000 km.

More recent work that incorporates mitochondrial markers as well as allozymes lends further support to the conclusion of ongoing long-distance gene flow in some species. Lessios et al. (1998) studied populations of the echinoid *Echinothrix diadema* separated by the Eastern Pacific Barrier (EPB), a span of ocean devoid of shallow water habitat for over 5400 km. Allozyme frequencies varied across the EPB, but suggested moderate ongoing gene flow. Numerous closely related mtDNA haplotypes were mixed among

(and sometimes shared by) distant populations again suggesting gene flow. The high overall variation seen also suggests that genetic connections were not solely due to a recent founder event. This inference of high ongoing connectivity could only be made using identity markers, a must for making a strong case for high levels of ongoing gene flow over great distances.

Conflicts between Methods, Scales, and Markers

Inferences drawn from genetic patterns may appear to conflict with direct observations of dispersing propagules. Different markers can also yield conflicting results. Even patterns from a single species may not agree with each other. While the cause of some of these conflicts remain unknown, some result from simple semantics and implicit assumptions about equilibrium and effective population sizes.

Too often, both ecological and evolutionary genetic studies are vague in their definition of time scales. An ecological field study monitoring local recruitment over a 10-yr time period would be 'long term'. In contrast, population genetic parameters that return 'rapidly' to equilibrium values may require time periods on the order of N_e generations, possibly thousands or even millions of years. As a result, populations that are demographically closed (as defined by largely independent recruitment events) may still show no genetic differentiation due to ongoing sporadic (but demographically inconsequential) interpopulation migration (e.g., Brown et al., 2001).

Different patterns of genetic differentiation may emerge at different spatial scales due to disequilibrium between high levels of connectivity in the past and lower levels, even complete isolation, in the present. Genealogical markers can provide insights as to whether history or ongoing forces prevail. Examination of the variance between genetic differentiation and geographic distance at different spatial scales (Slatkin, 1993; Hellberg, 1995; Hutchinson and Templeton, 1999) can also reveal patterns that change from panmixia (open populations) at very small scales, to an equilibrium pattern consistent with stepping stone gene flow at intermediate scales, to large-scale nonequilibrium condition dominated by history (corals: Hellberg, 1995; crabs: Lavery et al., 1995; reef fish: Planes et al., 1996).

Conflicts can also arise when some markers are subject to natural selection and others are not. Lewontin and Krakauer (1973) proposed that when the alleles at one locus conflict with a geographic pattern shared by many other loci, selection on the outlier locus can be implied. Although other forces can produce significant deviations among loci (Nei and Maruyama, 1975; Robertson, 1975), this approach can at least identify 'candidates' for loci under selection. The conflict between allozyme markers and mtDNA RFLPs in oysters was mentioned above (see Abrupt Genetic Change at a Geographical Barrier). Subsequent work using nuclear RFLPs (Karl and Avise, 1992) found a pattern that agreed with the mtDNA break, implying that the geographically homogeneous allozymes were under selection and that dispersal across the Cape Canaveral barrier was far more limited than allozyme frequencies implied. The Atlantic cod (*Gadus morhua*) shows a similar pattern (Pogson et al., 1995).

However, the conclusion that allozymes are consistently under broad scale stabilizing selection that will result in overestimates of gene flow cannot be taken as the universal cause of conflicts between genetic markers. An additional survey of nuclear RFLPs in oysters across Cape Canaveral found no evidence for a genetic break (McDonald et al.,

1996), thus conflicting with the data of Karl and Avise (1992). Both of these studies employed anonymous nuclear RFLPs which, although less likely than allozymes to be under selection, are nonetheless frequency markers. Nuclear sequence studies of the loci encoding allozymes may be necessary to sort out the complex and fascinating patterns seen in oysters. Selection may also increase differentiation of allozyme markers among populations, as appears to be the case for *Mytilus* (see Geographic Clines) and other marine examples (Johannesson et al., 1995; Schneider-Broussard et al., 1998, Schmidt and Rand, 1999; Lemaire et al., 2000).

Conclusions and Future Prospects

Marine population biologists have looked to genetic markers for insights into questions of larval dispersal that have been difficult to approach by more direct means (although see Thorrold et al., this issue). Early studies using allozymes revealed some striking breaks in population connectivity, even between sites separated by only a few kms (see Closed Populations), as well as instances of genetic similarity over staggeringly long distances (see Broad-Scale Homogeneity). Interpretation of these results requires caution, however, because allozymes, as frequency markers, provide no genealogical information and some loci have themselves been targeted by selection (see Geographical Clines). Results based upon more geographically explicit genetic models (see Isolation by Distance) and genealogical data (see Abrupt Genetic Change at a Geographical Barrier) suggest many patterns of geographical genetic differentiation reflect events in the distant past (thousands to millions of years ago) more than ongoing patterns of dispersal. That larvae collected over time at the same site show inter-class differentiation exceeding that for all known adult populations raises the possibility that the effective population size of marine species may be orders of magnitude lower than usually thought (see Chaotic Genetic Patchiness). Such a conclusion would have profound implications for every aspect of marine population biology.

Much of the work ahead for marine population geneticists, then, will explore this more dynamic, nonequilibrium view of the connections among populations. Repeated geographical sampling of larvae will be required to address questions raised by patterns of chaotic genetic patchiness. Coalescent methods to estimate N_e (Fu, 1994) may offer insights into effective population size. Methods for analyzing intraspecific genealogical data are growing rapidly, and should help resolve whether patterns are due to ongoing gene flow, historical events, or some combination of the two. In those species where ongoing gene flow dominates (most likely coastal species with pelagic larvae), sampling regimes will have to be structured differently to obtain reasonable estimates of the magnitude of movements between populations (Palumbi, 2002).

Finally, laboratory techniques and analytical methods (Skibinski, 2000) have advanced to a point where allozyme markers might fruitfully be revisited by studying the DNA sequence variation underlying particular loci. Such variation could reveal whether particular markers were under selection, the type of selection and amino acid residues involved, and how far the effects of selection extend from a particular selected site. Such data could also be used to evaluate proposed explanations for conflicts between different types of genetic markers and perhaps provide insights into the long-term effective population size of marine populations.

Acknowledgments

Thanks to I. Baums, R. Cowen, T. McGovern, M. Taylor, and R. Warner for comments on the manuscript. This work was conducted as part of the Working Group entitled "Open vs. Closed Marine Populations: Synthesis and Analysis of the Evidence" supported by the National Center for Ecological Analysis and Synthesis (NCEAS), a Center funded by NSF Grant DEB-94-21535, the University of California, Santa Barbara, the California Resources Agency, and the California Environmental Protection Agency. MEH supported by NSF (DEB-0075382).

Literature Cited

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard, Cambridge, Massachusetts.

_____, J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann. Rev. Ecol. Syst. 18: 489–522.

Ayre, D. J., J. Reid and J. Wishart. 1991. Genetic subdivision within the eastern Australian population of the sea anemone *Actinia tenebrosa*. Mar. Biol. 109: 379–390.

Benzie, J. A. H. 1992. Review of the genetics, dispersal, and recruitment of crown-of-thorns starfish (*Acanthaster planci*). Aust. J. Mar. Freshw. Res. 43: 597–610.

_____ and J. A. Stoddart 1992. Genetic-structure of outbreaking and non-outbreaking crown-of-thorns starfish (*Acanthaster planci*) populations on the Great Barrier Reef. Mar. Biol. 112: 119–130.

_____ and S. T. Williams. 1997. Genetic structure of giant clam (*Tridacna maxima*) populations in the West pacific is not consistent with dispersal by present-day ocean currents. Evolution 51: 768–783.

Billingham, M. J. and D. J. Ayre. 1996. Genetic subdivision in the subtidal, clonal sea anemone *Anthothoe albocincta*. Mar. Biol. 125: 153–163.

Birky, C. W., P. Fuerst and T. Maruyama. 1989. Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121: 613–627.

Borsa, P., A. Blanquer and P. Berrebi. 1997. Genetic structure of the flounders *Platchthys flesus* and *P. stellatus* at different geographic scales. Mar. Biol. 129: 233–246.

Bowen, B. W., A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs and J. C. Avise. 1992. Global population structure and natural history of the green turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution 46: 865–881.

Brown, A. F., L. M. Kann and D. M. Rand. 2001. Gene flow versus local adaptation in the acorn barnacle, *Semibalanus balanoides*: Insights from mtDNA polymorphisms. Evolution. 55: 1972–1979.

Buroker, N. E. 1983. Population genetics of the American oyster *Crassostrea virginica* along the Atlantic coast and the Gulf of Mexico. Mar. Biol. 75: 99–112.

Burton, R. S. 1997. Genetic evidence for persistence of marine invertebrate populations in an ephemeral environment. Evolution 51: 993–998.

_____ and M. W. Feldman. 1981. Population genetics of *Tigriopus californicus*: II. Differentiation among neighboring populations. Evolution 35: 1192–1205.

_____ and B.-N. Lee. 1994. Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus.* Proc. Nat'l. Acad. Sci. 91: 5197–5201.

Cunningham, C. W. and T. M. Collins. 1998. Beyond area relationships: extinction and recolonization in molecular marine biogeography. Pages 297–321 *in* R. DeSalle and B. Shierwater, eds. Molecular approaches in ecology and evolution. Birkhauser, Basel.

Edmands, S., P. E. Moberg and R. S. Burton. 1996. Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. Mar. Biol. 126: 443–450.

Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton, New Jersey.

Fu, Y. X. 1994. Estimating effective population size or mutation rate using the frequencies of mutations of various classes in a sample of DNA sequences. Genetics 138: 1375–1386.

Gaines, S. D. and M. D. Bertness. 1992. Dispersal of juveniles and variable recruitment in sessile marine species. Nature 360: 579–580.

Gold, J. R., L. R. Richardson, C. Furman and F. Sun. 1994. Mitochondrial DNA diversity and population structure in marine fish species from the Gulf of Mexico. Can. J. Fish. and Aquat. Sci. (suppl. 1): 205–214.

Goldstein, D. B., A. R. Linares, M. W. Feldman and L. L. Cavalli-Sforza. 1995. An evaluation of genetic distances for use with microsatellite loci. Genetics 139: 463–471.

Gonzalez-Villaseñor, L. I. and D. A. Powers. 1990. Mitochondrial-DNA restriction-site polymorphisms in the teleost *Fundulus heteroclitus* support secondary intergradation. Evolution 44: 27–37.

Grosberg, R. K. and C. W. Cunningham. 2001. Genetic structure in the sea from populations to communities. Pages 61–84 *in* M. D. Bertness, S. D. Gaines and M. E. Hay, eds. Marine community ecology. Sinauer, Sunderland, Massachusetts.

Hare, M. P. and J. C. Avise. 1996. Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). Evolution 50: 2305–2315.

Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates. Bull. Mar. Sci. 39: 550–564.

_____. 1994a. Population genetics of marine organisms. U.S. GLOBEC News 6: 1–3, 11.

_____. 1994b. Temporal and spatial genetic structure of marine animal populations in the California Current. Cal. Coop. Oceanic Fish. Invest. Rep. 35: 73–81.

_____. 1994c. Does variance in reproductive success limit effective population size of marine organisms? Pages 122–134 *in* A. Beaumont, ed. Genetics and evolution of aquatic organisms. Chapman & Hall, London.

_____, M. L. Tracey and K. Nelson. 1982. Genetics. Pages 297–403 *in* L. G. Abele, ed. The biology of crustacea, vol. 2. Academic Press, New York.

Hedrick, P. W. 1999. Highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313–318.

Hellberg, M. E. 1994. Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. Evolution 48: 1829–1854.

_____. 1995. Stepping-stone gene flow in the solitary coral *Balanophyllia elegans*: equilibrium and nonequilibrium at different spatial scales. Mar. Biol. 123: 573–582.

_____, D. P. Balch and K. Roy. 2001. Climate-driven range expansion and morphological evolution in a marine gastropod. Science 292: 1707–1710.

Hilbish, T. J. 1985. Demographic and temporal structure of an allele frequency cline in the mussel *Mytilus edulis*. Mar. Biol. 86: 163–171.

_____ and R. K. Koehn. 1985. The physiological basis of natural selection at the *Lap* locus. Evolution 39: 1302–1317.

_____. 1996. Population genetics of marine species: The interaction of natural selection and historically differentiated populations. J. Exp. Mar. Biol. Ecol. 200: 67–83.

Hoeh, W. R., K. H. Blakley and W. M. Brown. 1991. Heteroplasmy suggests limited biparental inheritance of *Mytilus* mitochondrial-DNA. Science 251: 1488–1490.

Hoskin, M. G. 1997. Effects of contrasting modes of larval development on the genetic structures of populations of three species of prosobranch gastropods. Mar. Biol. 127: 647–656.

Hutchison, D. W. and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53: 1898–1914.

Jiang L., W. L. Wu and P. C. Huang. 1995. The mitochondrial DNA of Taiwan abalone *Haliotis diversicolor* Reeve, 1846 (Gastropoda: Archaeogastropoda: Haliotidae). Molec. Mar. Biol. Biotech. 4: 353–364

Johannesson, K., B. Johannesson and U. Lundgren. 1995. Strong natural selection causes microscale allozyme variation in a marine snail. Proc. Nat'l. Acad. Sci. 92: 2602–2606.

Johnson, M. S. and R. Black. 1982. Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. Mar. Biol. 70: 157–164.

_____ and _____. 1984. Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38: 1371–1383.

_____ and _____. 1998. Effects of isolation by distance and geographical discontinuity on genetic subdivision of *Littorina cingulata*. Mar. Biol. 132: 295–303.

Karl, S. A. and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science 256: 100–102.

Koehn, R. K., R. I. E. Newell and F. Immermann. 1980. Maintenance of an aminopeptidase allele frequency cline by natural selection. Proc. Nat'l. Acad. Sci. 77: 5385–5389.

_____ and J. F. Siebenaller. 1981. Biochemical studies of amino-peptidase polymorphism in *Mytilus edulis*. II. Dependence of reaction rate on physical factors and enzyme concentration. Biochem. Genetics 19: 1143–1162.

Kordos, L. M. and R. S. Burton. 1993. Genetic differentiation of Texas Gulf Coast populations of the blue crab *Callinectes sapidus*. Mar. Biol. 117: 227–233.

Lavery, S., C. Moritz and D. R. Fielder. 1995. Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*. Molec. Ecol. 5: 557–570.

Lemaire, C., G. Allegrucci, M. Naciri, L. Bahri-Sfar, H. Kara and F. Bonhomme. 2000. Do discrepancies between microsatellite and allozyme variation reveal differential selection between sea and lagoon in the sea bass (*Dicentrarchus labrax*)? Molec. Ecol. 9: 457–467.

Lessios, H. A., B. D. Kessing and D. R. Robertson. 1998. Massive gene flow across the world's most potent marine biogeographic barrier. Proc. Roy. Soc. London B 265: 583–588.

_____, J. R. Weinberg and V. R. Starczak, 1994. Temporal variation in populations of the marine isopod *Excirolana*: how stable are gene frequencies and morphology. Evolution 48: 549–563.

Lewis, R. I. and J. P. Thorpe. 1994. Temporal stability of gene frequencies within genetically heterogeneous populations of the queen scallop *Aequipecten* (*Chlamys*) *operularis.* Mar. Biol. 121: 117–126.

Lewontin, R. C. and J. Krakauer. 1973. Distributions of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics 74: 175-195.

Magoulas, A., N. Tsimenides and E. Zouros. 1996. Mitochondrial DNA phylogeny and the reconstruction of the population history of a species: The case of the European anchovy (*Engraulis encrasicolus*). Molec. Biol. Evol. 13: 178–190.

McDonald, J. H., B. C. Verrelli and L. B. Geyer. 1996. Lack of geographic variation in anonymous nuclear polymorphisms in the American oyster, *Crassostrea virginica*. Molec. Biol. Evol. 13: 1114–1118.

Moberg, P. E. and R. S. Burton. 2000. Genetic heterogeneity among recruit and adult red urchins, *Stronglyocentrotus franciscanus*. Mar. Biol. 136: 773–784.

Morgan, S. G. 1995. Life and death in the plankton: larval mortality and adaptation. Pages 279–321 *in* L. McEdward, ed. Ecology of marine invertebrate larvae. CRC Press, Boca Raton, Florida.

Nei, M. and T. Maruyama. 1975. Lewontin-Krakauer test for neutral genes. Genetics 80: 395.

Neigel, J. E. 1997. A comparison of alternative strategies for estimating gene flow from genetic markers. Ann. Rev. Ecol. Syst. 28: 105–128.

_____ and J. C. Avise. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pages 515–534 *in* E. Nevo E and S. Karlin, eds. Evolutionary process and theory. Academic: New York.

Nielson, R. and M. Slatkin. 2000. Likelihood analysis of ongoing gene flow and historical association. Evolution 54: 44–50.

Nesbo, C. L., E. K. Rueness, S. A. Iversen, D. W. Skagen and K. S. Jakobsen. 2000. Phylogeography and population history of Atlantic mackerel (*Scomber scombrus* L.): a genealogical approach reveals genetic structuring among the eastern Atlantic stocks. Proc. Roy. Soc. London B 267: 281–292.

Palumbi, S. R. 1994. Genetic divergence, reproductive isolation, and marine speciation. Ann. Rev. Ecol. Syst. 25: 547–572.

_____. 1996. The polymerase chain reaction. Pages 205–247 *in* D. M. Hillis, C. Moritz and B. K. Mable, eds. Molecular systematics, 2nd ed. Sinauer: Sunderland, Massachusetts.

_____. 2002. Population genetics, demographic connectivity and the design of marine reserves. Ecol. Appl. (in press)

_____, G. Grabowshy, T. Duda, L. Geyer and N. Tachino. 1997. Speciation and population genetics structure in tropical Pacific sea urchins. Evolution 51: 1506–1517.

_____ and A. C. Wilson. 1990. Mitochondrian DNA diversity in the sea urchins *Strongylocentrotus purpuratus* and *S. droebachiensis*. Evolution 44: 403–415.

Parrish, R. H., C. S. Nelson and A. Bakun. 1981. Transport mechanisms and the reproductive success of fishes in the California Current. Biol. Oceanogr. 1: 175–203.

Planes, S., R. Galzin and F. Bonhomme. 1996. A genetic metapopulation model for reef fishes in oceanic islands: the case study of the surgeonfish, *Acanthurus triostegus*. J. Evol. Biol. 9: 103–117.

Pogson, G. H., K. A. Mesa and R. G. Boutilier. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua* — a comparison of allozyme and nuclear RFLP loci. Genetics 139: 375–385.

_____, C. T. Taggart, K. A. Mesa and R. G. Boutilier. 2001. Isolation by distance in the Atlantic cod, *Gadus morhua*, at large and small geographic scales. Evolution 55: 131–146.

Reeb, C. A. and J. C. Avise. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica*. Genetics 124: 397–406.

Riginos, C. and M. W. Nachman. 2001. Population subdivision in marine environments: the contributions of biogeography, geographical distance and discontinuous habitat to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. Mol. Ecol. 10: 1439–1453.

Robertson, A. 1975. Lewontin-Krakauer test for neutral genes — comment. Genetics 80: 396.

Ropson, I. J., D. C. Brown and D. A. Powers. 1990. Biochemical genetics of *Fundulus heteroclitus* (L.). VI. Geographical variation in the gene frequencies of 15 loci. Evolution 44: 16–26.

Schmidt, P. S. and D. M. Rand. 1999. Intertidal microhabitat and selection at *MPI*: interlocus contrasts in the northern acorn barnacle, *Semibalanus balanoides*. Evolution 53: 135–146.

Schneider-Broussard, R., D. L. Felder, C. A. Chlan and J. E. Neigel. 1998 Tests of phylogeographic models with nuclear and mitochondrial DNA sequence variation in the stone crabs, *Menippe adina* and *Menippe mercenaria*. Evolution 52: 1671–1678

Singh, S. M. and R. H. Green. 1984. Excess of allozyme homozygosity in marine molluscs and its possible biological significance. Malacologia 25: 569–582.

Skibinski, D. O. F. 2000. DNA tests of neutral theory: applications in marine genetics. Hydrobiologia 420: 137–152.

Slatkin, M. 1985. Rare alleles as indicators of gene flow. Evolution 39: 53–65.

_____. 1993. Isolation by distance in equilibrium and nonequilibrium populations. Evol. 47: 264–279.

_____. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457–462.

_____ and N. H. Barton. 1989. A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43: 1349–1368.

Sunnucks, P. 1999. Efficient genetic markers for population biology. Trends Ecol. Evol. 15: 199–203.

Thorrold, S. R., G. P. Jones, M. E. Hellberg, R. S. Burton, S. E. Swearer, J. E. Neigel, S. G. Morgan and R. R. Warner. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers: can we do it right? Bull. Mar. Sci. (this issue)

Vrijenhoek, R. C. 1997. Gene flow and genetic diversity in naturally fragmented metapopulations of deep-sea hydrothermal vent animals. J. Hered. 88: 285–293.

Wakeley, J. 1996. Distinguishing migration from isolation using the variance of pairwise differences. Theor. Pop. Biol. 49: 369–386.

Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J. Hered. 89: 438–450.

Watts, R. J., M. S. Johnson and R. Black. 1990. Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in western Australia. Mar. Biol. 105: 145–152.

Winans, G. A. 1980. Geographic variation in the milkfish *Chanos chanos*. I. Biochemical evidence. Evolution 34: 558–574.

Wright, S. 1943. Isolation by distance. Genetics 28: 114–138.

Zouros, E. and D. W. Foltz. 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. Malacologia 25: 583–592.

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