TEMPORAL AND SPATIAL GENETIC STRUCTURE OF MARINE ANIMAL POPULATIONS IN THE CALIFORNIA CURRENT

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ABSTRACT

The hope that "biotechnology" will permit the identification of geographic sources of recruitment to most marine animal populations is not well supported either by logic or fact. First, population genetics tells us that dispersal among geographic populations is expected, at equilibrium, to eliminate the very molecular genetic differences that are supposed to permit identification of geographic provenance. Second, studies of allozymes and mitochondrial DNA have clearly shown that fish and invertebrate species with planktotrophic larvae are genetically quite similar over large regions, though not necessarily throughout their whole ranges. Genetic studies are, nevertheless, contributing new insights into the structure and biology of marine animal populations.

One new insight is that sharp genetic subdivisions can occur in continuously distributed species, particularly those spanning biogeographic boundaries. An even more widespread observation is of very slight but significant microgeographic genetic heterogeneity embedded within broad regions of genetically very similar populations. Examples of the latter from the California Current are presented for the barnacle *Balanus glandula* and the northern anchovy *Engraulis mordax*. Microgeographic heterogeneity holds interest for biological oceanographers and fisheries scientists because it contradicts the logic of population genetics as well as commonly held notions about the structure of zooplankton populations. Evidence suggests that genetic heterogeneity on microgeographic scales results from temporal variation in the genetic composition of recruits. This temporal variation could be a consequence of either selection on larval populations or large variance in the reproductive success of individuals, owing to chance matching of reproductive activity with windows of oceanographic conditions conducive to fertilization, larval development, retention, and recruitment. In support of the latter hypothesis, effective sizes for natural oyster populations are estimated to be only small fractions of breeding population numbers. The temporal aspect of population genetic structure forges a strong interdisciplinary bridge to oceanographic research aimed at elucidating the temporally and spatially varying factors affecting recruitment.

RESUMEN

Ni la lógica ni los hechos sustentan la expectativa de que la "biotecnología" permitirá identificar las fuentes geográficas de reclutamiento en la mayoría de las poblaciones de animales marinos. En primeras, la genética poblacional nos indica que en condiciones de equilibrio se espera dispersión entre poblaciones geográficas para eliminar las mismas diferencias moleculares genéticas que supuestamente permitirían la identificación del sitio geográfico de origen. En segundas, los estudios de alocimos y ADN de la mitocondria han mostrado claramente que las especies de peces e invertebrados con larvas planctotróficas son genéticamente muy similares a lo largo de grandes trechos (aunque no necesariamente a través de todo su rango). Sin embargo, los estudios genéticos están contribuyendo conocimientos nuevos de la estructura y biología de las poblaciones de animales marinos.

Una perspectiva nueva es que en especies distribuidas continuamente pueden ocurrir subdivisiones genéticas muy marcadas, particularmente en aquellas especies cuyos rangos incluyen fronteras biogeográficas. Otra observación aun más común es la sutil pero importante heterogeneidad microgeográfica genética que ocurre embebida dentro de regiones más extenidas de poblaciones muy similares genéticamente. Se presentan ejemplos de este último caso para el cirripedio *Balanus glandula* y la anchoveta norteña *Engraulis mordax*. La heterogeneidad microgeográfica es interesante para los oceanólogos biólogos y los investigadores pesqueros debido a que contradice tanto la lógica de la genética poblacional como nociones comunes de la estructura de las poblaciones de zooplancton. La evidencia sugiere que la heterogeneidad genética en escalas microgeográficas resulta de la variación temporal de la composición genética de los reclutas. Esta variación temporal podría ser resultado de selección en poblaciones de larvas o gran varianza en el éxito reproductivo de los individuos. Esto último podría ser debido a sincronización aleatoria de la actividad reproductiva con periodos de condiciones oceanográficas conducentes a la fertilización, desarrollo larval, retención y reclutamiento. En apoyo a esta última hipótesis, se estima que los tamaños efectivos de las poblaciones naturales de ostras son muy pequeños en relación a los tamaños de las poblaciones de criadores. El aspecto temporal de la estructura genética poblacional forja un fuerte vínculo interdisciplinario hacia la investigación oceanográfica que se enfoca en dilucidar los factores dinámicos temporales y espaciales que afectan el reclutamiento.

INTRODUCTION

Identification of the geographic sources of recruits to marine animal populations is important to understanding the coupling of physical and biological processes governing the distribution and abundance of zooplankton (GLOBEC 1991). The notion that genes or gene products might provide inborn markers or tags of provenance has thus proven attractive to oceanographers, particularly in light of the burgeoning promise of biotechnology for precision and accuracy of individual genetic identification (Cullen 1988; Morse 1990; Powers et al. 1990; Incze and Walsh 1991). Origins of recruits to marine animal populations may be more difficult to ascertain than anticipated, however, for both theoretical and empirical reasons.

Migration among populations is a potent, systematic means of homogenizing the gene pools of conspecific populations. For simplicity, assume migrants to a local population i are drawn at random from all other conspecific populations, and that p is the average frequency of an allele at a locus for this species. Over a single generation, then, the change in local allelic frequency, p_i, as a function of p and the proportion of immigrants into the population, m, is given by (Wright 1931):

$$\Delta p_i = m(p - p_i).$$

At equilibrium, $\Delta p_i = 0 = (p - p_i)$, so that allelic frequencies in a local population under constant migration pressure become indistinguishable from those in other conspecific populations. Of course, the effects of gene flow can be modeled more complexly, for example, by taking into account the stochastic effects of finite subpopulation size, the dimensionality and continuity of an organism's distribution, or the effects of selection, but migration remains a potent homogenizing influence unless diversifying selection at a locus is quite strong. For selectively neutral genes, one reproductively successful immigrant in each subpopulation every other generation is enough to maintain cohesiveness of allelic frequencies across all subpopulations (Wright 1931). Thus, marine animals that are either wholly pelagic or have planktonically dispersing larvae (i.e., zooplankton *sensu latu*) are expected to show little genetic differentiation over large geographic areas.

Spatial variance in allelic frequencies is typically quantified by Wright's (1931) standardized variance measure, $F_{ST} = \sigma^2/p(1-p)$, where p is the average frequency of an allele in the total population under consideration, σ^2 is the variance of p among localities within that total population, and the denominator $p(1-p)$ is the maximum variance that would obtain if localities were each fixed for one of the alternate alleles in a ratio of $p:(1-p)$. Most zooplankton species are expected to have $F_{ST} < 0.05$ over large regions.

A substantial number of studies of marine animals have confirmed the expectation of low spatial genetic variance (e.g., Berger 1973; Koehn et al. 1976; Winans 1980; Johnson and Black 1982, 1984; Graves et al. 1984; Watts et al. 1990; reviews or summaries by Burton 1983; Gyllensten 1985; Hedgecock 1986, 1987; Utter and Ryman 1993). Marine fish and invertebrates with planktonic larvae generally maintain very similar allelic frequencies over large regions (500–2000 km) so that geographic variation in allelic frequencies (F_{ST}) typically accounts for only a few percent of the total genetic diversity of these species. Fauna of the California Current are no exception to this generalization (mussels, Levinton and Suchanek 1978; pandalid shrimp, Berthélémy 1978; Dungeness crab, Nelson and Hedgecock 1980, Soulé and Tasto 1983; barnacles, Hedgecock 1982, unpubl. obs.; herring, Grant and Utter 1984; nine species of marine shore fishes, Waples 1987; sardines and anchovies, Hedgecock et al. 1989; sea urchins, Palumbi and Wilson 1990; dover sole, Vetter, data presented at CalCOFI Conference, 1993). With so little genetic variation among geographically widespread populations, it is impossible to ascertain the provenance of population samples (Utter and Ryman 1993), much less of individuals. The hope that detecting individual DNA differences will rectify this is futile because dispersal ensures that all genetic variants are eventually broadly distributed.

However, two qualifications to the general rule that marine animals with planktonically dispersing life stages are genetically very similar over large regions must be discussed. The first qualification is that marine animal species, though genetically very similar over large areas, are not necessarily homogeneous over their entire ranges. Rather, zooplankton species may be genetically subdivided on macrogeographic scales, particularly if they range across boundaries between biogeographic provinces. The second qualification is that very slight but statistically significant and persistent heterogeneities of allelic frequencies have frequently been observed on microgeographic scales, embedded within the large regions over which dispersal maintains an otherwise high level of genetic similarity as described above. Such observations contradict the logic of population genetics that gene flow should prevent such heterogeneity, as well as commonly held notions about population mixing in the sea. Study of the causes of microgeographic variation provides a direct link between population genetics and the ecological and oceanographic processes affecting recruitment.

MACROGEOGRAPHIC SUBDIVISION AND PHYLOGEOGRAPHY

Evidence for genetic divergence among large geographic subpopulations has been reported for many marine animals with planktonically dispersing larvae (Mulley

and Latter 1981a,b; Buroker 1983; Grant and Utter 1984; Avise et al. 1987; Grant et al. 1987; Hedgecock 1987; Ovenden et al. 1990; Palumbi and Wilson 1990; Reeb and Avise 1990; Benzie and Stoddart 1992a,b; Karl and Avise 1992; Macaranas et al. 1992). The degree of subdivision depends on the genetic markers employed, and varies from a small proportion of total genetic diversity to substantial genetic differences suggesting ancient evolutionary separations, warranting in some cases systematic study and possibly taxonomic recognition. Very often the genetic divergence is associated with an obvious barrier to dispersal—land masses, divergent currents systems, impassible basins, etc.—but recent studies have revealed unexpectedly large genetic discontinuities in continuously distributed populations (Reeb and Avise 1990; Karl and Avise 1992; Burton 1994). These discontinuities are sometimes remarkably sharp, evidently reflecting long-standing barriers to dispersal and gene flow, and are often associated with known biogeographic boundaries. The ability to correlate intraspecific genetic variation, particularly DNA sequence divergence, with geography has given rise to a new discipline, phylogeography, which bridges population genetics, systematics, and biogeography (Avise et al. 1987; Avise 1989; Neigel 1994).

Phylogeographic studies of California Current fauna are likely to provide new insight into oceanographic constraints to dispersal across biogeographic boundaries, such as Points Conception and Eugenia (see Burton 1994). However, the depth of population history must be appreciated in these studies. Genetic divergence, which accumulates over an evolutionary time scale, may not necessarily accord well with present physical oceanographic conditions or shorter-term ecological processes.

MICROGEOGRAPHIC HETEROGENEITY—"CHAOTIC PATCHINESS"

An unsolved paradox concerning the genetics of marine animals that disperse by means of planktonic larvae is the occurrence of slight but significant local or microgeographic population structure despite apparently high gene flow (Johnson and Black 1982; Burton 1983). Lack of microgeographic patterning of allelic frequencies among population of the limpet *Siphonaria* sp. led Johnson and Black (1982) to describe this variation as "chaotic patchiness." Two striking examples of this phenomenon in the California Current are provided by allozyme studies of the barnacle *Balanus glandula* (Hedgecock 1982, 1986, unpubl.) and the northern anchovy *Engraulis mordax* (Hedgecock et al. 1989; Hedgecock 1991; Hedgecock et al. 1994).

Balanus glandula is among the most polymorphic of crustaceans that have been analyzed for allozyme variation. The proportion of loci polymorphic in a Bodega Bay, California, population is 19 of 27 loci (70%); the average number of alleles per locus is 2.41; and the average percentage of loci heterozygous per individual is 21.4% (Hedgecock et al. 1982). In order to assess the genetic consequences of larval dispersal—the larval phase of *B. glandula* lasts perhaps up to four weeks in the plankton (Barnes and Barnes 1956; Strathmann 1982; J. D. Standing, pers. comm.)—a survey of the most polymorphic allozymes was made for samples of 17 *B. glandula* populations, mostly from central California but including 1 from Alaska and 3 from the Southern California Bight.

Complete data are available for 5 allozyme loci and ten sampling localities, nine in north-central California and one in the Southern California Bight (table 1). A hierarchical population analysis was made by grouping sampling localities into four regions and calculating spatial variance components and *F*-statistics for comparisons of locality to region (F_{LR}), locality to total (F_{LT} equal to the F_{ST} statistic defined in the Introduction), and region to total (F_{RT}) (table 2A). There is little variance in the frequencies of alleles from north-central to southern California (mean $F_{LT} = 0.023$); variation among regions, which includes population samples from two biogeographic provinces and a substantial divergence of *Got-2* allelic frequencies (cf. locality 10 to the others in table 1), is no greater on average than variation among localities within regions ($F_{RT} = 0.011$, $F_{LR} = 0.012$). A similar analysis for twelve localities, including the Alaska sample and an additional sample from the Southern California Bight, but for only 3 of the 5 loci, gave similar results. Variation among individuals within single, 0.25 m^2 samples accounted for 96% of total genetic diversity in the species, whereas differences among population samples accounted for only 4% of total genetic diversity. On this basis, the population genetic structure of *B. glandula* fits the generalization that geographically distant populations are genetically very similar, most likely because of gene flow via larval dispersal.

Despite this picture of genetic similarity, statistical tests of the homogeneity of allelic frequencies at 4 polymorphic loci reveal slight, but significant differences in allelic frequencies (table 2B), sometimes over short distances (figure 1). As in *Siphonaria*, these slight but significant differences in allelic frequencies have no discernable pattern, and genotypes show no obvious microgeographic clustering in careful mapping studies (Standing and Hedgecock, unpubl.). If gene flow via larval dispersal makes gene frequencies from Alaska to southern California very similar, why does it not produce statistically homogeneous populations on a local or microgeographic level?

On the basis of meristic, morphometric and transferrin-electrophoretic data (Vrooman et al. 1981) and

TABLE 1
Allelic Frequencies for Five Loci in Ten Samples of *Balanus glandula* Populations

	Sampling localities[a]									
Locus	1	2	3	4	5	6	7	8	9	10
Got-1										
N[b]	38	48	31	45	48	45	48	14	47	48
109	.026	.010	.000	.044	.021	.022	.021	.000	.032	.000
104	.092	.104	.113	.100	.146	.156	.094	.000	.117	.083
100	.500	.583	.677	.556	.552	.522	.531	.393	.574	.521
94	.368	.281	.210	.289	.260	.289	.344	.607	.266	.396
89	.013	.021	.000	.011	.021	.011	.010	.000	.011	.000
Got-2										
N	48	48	36	48	48	45	48	90	48	48
104	.708	.615	.736	.625	.573	.578	.615	.539	.677	.135
100	.292	.365	.264	.354	.427	.422	.385	.461	.323	.667
Other[c]	.000	.021	.000	.021	.000	.000	.000	.000	.000	.198[d]
Gpi										
N	48	48	47	48	48	46	48	93	48	48
106	.031	.010	.064	.031	.021	.043	.083	.091	.042	.010
104	.042	.063	.074	.073	.042	.043	.052	.059	.063	.063
100	.490	.479	.457	.354	.521	.413	.438	.425	.469	.302
98	.375	.354	.330	.417	.344	.435	.365	.355	.375	.563
95	.031	.073	.043	.031	.042	.022	.063	.043	.052	.010
93	.031	.010	.021	.063	.031	.043	.000	.027	.000	.042
Other	.000	.010	.011	.031	.000	.000	.000	.000	.000	.010
Mdh										
N	48	48	48	48	48	48	48	92	48	48
106	.000	.000	.000	.000	.000	.000	.021	.011	.000	.000
100	.969	1.000	.969	.990	.958	.948	.969	.957	.958	1.000
95	.031	.000	.031	.010	.042	.052	.010	.033	.042	.000
Mpi										
N	48	48	48	48	48	46	48	92	48	48
110	.094	.083	.073	.073	.073	.141	.063	.076	.052	.073
107	.292	.292	.229	.229	.125	.283	.292	.245	.240	.146
103	.167	.177	.177	.250	.260	.152	.188	.272	.177	.260
100	.354	.313	.250	.344	.323	.326	.292	.337	.396	.365
95	.094	.115	.219	.396	.188	.087	.135	.065	.125	.125
93	.000	.010	.031	.000	.010	.000	.031	.000	.000	.000
Other	.000	.010	.021	.010	.021	.011	.000	.005	.010	.031

[a]Key to sampling localities (all in California): 1, Fort Bragg; 2, Point Arena; 3, Gualala Point; 4, Salt Point; 5, Bodega Harbor jetty, high intertidal; 6, Bodega Harbor jetty, mid intertidal; 7, Bodega Harbor jetty, low intertidal; 8, Bodega Harbor, Gaffney Point; 9, San Francisco Bay; 10, Point Latigo.
[b]Number of individuals studied.
[c]Some rare alleles are pooled as "Other."
[d]A unique *97* allele at *Got-2* was found at this frequency in Point Latigo.

by analogy to concepts of population structure for the California sardine *Sardinops sagax caeruleus* (Radovich 1982), the northern anchovy *Engaulis mordax* is thought to comprise three geographic stocks—a northern population spawning in the Columbia River plume, a central population spawning primarily in the Southern California Bight, and a southern population spawning off of Punta Eugenia and in Magdalena Bay, Baja California Sur. Allozyme and morphometric studies of aged and sexed specimens from the central stock, which were collected by NMFS spawning biomass cruises from 1982 to 1985 (a total of over 3000 fish), revealed substantial genetic polymorphism and morphometric and life-history variation (Hedgecock et al. 1989; Hedgecock 1991). Detailed analyses of the allozyme data and of the morphometric data for the larger collections in 1984 and 1985 are presented elsewhere in this volume (Hedgecock et al. 1994; Nelson et al. 1994).

Like other members of the Clupeiformes that have been analyzed by protein electrophoretic methods, the northern anchovy has substantial levels of genetic variation. In a survey of 39 protein-coding loci, about 40% of the loci were polymorphic, and individuals were heterozygous, on average, at 7.5% of loci (Hedgecock et al. 1989). An initial survey of genetic variation for the 11 most polymorphic loci, among samples taken from Half Moon Bay to Santa Monica Bay in early 1982, revealed a typically small allele-frequency variance (mean $F_{ST} = 0.032$). Nevertheless, log-likelihood ratio tests of the independence of allele-frequencies and locality indicated that 5 loci (*Gpi*, *Hbdh-2*, *Lgg*, *Pgm*, and *Xdh*) had significantly heterogeneous allele-frequencies.

Similar results—low F_{ST} values but statistically significant heterogeneity of allelic frequencies—were obtained in each of four subsequent population surveys made in December 1982 and the winters of 1983, 1984,

TABLE 2
Spatial Variation for Five Loci among Samples from 10 Populations of *Balanus glandula*

A. Variance components and *F*-statistics for hierarchical analysis

X	*Y*	Variance component	F_{XY}
Locality	Region	.02924	.011
Locality	Total	.06023	.023
Region	Total	.03099	.012

B. Contingency chi-square analysis for each locus

Locus	Number of alleles	Chi-square	d.f.	*P*
Got-1	5	38.165	36	0.375
Got-2	3	215.295	18	0.0
Gpi	7	76.091	54	0.021
Mdh	3	24.665	18	0.105
Mpi	7	105.96	54	0.0
Totals		460.176	180	0.0

Variance components are corrected for sampling error. Regions are: (1) northern California coast, north of Russian River (four localities); (2) Bodega Harbor, Calif. (four localities); (3) San Francisco Bay (one locality); (4) Southern California Bight (one locality). Probabilities for contingency chi-square estimated from 1000 Monte Carlo runs of resampled matrix (Zaykin and Pudovkin 1993).

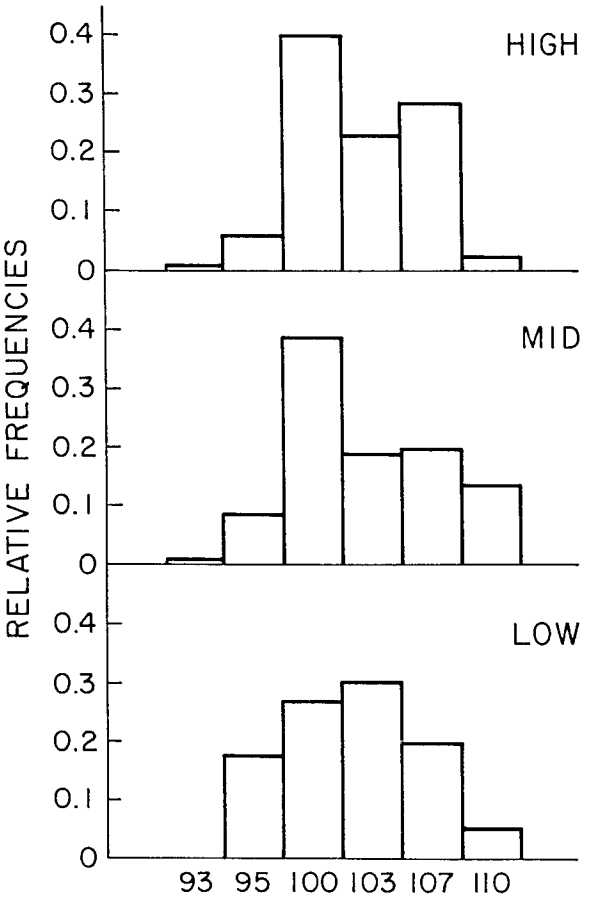

Figure 1. An example of microgeographic genetic heterogeneity in the barnacle *Balanus glandula*. Frequencies of six electrophoretically detectable alleles at the mannose-phosphate isomerase gene, *Mpi*, across an intertidal transect. Samples of 48 individuals were collected from each of high, mid, and low positions in the barnacle zone, spanning about 1 m of intertidal height, on the jetty at Bodega Harbor, California. These samples were collected one year before samples 5–7, table 1, with which they can be compared. Allelic frequencies are very significantly heterogeneous over this local transect (χ^2 = 24.644, 10 d.f., $p < 0.003$ by pseudo-probability method of Zaykin and Pudovkin 1993).

and 1985 (Hedgecock et al. 1994). F_{ST} ranged from only 0.005 to 0.020 in these surveys, somewhat lower than that reported for the early 1982 survey, owing primarily to exclusion of two enzymes, HBDH and XDH, that appeared to be influenced by liver tissue degradation (Hedgecock et al. 1989). Still, in each of the five surveys, 4 or 5 loci show significant heterogeneity of allelic frequencies among samples, although the loci showing this heterogeneity are not necessarily the same from year to year. With the exception of *Idh-1*, which was studied in only four of the five surveys, each locus shows significant heterogeneity of allelic frequencies in at least one survey; conversely, each locus has homogeneous allelic frequencies in at least one survey. Lack of consistency in the loci contributing to heterogeneity, and lack of spatial patterning of allelic frequencies result in a picture of "chaotic patchiness" in the genetic structure of the central stock of northern anchovy.

Genetic differentiation of barnacle populations on a local scale, despite a potential for high gene flow by pelagic larvae, can be explained either by differential survival of genotypes after recruitment or by temporal variation in the genetic composition of recruits. The same two hypotheses might apply to northern anchovy, which can disperse at all life stages, but would require additional hypotheses concerning the long-term cohesion of schools or homing to natal waters. Although differential survival may account for clinal patterns of variation (e.g., Koehn et al. 1980), which may be consistent with environmental gradients and natural selection for appropriate physiological responses, it does not explain well the chaotic patchiness of most microgeographic genetic variation observed to date. On the other hand, temporal variation in the genotypes of recruits has been demonstrated as a cause of microgeographic heterogeneity in limpets (Johnson and Black 1982, 1984) and sea urchins (Watts et al. 1990). These studies show, moreover, that temporal variance in allelic frequencies is exceeded by spatial variance only on scales of several hundreds to thousands of kilometers.

Although Johnson and Black (1984) believe that temporal genetic variance arises from temporally and spatially varying selection on larvae, an alternative explanation is that temporal genetic variance is a by-product of a large sampling error engendered by sweepstakes reproductive success on the part of a minority of individuals (Hedgecock 1994). The high fecundities and mortalities of early life stages of most marine animals create

the potential for a large variance in the number of offspring that individuals contribute to the next generation of reproducing adults. Such variance in reproductive success would, in turn, limit the effective population sizes of these species by several orders of magnitude, according to the relationship (Crow and Kimura 1970; Crow and Denniston 1988):

$$N_e = (4N-4)/(V_k + 2),$$

where N_e is the effective population size, N is the number of breeding adults, V_k is the variance in offspring number per parent, and the population is assumed to be dioecious and demographically stable. Whereas in terrestrial animals V_k is often binomial or Poisson and the ratio N_e/N is nearly 1.0, in marine species V_k may be orders of magnitude larger than binomial or Poisson, and the N_e/N ratio may be a small fraction.

This hypothesis makes two testable predictions. First, random genetic drift, which is a function of the effective population size, ought to be measurable if N_e is limited by large V_k. Second, to the extent that specific cohorts of larvae or new recruits represent the reproductive output of a minority of individuals, they should have less genetic diversity than that which exists in the total adult population. Thus, studies of temporal genetic change in adult populations and of the genetic composition of pelagic larval populations are promising approaches to testing alternative explanations of chaotic patchiness and temporal genetic change in marine animal populations.

TEMPORAL GENETIC CHANGE AND OCEANOGRAPHY

Analysis of temporal genetic change is a powerful means of measuring random genetic drift, estimating effective population numbers, and testing hypotheses about population genetics. The method is particularly robust over intervals of two to ten generations and when N_e is truly finite (Waples 1989) and has proved illuminating in the study of isolated, hatchery-propagated stocks of fish and shellfish (Hedgecock and Sly 1990; Waples and Teel 1990; Hedgecock et al. 1992). Application of the temporal method to natural populations now appears useful in testing the hypothesis that variance in reproductive success limits effective population numbers of many marine animals.

The analysis is based on the inverse relationship between observed temporal change in the frequencies of alleles and the effective size of an isolated population, N_e:

$$E(F) = t/(2N_e) + 1/(2S_0) + 1/(2S_t),$$

where $E(F)$ is the expected variance, owing to random drift of allelic frequencies, between an initial sample (taken without replacement) of S_0 individuals and a second sample of S_t individuals taken (without replacement) after an interval of t generations. Estimates of temporal variance are made from data (Pollak 1983), standardized to eliminate the effect of differences in initial allelic frequencies, and then averaged across loci, weighted by the number of independent alleles at each locus, to yield an estimate, $\hat{F}_K$ of $E(F)$ (see Hedgecock et al. 1992). Rearrangement of this equation yields an estimator, $\hat{N}_K$, of the effective population number:

$$\hat{N}_K = t/(2[\hat{F}_K - 1/(2S_0) - 1/(2S_t)]).$$

The terms $1/(2S_0)$ and $1/(2S_t)$ are harmonic mean sample sizes per locus, weighted by numbers of independent alleles per locus; temporal variance is thus corrected for sampling error.

Temporal genetic analysis has been applied to data from several natural populations of oysters (table 3). The Dabob Bay, Washington, population of Pacific oysters is a semi-isolated, naturalized population, which was established by repeated introductions from Japan over several decades. Mean effective size of this population over a period of 19 years is estimated to be about 400, in contrast to annual harvests on the order of 10^7–10^8 oysters. Estimating temporal variance and effective size for local populations of the American oyster appears to violate a basic assumption of temporal genetic analysis that the population under study be isolated so that immigration plays no role in changing allelic frequencies. Nevertheless, temporal genetic variance over two generations (corrected for sampling error) in three Delaware and Chesapeake Bay localities is as large as or larger than spatial genetic variance along the entire Atlantic seaboard (F_{ST} = 0.029; calculated from data of Buroker 1983). Actual temporal variance for the Chesapeake Bay site, 0.067, is greater than spatial genetic variance over the range of the species, from Canada to Mexico (F_{ST} = 0.039; Buroker 1983). Partial isolation of these oyster populations cannot be explained by immigration and are better explained by random genetic drift in partially isolated estuarine populations maintained by larval retention (cf. Hedgecock 1982). Partial isolation of major estuarine populations would help explain the evolution of local physiological races of oysters (Loosanoff and Nomejko 1951; Hedgecock and Okazaki 1984). Lack of temporal change for the Long Island site may be attributed to relatively greater gene flow into the more oceanic Long Island Sound.

Another major assumption of temporal genetic analysis is that the genetic markers are not affected by natural selection, so that changes of allele-frequencies over time are attributable strictly to random genetic drift. The validity of this assumption for allozymes can be verified in two ways. If allozymes are selectively neutral, then $n\hat{F}/E(\hat{F})$ is distributed as a chi-square variable with n

TABLE 3
Mean Temporal Variances in Allelic Frequencies, F_K, and Estimated Effective Population Numbers, N_K, for Populations of Pacific and American Oysters

A. Pacific oysters *Crassostrea gigas* from Dabob Bay, Washington (after Hedgecock 1994)

t	l	F_K	Sampling variance	Actual variance	lCL	N_K	uCL
1	6	0.0234	0.0114	0.0120	13.4	41.7	218.8
2	11	0.0192	0.0172	0.0020	63.6	511.6	∞
6	5	0.0237	0.0148	0.0089	68.0	336.7	∞
7	5	0.0206	0.0136	0.0070	93.0	501.9	∞
9	6	0.0340	0.0141	0.0199	77.1	226.0	804.7
9.5	6	0.0252	0.0139	0.0113	115.0	418.7	9293

B. Four populations of the American oyster *Crassostrea virginica* sampled two generations apart (after Hedgecock et al. 1992)

Locality	l	F_K	Sampling variance	Actual variance	lCL	N_K	uCL
Long Island	6	0.0158	0.0162	−0.0004	62.3	∞	∞
Delaware Bay	6	0.0424	0.0127	0.0296	13.8	33.8	79.4
Chesapeake Bay	4	0.0974	0.0304	0.0670	4.5	14.9	48.2
James River	6	0.0433	0.0100	0.0333	13.5	30.0	60.8

Interval length in generations is t; l is the number of loci studied; sampling variance is the harmonic mean of sample sizes per locus in the two populations compared; actual variance is F_K minus sampling variance; and lCL and uCL are the lower and upper 95% confidence limits on N_K.

degrees of freedom corresponding to the number of independent loci sampled. Agreement of the observed distribution with the chi-square distribution provides a test of the assumption of selective neutrality, as well as a means for calculating confidence limits on $\hat{N}_K$ (Waples 1989; table 3). An independent test of selective neutrality compares the actual loss of alleles over time to that predicted by population genetic theory assuming $N_e = \hat{N}_K$. Both tests have indicated that temporal genetic change in these oyster populations is caused by random genetic drift (Hedgecock et al. 1992; Hedgecock 1994).

These observations of random genetic drift confirm the first prediction of the hypothesis that variance in reproductive success is large enough in certain marine animal populations to limit effective population numbers to fractions of actual abundance. The observations are also consistent with the studies of Johnson and colleagues, indicating that temporal genetic change is not unusual in marine animal populations. Still, many more temporal genetic studies are needed to confirm the generality of these observations.

A second prediction of the hypothesis is for lower genetic diversity in particular cohorts of larvae or newly recruited juveniles than exists in the spawning adult stock. This prediction may be verified in the future by detailed comparisons of genetic diversities among adults, larvae, and juveniles. Because mitochondrial DNA appears to be predominantly maternally inherited in animals, polymorphisms in this genome may be ideal genetic markers for studies of larval broods. Advances in molecular biology, particularly in the development of enzymatic amplification of DNA by the polymerase chain reaction (PCR), now make possible population genetic studies of marine larvae (Banks et al. 1993), which have not generally been amenable to allozyme analysis. We are presently carrying out a detailed genetic study of oyster larvae in Dabob Bay, an ideal locality because temporally well-separated larval cohorts can be readily identified in plankton samples during a spawning season.

As can now be appreciated from satellite imagery (Roughgarden et al. 1988, 1991), oceanographic processes and conditions that affect the reproduction of marine animal life vary not only among years but also within and among seasons and over mesoscale distances. Temporal and spatial oceanographic variability has been correlated broadly with community structure (Parrish et al. 1981) and more narrowly with overall or regional recruitment success for a variety of taxa (Ebert and Russell 1988; Roughgarden et al. 1988). Nevertheless, the extent to which variability of the marine environment might also enhance variance in offspring numbers among conspecific individuals must now be considered.

To the extent that large variance in reproductive success in marine animals is mediated by oceanographic conditions and processes, there is a strong and direct linkage between population genetics and oceanography. This linkage must be forged if we are to understand broader questions about marine populations, such as their responses to global climate change (Incze and Walsh 1991). At the operational level, detailed studies of genetic diversities within and between cohorts of larvae might provide useful information, for example, on the spatial and temporal dimensions of windows of oceanographic conditions conducive to reproduction and recruitment. Such studies will require sample sizes of thousands of indi-

viduals, however, so that appropriate molecular methods will have to be developed for rapid and efficient processing of population samples. This almost certainly means going beyond the tedious direct sequencing of PCR products in every individual to the application of secondary methods for mass screening of particular nucleotide polymorphisms (e.g., Stoneking et al. 1991) or length variants at simple repeat-sequence loci (Weber and May 1989; Frégeau and Fourney 1993).

Within populations, large V_k might make population responses to selection pressures more complex and indeterminate than is presently appreciated by modelers of population dynamics. On the other hand, adaptive divergence among populations with the potential for gene exchange via dispersing pelagic larvae might be facilitated by a coupling of large V_k with mechanisms of larval retention, as perhaps illustrated by the evolution of physiological races of American oysters along the eastern U.S. seaboard. Finally, speciation in the sea may be more understandable if effective numbers of marine organisms are orders of magnitude smaller than abundance and if marine species are therefore subject to shifting-balance evolutionary processes.

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