Mar. Biotechnol. 2, 449–455, 2000
DOI: 10.1007/s101260000020

MARINE BIOTECHNOLOGY

Comparison of Four Mendelian Loci of the California Sea Hare (*Aplysia californica*) from Populations of the Coast of California and the Sea of Cortez

Mónica Medina* and Patrick J. Walsh

Rosenstiel School of Marine and Atmospheric Science, Division of Marine Biology and Fisheries, National Resource for Aplysia, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, U.S.A.

Abstract: *Aplysia californica* is a species widely used in neurobiology, and specimens are collected from a wide range of places along its distribution range. *A. californica* is endemic to the coast of California and the Gulf of California. On the west coast, this is an unusual distribution range relative to other benthic species from that region. Four polymorphic nuclear Mendelian markers were identified (three single-copy nuclear DNA loci and one microsatellite) for an initial survey of genetic variation of wild populations. F_{ST} values not significantly different from 0 (overall $F_{ST} = 0.0148$) suggest there was no geographic genetic population subdivision in 177 individuals examined.

Key words: *Aplysia californica,* gene flow, mendelian markers, population structure, California coast.

INTRODUCTION

The California sea hare (*Aplysia californica*) is a large opisthobranch mollusk, endemic to the west coast of North America from Northern California to the Gulf of California (MacFarland, 1966). *A. californica* is a species of considerable interest to neurological and biomedical research because it has been widely used as a model organism for the study of learning behavior (Kandel, 1979). Despite the economic and scientific importance of this species, many aspects of its basic biology are not well understood, including the genetic composition and structure of natural populations that serve as source populations for hatcheries and research.

Received December 3, 1999; accepted March 3, 2000.

*Present address: Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543 USA; telephone 508-289-7377; fax 508-457-4727; e-mail medina@evol5.mbl.edu

Biogeographic history has an important effect on genetic structure of benthic marine taxa in most oceans (Avise, 1992; Benzie, 1994). Several marine species with high dispersal capabilities from the Atlantic coast of North America show geographic discontinuities in mitochondrial DNA haplotypes and nuclear genotype frequencies associated with the biogeographic transition zone at Cape Canaveral (Avise, 1992). These observations have been interpreted as evidence for common vicariant events of population separation in the different taxa analyzed (Avise, 1992). Similarly, Point Conception on the coast of California is also known as a major faunal barrier for benthic species (Briggs, 1974; Pielou, 1992). However, no correlation has been found between this biotic transition zone and a phylogeographic break in any of the species examined so far (reviewed in Burton, 1998).

The geographic situation of the Baja California peninsula is somewhat similar to the Florida peninsula. The tem-

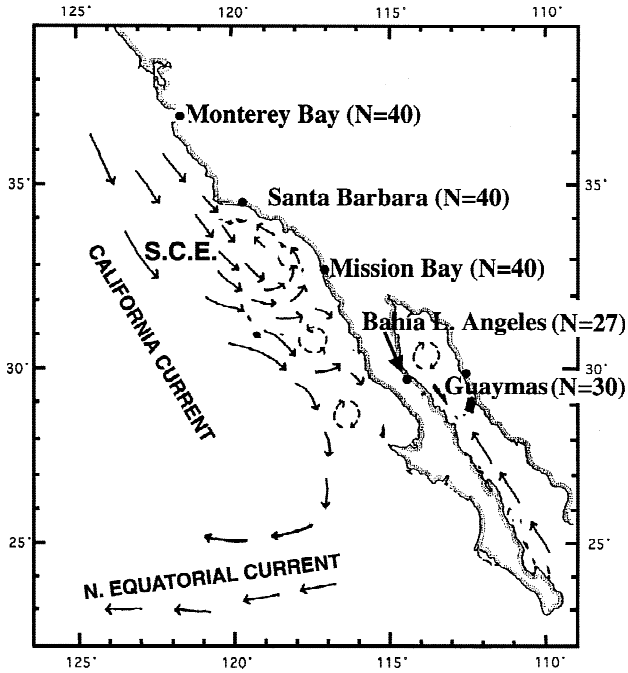

Figure 1. Summer surface circulation patterns in the Northeastern Pacific, modified from Waples (1987). On the Pacific coast, the California current and the Southern California eddy (S.C.E.) are predominant. The S.C.E. develops into the Davidson poleward current in the winter months. In the Gulf of California, there is a northward surface flow in the summer months that shifts to a southward flow in the winter. The five collection locales with the corresponding sample sizes are also depicted on the map.

perate fauna has a southernmost limit on the Pacific coast of the peninsula, whereas the Gulf of California is mainly inhabited by subtropical fauna. *Aplysia californica* is one of the few benthic species that expands its distribution range over the whole Pacific coast and into the Gulf of California. This geographic range makes *A. californica* a suitable experimental species to address questions of genetic isolation due to possible biogeographic barriers in the Northeastern Pacific, such as local circulation patterns, land barriers, and differences in water temperature.

In the Northeastern Pacific, the major circulation components are the California current system (Schwartzlose, 1963), which is mainly made of a southward flow (Figure 1), a seasonal gyre (Southern California eddy), and a seasonal poleward current (Davidson current). At the same time, the Gulf of California shows local and seasonal circulation patterns associated with its geographic orientation (Figure 1) (Alvarez and Schwartzlose, 1979). Some of the recruitment strategies of *Aplysia californica* have been described in detail, and it has been shown that changes in surface circulation affect new recruits (Pennings, 1990, 1991a, 1991b). This species exhibits a semiannual life cycle with a planktonic life stage of approximately 35 days (Kandel, 1979; Pennings, 1991a, 1991b).

The recruitment patterns of this species have been studied in the Channel Islands, where it shows two recruitment peaks (Pennings, 1991a), one in late winter, and a second in the middle of the summer. On the basis of ocean current patterns, Pennings (1991a) proposed that winter recruits come from local populations entrapped in the Southern California eddy, while summer recruits come from Baja California populations. Because reproductive activity is cued by high temperature (Pennings, 1991a), the populations in Baja spawn earlier in the year than the northern populations, and the larvae may be carried northward in the prevailing currents. The populations in the Gulf of California are seasonal: they are present only in late winter and early spring and start to die off as soon as the macroalgae populations decrease (Antonio Rosendiz, Universidad de Baja California, personal communication). Thus, gulf populations of *A. californica* are replaced yearly, probably from populations on the Pacific Coast. The relatively long-lived larval stage of this species can disperse widely with oceanographic currents; however, currents and zoogeographic transition zones can also be significant barriers to gene flow (Waples, 1987; Avise, 1992; Bucklin et al., 1996).

Because *Aplysia californica* is an important research animal and because of its exceptional distribution range relative to other benthic species in the Northeastern Pacific, the goals of this investigation were (1) to determine if there was any population subdivision in *A. californica,* and (2) to examine the role of currents and biogeographic zones in shaping genetic structure in this species.

Materials and Methods

Collection Sites and DNA Isolation

Tissue samples were collected from *A. californica* populations from the coast of California in the summer of 1992, and from the Gulf of California in the spring of 1994 (Figure 1). Total DNA was isolated by standard sodium dodecyl sulfate (SDS)/proteinase K digestion (Sambrook et al., 1989) and resuspended in TE buffer to an approximate concentration of 250 ng/μl.

Nuclear Markers

Two types of single locus Mendelian markers were used in this study: restriction fragment length polymorphism (RFLP) analysis of anonymous single-copy nuclear DNA (scnDNA-RFLP), as described in Karl and Avise (1993), and a microsatellite locus (Tautz, 1989).

Single-Copy Nuclear Markers

A partial DNA library was constructed and screened for low-copy DNA clones, which were then sequenced to develop species-specific primers (Karl and Avise, 1993). *Aplysia californica* genomic DNA was digested to completion with the restriction enzyme *Mbo*I, and DNA fragments of between 500 and 2000 bp were collected by electroelution (Ausubel et al., 1990). The size-selected *A. californica* genomic fragments were cloned into the plasmid pBX (modified pBS, Stratagene), and the ligation mix was transfected into *Escherichia coli* DH5-α. The recombinant plasmids were digested with enzymes flanking the cloning site in the polylinker and examined on agarose gels to verify the presence of DNA inserts.

Copy numbers of the cloned *Aplysia californica* inserts were estimated by slot blot analysis with hybridization to labeled total genomic *Aplysia* DNA as described in Karl and Avise (1993). Approximately 1 μg of each recombinant plasmid was denatured, applied onto nylon membranes (Hybond-N, Amersham) using a slot blot apparatus, and fixed by UV cross-linking. Genomic DNA was labeled, by random priming with deoxygenin (Boehringer-Mannheim), and the filters were hybridized with total *A. californica* DNA. The hybridization signal was detected by chemiluminescence (Boehringer-Mannheim Genius kit), following stringency conditions described in Silberman and Walsh (1992). Low-copy clones (those with little or no hybridization to total DNA) were screened against *Aplysia* mtDNA by slot blot analysis (as described above) to confirm the nuclear nature of the insert. MtDNA was isolated from gonad tissue by differential centrifugation as described in Silberman and Walsh (1992). Twenty-seven single-copy nuclear clones were isolated.

Low-copy or single-copy clones were partially sequenced and then analyzed to select suitable sequence for the design of amplification primers. Primer pairs were designed for nine clones, of which only four primer pairs produced a clean polymerase chain reaction (PCR) product for scnDNA loci and were used in this study (Table 1).

Table 1. Primers for the Four Nuclear Loci Used in This Study

Primer*	Sequence	Size (bp)†
scnDNA		
MP1F	5′-CCACCGCAGTACTGCCTGCAATCACGGAG-3′	600
MP1R	5′-GCATGGCCAGGAGTTGAAGCCATACT-3′	
MP44F1	5′-CAGTTACGGTACAGTTCTATTTGG-3′	1600
MP44R2	5′-CCAAGTCGTACGGACACGATAG-3′	
MP1bF145	5′-GCGGGGGCCTGTTGTAAAAATC-3′	450
MP1bR423	5′-AATGAAGGCTGTGCGAAATAAT-3′	
Microsatellite		
18RF	5′-TCACACCTTCGTCGGCACAAT-3′	300
18RR	5′-GTCGAGAGTTCAGTTCCATCC-3′	

*F indicates forward; R, reverse.
†Approximate fragment size.

Approximately 50 ng of genomic DNA was used for amplification, and PCR parameters varied depending on the primers in use. The conditions were generally an initial step of 2 minutes at 94°C, followed by 30 cycles of 30 seconds at 94°C, 30 seconds of annealing, and 30 seconds at 72°C. The annealing temperature was 50°C for loci *MP1* and *MP44,* and 47°C for locus *MP1B.* Genetic variation was assessed by RFLP analysis of the resulting PCR fragments generated from different individuals with 31 enzymes: *Alu*I, *Ase*I, *Bam*HI, *Bcl*I, *Bgl*II, *Bst*NI, *Bst*UI, *Bst*YI, *Cfo*I, *Dde*I, *Dra*I, *Eco*RI, *Eco*RV, *Hae*III, *Hha*I, *Hin*dIII, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I, *Mbo*I, *Mse*I, *Msp*I, *Nla*III, *Pst*I, *Pvu*II, *Rsa*I, *Sau*96I, *Ssp*I, *Taq*I, and *Xba*I.

Microsatellite Markers

For the development of the microsatellite markers, one of the highly repetitive clones from the partial DNA library (clone 18) was sequenced and two microsatellites were found on each end of the clone. Using the flanking sequence on both ends of the repeat region, amplification primers were developed. PCR conditions were similar to those described above, except for temperature variations in the annealing step. One of the primers was end-labeled by T4 polynucleotide kinase (Pharmacia) with ^{33}P-γ-ATP, and used in PCR under similar conditions as the scnDNA loci (58°C annealing for *MSR18*). PCR products were run on 6% denaturing polyacrylamide gels against an M13 sequence ladder, and polymorphisms were detected in the form of length variation.

Statistical Analysis

Allele frequencies and genetic distances were calculated using BIOSYS Version 1.7 (Swofford and Selander, 1981). All loci were analyzed in a single data set, and the microsatellite locus was subsequently evaluated separately (see below). Descriptive statistics were performed for all loci in the GDA package, Version d10 (Lewis and Zaykin, 1997), using the *F*-statistic analysis to determine the level of population subdivision (Wright, 1965). The F_{ST} parameters used were *F* (F_{IT} = total inbreeding), *f* (F_{IS} = inbreeding within populations) and θ (F_{ST} = inbreeding between populations) (Weir and Cockerham, 1984), which take into account differences in sample size. *F* statistics confidence intervals were calculated by bootstrapping over loci, and mean and standard deviations per locus were calculated by jackknifing over populations (Lewis and Zaykin, 1997). The relationship

$$F_{ST} = 1/(4Nm + 1) \quad (1)$$

was used to estimate the number of effective migrants per generation, where *N* is the local population size and *m* is the migration rate (Slatkin, 1987). Nei's genetic distance between populations was also used as another parameter to look at genetic differentiation (Nei, 1978).

The R_{ST}CALC program (Goodman, 1997) was used to estimate R_{ST} and $(\delta\mu)^2$ for the microsatellite locus (*MS18R*). In this program the R_{ST} estimate incorporates different sample sizes in the calculation. The number of migrants was estimated with equation 1, using R_{ST} as the F_{ST} estimator.

Results

Polymorphic Loci

Three of the successfully amplified scnDNA loci were polymorphic for at least one restriction site. From the microsatellite sequences one polymorphic locus was identified. Table 2 presents allele frequencies for all populations. The observed mean heterozogosities agreed with those expected under Hardy-Weinberg equilibrium for most loci. Confidence intervals for θ (F_{ST}) were not significantly different from 0 (Table 3), which suggests no departure from panmixia (random mating). The overall θ (F_{ST}) value (0.0148) corresponded to *Nm* between populations of approximately 17 migrants per generation. Genetic distances among the different populations were also small (mean Nei's *D* = 0.012, values below diagonal in Table 4).

Table 2. Allele Frequencies for All Populations and All Loci*

Locus and Allele	Population				
	MB	SB	MP	GU	BA
MP1					
(*N*)	40	39	39	30	22
A	0.813	0.846	0.782	0.767	0.909
B	0.125	0.103	0.179	0.150	0.091
C	0.050	0.051	0.026	0.083	0.000
D	0.000	0.000	0.013	0.000	0.000
E	0.013	0.000	0.000	0.000	0.000
MP44					
(*N*)	33	30	28	29	—†
A	0.561	0.683	0.571	0.621	—
B	0.015	0.000	0.000	0.000	—
C	0.379	0.250	0.393	0.328	—
D	0.015	0.000	0.000	0.000	—
F	0.000	0.033	0.000	0.017	—
G	0.030	0.033	0.018	0.000	—
H	0.000	0.000	0.000	0.017	—
I	0.000	0.000	0.000	0.017	—
J	0.000	0.000	0.018	0.000	—
MP1B					
(*N*)	37	37	32	24	21
A	0.892	0.932	0.813	0.854	0.976
B	0.014	0.000	0.000	0.000	0.000
C	0.095	0.068	0.125	0.125	0.000
D	0.000	0.000	0.000	0.021	0.000
E	0.000	0.000	0.031	0.000	0.024
F	0.000	0.000	0.031	0.000	0.000
MS18R					
(*N*)	38	30	28	30	19
285	0.118	0.117	0.232	0.217	0.421
297	0.184	0.167	0.089	0.250	0.158
317	0.684	0.717	0.679	0.483	0.421
325	0.000	0.000	0.000	0.050	0.000
357	0.013	0.000	0.000	0.000	0.000

*First column lists each locus and the corresponding alleles. First line lists sample size for each population. Alleles are represented by letters in the scnDNA loci and by fragment size for the microsatellite.
MB indicates Mission Bay; SB, Santa Barbara; MP, Monterey Point; GU, Guaymas; BA, Bahía de Los Angeles.
†Degraded DNA prevented amplification of locus MP44 in the population from Bahía de Los Angeles.

Microsatellite Locus

Studies have shown that microsatellite loci seem to evolve following a stepwise mutation model (SMM) (Slatkin, 1995; Goodman, 1997); therefore, we gave special consideration

Table 3. *F* Statistics Using the Estimates of Weir and Cockherham (1984) for Each Locus and Overall*

Locus	*f*	*F*	θ
MP1	−0.0537	−0.0528	0.0009
MP44	0.0350	0.0336	−0.0015
MP1B	0.1967	0.2066	0.0123
MS18R	0.1548	0.1873	0.0385
Overall	0.0789	0.0925	0.0148
95% CI†	−0.0101–0.1767	−0.0071–0.1973	−0.0006–0.0326

*$f = F_{IS}$ (inbreeding within populations), $F = F_{IT}$ (individuals within populations-total inbreeding), $\theta = F_{ST}$ (between populations).
†Bootstrapping over loci was used to obtain 95% confidence intervals.

to the microsatellite locus (*MS18R*). In the SMM, alleles can increase or decrease by one unit (Kimura and Ohta, 1978), which is the nature of microsatellites (Estoup et al., 1995). This mutational process can lead to alleles that are identical in size and sequence but do not share a common ancestor. Thus, convergence, parallelism, or reversal to the same allele size (size homoplasy) play an important role in the evolution of repeated DNA loci microsatellites (Estoup et al., 1995). Because size homoplasy can confound identification of alleles identical by descent, new estimates of genetic differentiation parameters have been recently developed for microsatellite loci (Slatkin, 1995; Goldstein et al., 1995) that take into account the mutation process of these repeat regions: R_{ST}, an analogue of the F_{ST} parameter (Slatkin, 1995); and $(\delta\mu)^2$ (Goldstein et al., 1995). Therefore, locus *MS18R* was analyzed separately using the genetic population parameters developed for a locus evolving under a SMM. The R_{ST} (F_{ST} analogue) value was 0.05135, slightly higher than the θ (F_{ST}) estimate for this locus (Table 3). This R_{ST} value corresponded to an *Nm* between populations of approximately 4.5 migrants per generation. We present the $(\delta\mu)^2$ distance matrix in Table 4 (values above diagonal).

Discussion

Benthic invertebrates are either sessile or slow moving as adults. Therefore, their main way of dissemination should be their pelagic larvae. *Aplysia californica* has a relatively long pelagic larval stage (~35 days), which can be prolonged in the absence of a suitable substrate to settle. This ability to extend its pelagic larval life makes *A. californica* capable of long-distance dispersal. The nuclear genome data from this study supported the initial hypothesis of high levels of gene flow in this species (~17 migrants per generation). The microsatellite locus that had the highest F_{ST} (R_{ST}) value (=0.0385) also produced a high *Nm* value (~4.5 migrants per generation). Simulation and empirical studies have shown that high levels of gene flow can act as a strong homogenizing force, and substantial local differentiation will occur only when population sizes are small and the number of migrants (*Nm*) between populations is less than 1 (Slatkin, 1987, 1989). Therefore the preliminary results obtained in this study seem to indicate that *A. californica* is a panmictic species. We can thus conclude that environmental factors (e.g., oceanic currents and geographic distance) do not seem to play an important overall role as barriers to gene flow in *A. californica.* The fact that the Bahía de Los Angeles population is slightly more distinct than any of the other populations (Table 4) may be an indication that some alleles are being retained in certain areas of the gulf despite the high levels of gene flow. This observation, however, could be an artifact caused by a small sample size and the fewer loci sampled; thus, information from additional loci would be necessary to test this hypothesis.

The genetic homogeneity found in *Aplysia californica* is concordant with findings from genetic studies of other benthic invertebrates of the Northeastern Pacific with planktonic larval stages (summarized in Burton, 1998). Thus zoogeographic transition zones seem to have a milder effect on the genetic population structure of species that extend over several zoogeographic regions on the Northeastern Pacific coast when compared with the Northwestern Atlantic coast. *A. californica* larvae are dispersed in a passive manner by the surface currents (Pennings, 1991a). The high levels of gene flow expected from this type of dispersal are supported by the lack of genetic structure in both types of nuclear markers. The genetic homogeneity of *A. californica* throughout its entire geographic range indicates that neither local gyres nor inversion of currents are important barriers to gene flow in this species. Even though it has been shown that several species ranges span over Point Conception with no clear genetic breakup, this study also shows that the Baja Peninsula seems not to be a major barrier to dispersal. Finally, any historic events that could have affected population structure in this species appear to have been overcome by high levels of gene flow. There are important implications of these findings for sea hare workers in the neu-

Table 4. Genetic Distance Measures Between Populations of *Aplysia californica* Based on the Four Loci Surveyed*

	Mission Bay	Santa Barbara	Monterey Point	Guaymas	Bahía de Los Angeles
Mission Bay	—	0.00025	0.01546	0.11314	0.50452
Santa Barbara	−0.0016	—	0.01181	0.10283	0.48248
Monterey Point	−0.0030	0.0047	—	0.04495	0.34333
Guaymas	0.0025	0.0056	0.0046	—	0.13983
Bahía de Los Angeles	0.0380	0.0378	0.0313	0.0123	—

*Negative values are an artifact of the distance estimator and should be considered equivalent to 0 (Nei, 1978).
Nei (1978) distance used below diagonal for all populations. $(\delta\mu)^2$ (Goldstein et al., 1995) distance used above diagonal for all populations, using locus *MS18R* only.

robiology and biomedical fields. Field-collected specimens seem to be part of a panmictic metapopulation; therefore, individuals collected from distant localities will likely be genetically similar.

The allele frequencies of the microsatellite locus included in the study (*MS18R*), was a major factor in the clustering of the Bahía de Los Angeles population separate from the other populations (Table 3). Additional microsatellite loci could potentially give a better understanding of population structure in *Aplysia californica* because of the higher polymorphism at these repeat regions. The four nuclear loci analyzed in this study could be used to provide *A. californica* investigators with a preliminary understanding of the genetic background of hatchery-reared specimens.

Acknowledgments

We are especially grateful to A. Rosendiz and L. Burillón, who collected the Baja California specimens. T. Capo and the National Resource for *Aplysia* provided hatchery animals. J. Silberman assisted in the construction of the genomic library and various laboratory procedures. S. Pennings provided insight throughout the entire project. We also thank K. Rosen, T. Collins, J. Fell, and two anonymous reviewers for their helpful comments on a previous draft of the manuscript. The material in this paper is part of the thesis submitted to the University of Miami, in partial fulfillment of requirements for the Ph.D. in marine biology and fisheries. Support for this project was funded by the National Institutes of Health (RR10294).

References

Alvarez, S., and Schwartzlose, R.A. (1979). Masas de agua del golfo de California. *Cienc Mar* 6:42–43.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (1990). *Current Protocols in Molecular Biology.* New York: Greene-Wiley.

Avise, J.C. (1992). Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos* 63:62–76.

Benzie, J.A.H. (1994). Patterns of gene flow in the Great Barrier Reef and Coral Sea. In: *Genetics and Evolution of Aquatic Organisms,* Beaumont, A.R. (ed.). London: Chapman & Hall, 67–79.

Briggs, J.C. (1974). *Marine Zoogeography.* New York: McGraw-Hill.

Bucklin, A., LaJeunesse, T.C., Curry, E., Wallinga, J., and Garrison, K. (1996). Molecular diversity of the copepod, *Nannocalanus minor:* genetic evidence of species and population structure in the North Atlantic Ocean. *J Mar Res* 54:285–310.

Burton, R.S. (1998). Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution* 52:734–745.

Estoup, A., Tailliez, C., Cornuet, J.M., and Solignac, M. (1995). Size homoplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris. Mol Biol Evol* 12:1074–1084.

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

Goodman, S.J. (1997). RST CALC: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. *Mol Ecol* 6: 881–885.

Kandel, E. (1979). *Behavioral Biology of Aplysia.* New York: W.H. Freeman and Company.

Karl, S.A., and Avise, J.C. (1993). PCR-based assays of mendelian polymorphisms from anonymous single-copy nuclear DNA: tech-

niques and application for population genetics. *Mol Biol Evol* 10: 342–361.

Kimura, M., and Crow, J.F. (1964). The number of alleles that can be maintained in a finite population. *Genetics* 49:725–738.

Kimura, M., and Ohta, T. (1978). Stepwise mutation model and distribution of allelic frequencies in finite populations. *Proc Natl Acad Sci USA* 75:2868–2872.

Lewis, P.O., and Zaykin, D. (1997). Genetic Data Analysis: computer program for the analysis of allelic data. Available at http://chee.unm.edu/gda/.

MacFarland, F.M. (1966). Studies of Opisthobranchiate mollusks of the Pacific Coast of North America: suborder Anaspidea. *Mem Calif Acad Sci* 6:12–32.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590.

Palumbi, S.R. (1995). Using genetics as an indirect estimator of larval dispersal. In: *Ecology of Marine Invertebrate Larvae,* Mc Edward, L. (ed.). Boca Raton, Fla.: CRC Press, 365–387.

Palumbi, S.R. (1996). Macrospatial genetic structure and speciation in marine taxa with high dispersal abilities. In: *Molecular Zoology: Advances, Strategies and Protocols,* Ferraris, J.D., and Palumbi, S.R. (eds.) New York: Wiley-Liss, 101–117.

Pennings, S.C. (1990). Multiple factors promoting narrow host range in the sea hare, *Aplysia californica. Oecologia* 82:192–200.

Pennings, S.C. (1991a). Reproductive behavior of *Aplysia californica* Cooper: diel patterns, sexual roles and mating aggregations. *J Exp Mar Biol Ecol* 149:249–266.

Pennings, S.C. (1991b). Spatial and temporal variation in recruitment of *Aplysia californica* Cooper: patterns, mechanisms and consequences. *J Exp Mar Biol Ecol* 146:253–274.

Pielou, E.C. (1992). *Biogeography.* Fla.: Krieger Publishing Company.

Sambrook, J., Fritsch, E., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory.

Schwartzlose, R.A. (1963). Nearshore currents of the western United States and Baja California as measured by drift bottles. *CalCOFI Rep* 7-1-60 to 6-3-62:15–22.

Silberman, J.D., and Walsh, P.J. (1992). Species identification of spiny lobster phyllosome larvae via ribosomal DNA analysis. *Mol Mar Biol Biotechnol* 1:195–205.

Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science* 236:787–792.

Slatkin, M. (1989). Population structure and evolutionary progress. *Genome* 31:196–202.

Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139:457–462.

Swofford, D., and Selander, R.B. (1981). BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J Hered* 12:281–283.

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res* 16:6463–6471.

Waples, R.S. (1987). A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution* 41:385–400.

Waples, R., and Rosenblatt, H. (1987). Patterns of larval drift in Southern California marine shore fishes inferred from allozyme data. *Fish Bull* 85:1–11.

Weir, B.S. (1996). *Genetic Data Analysis II.* Sunderland, Mass.: Sinauer.

Weir, B.S., and Cockerham, C.C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370.

Wright, S. (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* 19:395–420.