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M. R. Chase · R. J. Etter · M. A. Rex · J. M. Quattro

Bathymetric patterns of genetic variation in a deep-sea protobranch bivalve, *Deminucula atacellana*

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Abstract The origin of the deep-sea benthic fauna is poorly understood and represents an enormous gap in our understanding of basic evolutionary phenomena. One obstacle to studying evolutionary patterns in the deep sea has been the technical difficulty of measuring genetic variation in species that are typically minute, rare, and must be recovered from extreme depths. We used molecular genetic techniques to quantify variation in the 16S rRNA mitochondrial gene within and among populations of the common protobranch bivalve *Deminucula atacellana* (Schenck, 1939). We analyzed 89 individuals from nine samples collected in the 1960s along a depth gradient from 1100 to 3800 m in the western North Atlantic. Genetic variability within populations is much lower than between populations, and peak haplotype numbers occur near the center of its depth distribution. Continental slope (<2500 m) and rise (>2500 m) populations were genetically distinct despite the lack of any obvious topographic or oceanographic features that would impede gene flow. These findings indicate that the deep-sea macrofauna can have strong population structure over small (134 km) spatial scales, similar to that observed in shallow-water and terrestrial organisms. This surprisingly high biodiversity at the genetic level affords the potential for adaptation and evolutionary diversification, the ultimate historical causes of high species diversity in the deep-sea benthos.

Introduction

The deep sea below 200 m covers two-thirds of the Earth's surface and supports extremely high biodiversity, possibly rivaling that of tropical rain forests (Grassle and Maciolek 1992). Intensive precision sampling has revealed diversity that averages 100 macrofaunal species per 0.09 m^2, most of which rely ultimately on sinking phytodetritus for food. Such an unusually high level of species coexistence on these very small scales presents significant challenges to contemporary ecological and evolutionary theory. While much has been learned about spatial trends of deep-sea diversity and their potential ecological causes, the evolutionary processes of population differentiation and speciation that generate this rich and endemic fauna remain largely unexplored. There have been several general and speculative discussions on the evolution of higher taxa in the deep sea (e.g. Sanders 1977; Rex and Warén 1982; Wilson and Hessler 1987), but few attempts to quantify patterns of geographic variation, the primary evidence for evolutionary trends in other environments (Gould and Johnston 1972; Slatkin 1987). The only explicit model of population differentiation in the deep sea (Etter and Rex 1990) is based on patterns of geographic variation in gastropod shell architecture.

To understand evolution in the deep-sea ecosystem, one must address the fundamental question of how populations diverge. What are the mechanisms leading to isolation and ultimately population differentiation and speciation? Palumbi (1994) suggests several possible causes of population structure in widely distributed marine organisms with good dispersal abilities. These include oceanographic and topographic features that limit dispersal, isolation by distance, adaptation to local selective pressures, and unique historical events that allow populations to diverge. There is considerable evidence for these mechanisms in shallow water, but little for their role in the deep sea (see Grassle 1985; Bucklin et al. 1987; France et al. 1992; France 1994; Craddock et al. 1995a, b; France and Kocher 1996b). The lack of data on the genetic structure of deep-sea populations has greatly limited our knowledge of what evolutionary processes are important and over what geographic and bathymetric scales they might operate. Several studies of

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M.R. Chase · R.J. Etter (✉) · M.A. Rex
Department of Biology, University of Massachusetts/Boston, Boston, Massachusetts 02125-3393, USA

J.M. Quattro
Department of Biology, University of South Carolina, Columbia, South Carolina 29208, USA

interpopulation genetic differences have been conducted on large hydrothermal vent species that can be collected live with submersibles, and on common large scavengers in soft-sediment habitats that can be trapped in abundance (Grassle 1985; Bucklin et al. 1987; France et al. 1992; Black et al. 1994; France 1994; Vrijenhoek et al. 1994; Craddock et al. 1995a, b; France and Kocher 1996b). Unfortunately, there remains a paucity of genetic studies on the macrofauna which represent the vast majority of deep-sea species.

There are severe logistical and technical difficulties associated with genetic analyses of macrofaunal species because of their small size (usually ≤1.0 mm), very low abundance and because they are recovered from great depths in small samples, which are bulk sorted to size fractions on board ship and later sorted microscopically in the laboratory. Most species do not survive the extreme temperature and pressure changes that occur as samples are brought to the surface. For those that do, the difficulty and time (weeks to months) required to sort and identify each specimen make it impractical to keep them alive or to freeze the samples. On recovery, samples are typically fixed for 24 to 48 h in 10% buffered formalin and then transferred to 70% ethanol. This method of preservation creates significant problems for molecular genetic analysis because it leads to degradation of proteins and nucleic acids (Karlsen et al. 1994). The effects of fixation coupled with the very small amount of tissue in macrofaunal individuals has made it extremely difficult to obtain usable DNA.

To determine how deep-sea populations are structured, we developed methods to extract, amplify and sequence mitochondrial DNA from formalin-fixed, deep-sea macrofaunal species (Chase et al. 1998). We apply these techniques to document bathymetric patterns of genetic variation within and among populations of the deep-sea protobranch bivalve *Deminucula atacellana*. This species is continuously distributed along a depth gradient (1100 to 3800 m) in the western North Atlantic, occurs throughout the North and South Atlantic, has the potential for wide dispersal (Rhind and Allen 1992), but shows a distinct genetic break between populations above and below 2500 m.

Materials and methods

*Deminucula atacellana (*Schenck, 1939) were collected during 1964 to 1973 along a bathyal gradient (1100 to 3800 m) in the western North Atlantic by the Woods Hole Oceanographic Institution's benthic sampling program (Sanders et al. 1965). We extracted, amplified, and sequenced 196 bp of the 16S rRNA mitochondrial gene from 89 individuals from nine different locations along the depth gradient (Table 1). Individuals from each station were included in the analysis until no new haplotypes were found, i.e. the asymptotic number of haplotypes was obtained. At some stations, this could not be done because of the low number of individuals (e.g. Stn. 62).

To extract DNA, whole individuals were placed in microfuge tubes with 200 µl of tissue lysis buffer ATL, from the QIAamp, tissue extraction kit (Qiagen, Chatsworth, California, USA) and incubated for 24 h at 55 °C. Then 5 µl of a 50 mg ml^{-1} solution of Proteinase K and an additional 95 µl of lysis buffer were added and incubation continued at 55 °C for an additional 72 h (Goelz et al. 1985). The extraction then followed the manufacturer's instructions, except that buffer AL and ethanol were increased from 200 to 300 µl. DNA was eluted with one 200-µl aliquot of 10 m*M* Tris pH 8.0.

Because fresh deep-sea specimens were not available for primer development, we targeted 16S rDNA by designing primers to conserved regions within shallow-water protobranchs. A forward primer (5′-AWR WGA CRA GAA GAC CCT-3′, Proto16F) internal to 16sar (Kocher et al. 1989; Palumbi et al. 1991) was designed by aligning two protobranch bivalve species, *Nucula proxima* and *Solemya velum*. A reverse primer 16R3, 5′-GCT GTT ATC CCT RNR GTA ACT-3′, internal to 16sbr (Kocher et al. 1989; Palumbi et al. 1991) was designed by aligning *N. proxima*, *S. velum* and *Homo sapiens*. These primers [Proto16F and 16R3 (anneal temperature 50 °C)] were used initially to amplify DNA isolated from preserved deep-sea specimens of *Deminucula atacellana*.

These primers were degenerate, required low annealing temperatures, and only worked sporadically. To improve amplification success, we developed species-specific primers for *Deminucula atacellana* by amplifying the whole 16sar and 16sbr fragment (557 bp) in two pieces. A primer internal to Proto16F and 16R3 was designed, Proto16R 5′-CYC YCA GTT GCC CCA ACT MAA-3′ from the DNA sequence of the 16S fragment amplified from *D. atacellana*. Template DNA from *D. atacellana* was amplified with Proto16R and 16sar (anneal temperature 55 °C), resulting in a 326-bp fragment. The fragments were sequenced, aligned and used to re-design a species-specific primer, Demi16F 5′- GAC GAG AAG ACC CTA TTG AGT -3′ in the same area as Proto16F but extending it several bases at the 3′ end to avoid similarity to human DNA. Template DNA from *D. atacellana* was then amplified with Demi16F and 16sbr (anneal temperature 60 °C) and the 330-bp fragment sequenced. Sequences were aligned and used to re-design a primer Demi16R, 5′-GAT TAC GCT GTT ATC CCT RTG-3′, conserved to *N. proxima* and *D. atacellana* in the same area as 16R3.

Developing the species-specific internal primers and working with small (<300 bp) mtDNA fragments were crucial steps in successfully working with formalin-fixed individuals. The species-specific primers Demi16F and Demi16R were used to amplify a 196-bp fragment of DNA from 89 individuals. Individuals were amplified in 50 µl reaction volumes consisting of 10 µl template (no dilution of stock DNA eluted from column), 50 m*M* KCl, 10 m*M* Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 m*M* $MgCl_2$, 0.2 µ*M* each dNTP, 20 pm each primer, 1.0 unit *Taq* (Promega, Madison, Wisconsin I, USA), an equal volume of Taqstart Antibody (Clonetech, Palo Alto, California, USA) and H_20 to 50 µl. Reactions were layered with mineral oil and heated to 95 °C for 2 min prior to five cycles of 95 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min, then 35 to 40 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. After amplification we confirmed and purified PCR (polymerase chain reaction) products by running them out on 1.5% agarose gels and purifying with QIAquick PCR purification kit (Qiagen, Chatsworth, California, USA). PCR products were sequenced with a *Taq* Dye Deoxy Termination cycle sequencing kit (PE Applied Biosystems, Foster City, California, USA), ethanol precipitated and run on an Applied Biosystems Model 373 automated DNA sequencer.

Sequences were aligned with SEQUENCER Version 3 (Gene Codes Corp., Ann Arbor, Michigan). We used modified co-ancestry coefficients calculated from AMOVA (Excoffier et al. 1992) using the algorithms in Arlequin (Schneider et al. 1996) for the genetic distances in our analyses.

We wanted to know whether depth or distance separating samples was more important in explaining any patterns of genetic variation. Previous work on deep-sea organisms indicated that phenotypic (Rex et al. 1988; Etter and Rex 1990; Rex and Etter 1990; France 1993) or genetic (Bucklin et al. 1987; France and Kocher 1996b) divergence among populations can be influenced by both, although changes in depth appear to be much more impor-

tant than horizontal displacement. We used Mantel tests to determine which might be more important in controlling population differentiation in *Deminucula atacellana*.

Results

We found considerable variation in the 16S rRNA locus within and among populations of *Deminucula atacellana*. The sequences are highly related with no gaps in the aligned data (Fig. 1). Among the 89 individuals sequenced, 12 haplotypes were identified with clear bathymetric patterns (Table 1). The most common haplotype (A) occurred at most stations, but showed a depth cline, decreasing in frequency from 1100 to 3800 m (Fig. 2). An unrooted network interrelating the 12 haplotypes (Fig. 3) indicates that most haplotypes can be related to the most commonly observed haplotype (A) by one or two mutations. Haplotype F from samples deeper than 3000 m is an exception, being eight mutations from haplotype A.

A tree based on the genetic distances among different stations (Fig. 4) indicates that the samples from deeper than 3000 m group together and are quite distinct from the shallower stations (< 2500 m). An analysis of molecular variance (AMOVA, Excoffier et al. 1992) indicated that populations above and below 2500 m (hereafter referred to as slope and rise, respectively) differed significantly ($P < 0.0001$). Practically all of the genetic variability surveyed was found between slope and rise populations (the among-region variance component explained 93.79% of the total molecular variance), reflecting both widescale differences in haplotype frequency and comparatively large interhaplotypic distances characterizing the sampled individuals from these two regions.

The large number of substitutions between the most common haplotypes in slope (haplotype A) and rise (haplotype F) populations suggests that the matriarchal lineages characterizing these samples have been isolated for a substantial period of evolutionary time. Although no molecular clock exists for the protobranch bivalve 16S rRNA gene, clocks based on the gastropod genus *Littorina* indicate that rates of sequence divergence average approximately 0.21% transitions and 0.084% transversions per million years (Reid et al. 1996). This clock may not be accurate for protobranchs, so we use it only to provide a very rough estimate of divergence time. Also, we only sequenced 196 bp while Reid et al. (1996) base their calculations on 444 bp. To be conservative, we base our calculations of divergence over 444 bp assuming that the 248 bp we have not sequenced would yield no new substitutions. This is not an unreasonable assumption since we are working with the hypervariable region, and we found few substitutions in the conserved region for those haplotypes in which a larger (500 bp) segment was sequenced. Given these reservations, shallow and deep lineages of *Deminucula atacellana* differ by 1.58% transitions, and 0.22% transversions, suggesting these lineages may have been isolated for 7.5 to 2.6 million years.

The degree of genetic divergence was strongly correlated with differences in both depth and horizontal distance among samples in all possible pairwise comparisons (Table 2; Fig. 5). The partial regression coefficient for geographic distance is higher, suggesting divergence might be more sensitive to absolute distances separating samples. Depth and distance separating samples are highly correlated (Table 2), however, making it difficult to determine which might be more important. Examination of more populations on larger spatial scales will determine the relative importance of depth and distance in explaining population differentiation.

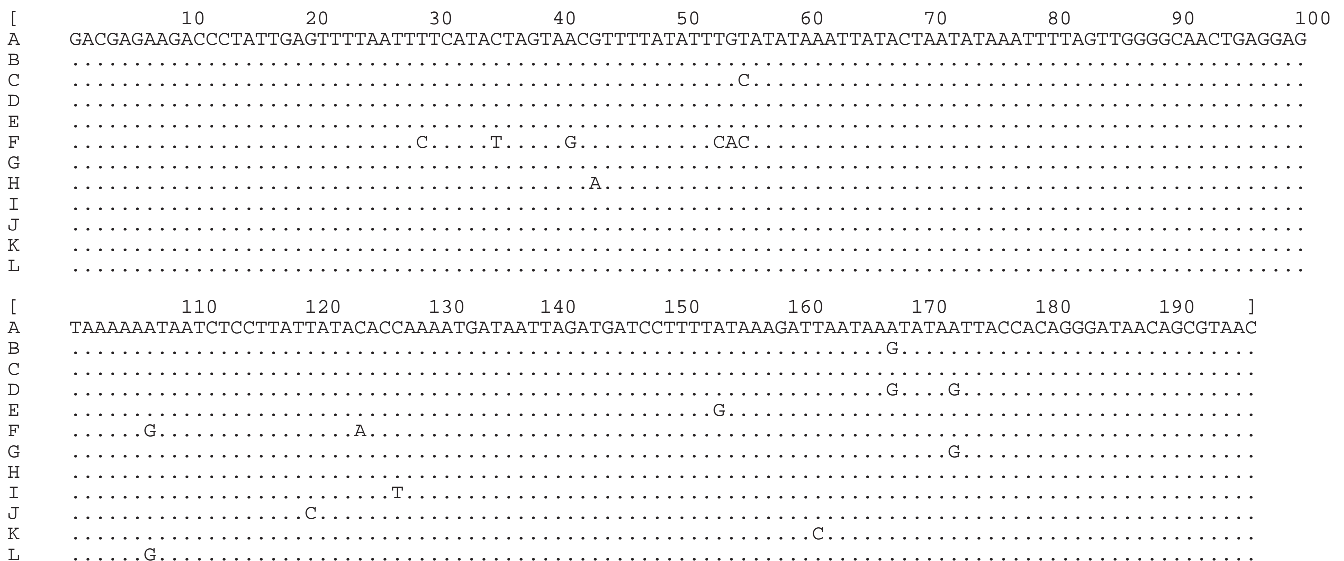

Fig. 1 *Deminucula atacellana*. Partial 16S rDNA gene sequences for the unique haplotypes. Both DNA strands of all unique haplotypes were sequenced to ensure accuracy. Gene Bank accession numbers are AF029093, AF029094, AF029095, AF029096, AF029097, AF029098, AF029099, AF029100, AF029101, AF029102, AF029103 and AF029104 for haplotypes A through L, respectively

Table 1 *Deminucula atacellana*. Sample locality and genetic data for specimens from the western North Atlantic . Station designations are Woods Hole Oceanographic Institution benthic samples. Last row in the table indicates the number of transitions and transversions for each haplotype relative to the most common haplotype A

Station number	Date	Latitude °N	Longitude °W	Depth (m)	Individuals sequenced	Number of haplotypes	Haplotype frequency											
							A	B	C	D	E	F	G	H	I	J	K	L
87	6 Jul 1965	39°48.7′	70°40.8′	1102	11	2	0.91											0.09
73	25 Aug 1964	39°46.5′	70°43.3′	1400	6	2	0.83	0.17										
209	22 Feb 1969	39°47.6′	70°49.9′	1600	14	4	0.71							0.14	0.07	0.07		
103	4 May 1966	39°43.6′	70°37.4′	2022	15	3	0.87	0.07	0.07									
115	16 Aug 1966	39°39.2′	70°24.5′	2040	6	2	0.83	0.17										
62	20 Aug 1964	39°26.0′	70°33.0′	2496	8	4	0.62			0.13	0.13						0.13	
340	24 Nov 1973	38°14.4′	70°20.3′	3305	4	1						1.00						
77	30 Jun 1965	38°00.7′	69°16.0′	3806	17	1						1.00						
85	5 Jul 1965	37°59.2′	69°26.2′	3834	8	2						0.88	0.13					
Transition/Transversion							0/0	1/0	1/0	2/0	1/0	7/1	1/0	1/0	1/0	1/0	1/0	1/0

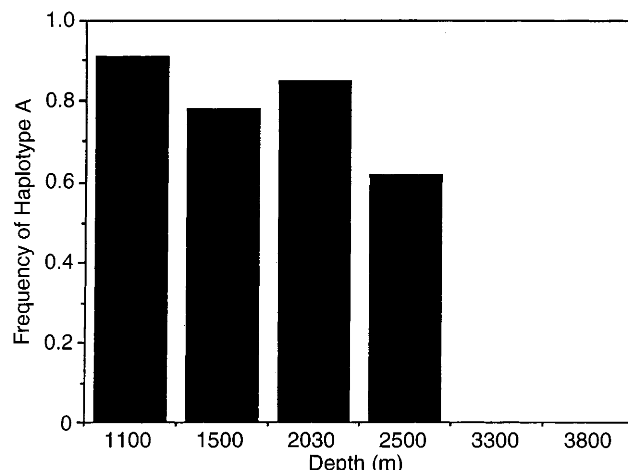

Fig. 2 *Deminucula atacellana*. Frequency of haplotype A for the 16S mtDNA fragment as a function of depth. Data from some stations were combined: 1500 m, Stns. 73 and 209; 2030 m, Stns. 103 and 115; 3800 m, Stns. 77 and 85

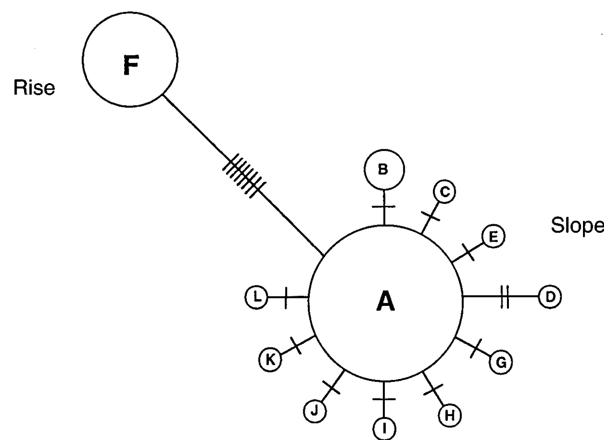

Fig. 3 *Deminucula atacellana*. An unrooted network indicating interrelationships among 12 haplotypes for the 16S mtDNA derived from 89 individuals along a depth gradient of 1100 to 3800 m. Each haplotype is represented by a circle. Area of the circle is directly proportional to the number of individuals possessing that haplotype. Length of the line connecting haplotypes is directly proportional to the number of substitutions separating the haplotypes. Number of substitutions is indicated by slashes across each line

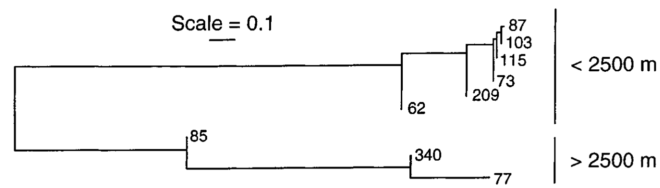

Fig. 4 *Deminucula atacellana*. Tree based on modified coancestry coefficients showing the relative genetic distances among samples. Note that stations above 2500 m group together and are separate from those deeper than 2500 m

Table 2 *Deminucula atacellana*. Results of Mantel tests which are based on 10 000 replications. Genetic distances are modified coancestry coefficients. Geographic distance and Depth difference are, respectively, the horizontal and vertical distances separating samples

Comparison	R^2	P-level
Genetic distance × Geographic distance	0.7328	33/10000
Genetic distance × Depth difference	0.5770	47/10000
Geographic distance × Depth difference	0.5730	45/10000

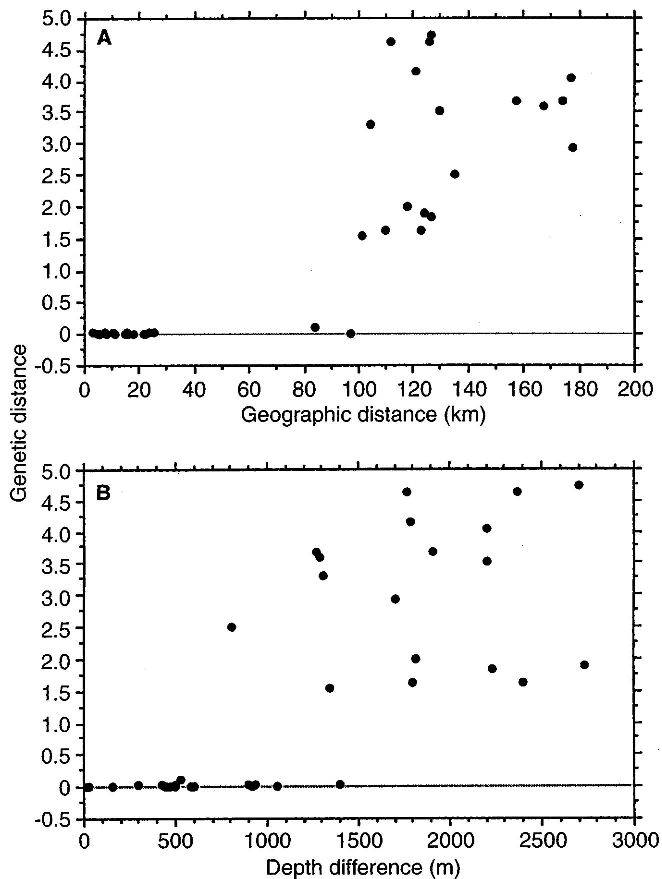

Fig. 5 *Deminucula atacellana*. Relationship between genetic distance and **A** geographic distance separating samples, or **B** depth separating samples. Mantel tests (see Table 2) were used for statistical analyses

Discussion and conclusions

The strong divergence between slope and rise populations on such a small scale (134 km) raises specific questions about the mechanism(s) leading to the differentiation among *Deminucula atacellana* populations. *D. atacellana* is widely distributed in both the North and South Atlantic and appears to have pelagic larvae that develop in the near-bottom waters (Rhind and Allen 1992). This potentially permits extensive long-distance dispersal and concomitant gene flow in cold deep currents. To maintain such an extensive geographic and bathymetric distribution, *D. atacellana* must be dispersing widely. Current velocities at these depths vary between 1.5 and 40 cm s^{-1} (Hogg 1983; Saunders 1983; Schmitz and McCartney 1993). Using a median value of 20 cm s^{-1} suggests that larvae passively dispersing in these currents would drift approximately 17 km d^{-1}. Even if larvae spend only a few days in the water column, populations separated by 134 km should experience extensive gene flow. The deep currents are also complex and well mixed in this region of the western North Atlantic with north, south, east, and west flows (Hogg 1983; Schmitz and McCartney 1993), suggesting that there is no obvious hydrodynamic reason why larvae could not disperse among the slope and rise stations. Given the high potential for migration coupled with the absence of any obvious topographic or oceanographic features that would prevent dispersal, it is surprising that such genetic differences exist over the small bathymetric (800 m) and geographic (134 km) scales separating these populations.

Others have also found strong bathymetric divergence in deep-sea organisms with good dispersal potentials (Bucklin et al. 1987; France and Kocher 1996b), but these were either on a much larger scale (among ocean basins) or involved comparisons among populations where topographic or oceanographic features probably influence dispersal. For example, Bucklin et al. (1987) documented allozyme variation in the amphipod *Eurythenes gryllus* around the Pacific seamount Horizon Guyot and found that basin and peak populations separated by 80 km horizontally and 3700 m vertically differed. They were unable to determine whether the difference was due to selection or limited gene flow, but current patterns are highly influenced by seamounts (Haidvogel et al. 1993) and may tend to limit dispersal between populations at different depths, especially in this case where the populations are separated by 3700 m. France and Kocher (1996b) quantified variation in mtDNA (16S rRNA gene) among *E. gryllus* from both the Atlantic and Pacific, including samples from the slope and base of the Horizon Guyot seamount. Samples from similar depth regimes tended to group together phylogenetically even if they were from different oceans, indicating that bathymetric variation is much greater than geographic. The bathymetric variation in *E. gryllus*, however, represents a much larger geographic scale compared to that observed in *Deminucula atacellana*. The genetic divergence found in *E. gryllus* between depth regimes was primarily among samples from different ocean basins within broad geographic regions (e.g. Northwest Atlantic), whereas *D. atacellana* exhibits similar levels of divergence within a single basin. The only exception to this for *E. gryllus* is the Horizon Guyot samples, which again exhibited strong bathymetric divergence.

How did the slope and rise populations of *Deminucula atacellana* diverge? One possible explanation is that

the distance separating these populations is sufficiently large to prevent or minimize gene flow. However, the distance is relatively small, less than 150 km, and the larvae of *D. atacellana* are thought to disperse widely (Rhind and Allen 1992) as noted above. Even if the larvae of a single individual dispersed only 10 km, a stepping-stone model should easily permit gene flow over these distances. Although we can not rule out isolation by distance, it seems unlikely given the dispersal potential of pelagic larvae and the current velocities and patterns in this region.

Producing larvae that have the potential to disperse over large distances does not ensure high levels of gene flow or homogeneous population structure (Hedgecock 1986; Jackson 1986; Reeb and Avise 1990). Isolation of gene pools may occur because the populations experience strong local selective pressures that prevent the successful exchange of individuals (e.g. Hilbish and Koehn 1985). For example, the depth gradient itself may act as a novel isolating mechanism in the deep sea because of the effects of hydrostatic pressure on the structure and function of enzymes (Hochachka and Somero 1984; Somero 1990). Populations of *Deminucula atacellana* living at such extremes of depth may require different enzymes as adaptations to pressure regimes, and this may prevent larvae from dispersing between slope and rise populations, effectively isolating these gene pools. Several studies have recently shown that larvae of some echinoderms living below 2000 m cannot develop above 2000 m, indicating that larval development may be sensitive to pressure (Young et al. 1995, 1996a, b). Because enzymes and proteins differ in their sensitivity to hydrostatic pressure (Hochachka and Somero 1984; Somero 1990), this potential isolating mechanism could operate throughout the entire depth range, and species may differ in their responses. Palumbi (1992) recently formulated an intriguing hypothesis that small mutations that alter proteins on the surface of sperm and eggs may act as isolating mechanisms in marine systems. In addition to genetic mutations, pressure may alter the three-dimensional structure of these proteins leading to incompatibilities between sperm and eggs, and ultimately reproductive isolation.

The genetic differences may also reflect local selective pressures favoring particular haplotypes. Sequence variation in mtDNA is typically thought to be neutral, but there is evidence for fitness differences among mtDNA variants in *Drosophila* spp. (Rand et al. 1994). Numerous potentially important selective agents vary with depth, including sediment characteristics (MacIlvaine and Ross 1979; Etter and Grassle 1992), hydrostatic pressure (Hochachka and Somero 1984), the nature and rates of nutrient input (Pace et al. 1987; Altabet et al. 1991; Turley et al. 1995) and the nature and intensity of biotic interactions (Rex 1983; Rex et al. 1997). Some of these have been shown to be important in producing population structure in other environments (reviewed by Palumbi 1994), but more research will be necessary to quantify their role in population differentiation of *Deminucula atacellana*.

Another interpretation for the large number of substitutions between slope and rise haplotypes may be that *Deminucula atacellana* represents two species occupying different depth regimes. The level of differentiation is in the lower range of what has been found for 16S mtDNA between molluscan congeners (Geller et al. 1993; ÓFoighil et al. 1995; Reid et al. 1996). However, we were unable to identify any conchological differences among individuals representing the different haplotypes, and previous taxonomic work concluded that the slope and rise populations are one species (Rhind and Allen 1992). In addition, some shallow-water mytilids exhibit inter-population genetic differences in 16S that far exceed those observed in *D. atacellana* (Geller et al. 1993). Since protobranch internal anatomy is highly conserved, sometimes with no detectable differences among species (Rhind and Allen 1992), patterns of genetic differentiation should prove especially useful for discerning evolutionary relationships.

It is interesting that the number of haplotypes appears to be highest between 1600 and 2500 m (Table 1). This is approximately where species diversity is greatest in protobranchs (Rex 1981) as well as in the benthic community as a whole (Rex 1981; Etter and Grassle 1992). The abiotic environment is also extremely heterogeneous (MacIlvaine and Ross 1979), has the greatest variability in sediment characteristics (Etter and Grassle 1992) and is transected by numerous submarine canyons. The biotic and abiotic environmental heterogeneity at mid-bathyal depths may act to maintain high genetic variation. This is also where *Deminucula atacellana* is most abundant and may simply reflect the large population size at intermediate depths. Whatever the mechanism for causing or permitting this variation, it is clear that deep-sea species can have reservoirs of high genetic variability that are available to be shaped and transformed geographically, and may provide the potential for speciation.

Our work indicates that formalin-fixed material can now be used to address fundamental evolutionary problems in typical deep-sea macrofauna, and presumably in collections of taxa from other habitats that have been preserved in a similar fashion. These techniques, along with others (Bucklin 1992; France and Kocher 1996a; Shedlock et al. 1997), will make it possible to use the extensive archived museum collections of deep-sea material to study evolutionary problems at the genetic level. Our protocols are designed to work with very small organisms (<1 mm) and to efficiently obtain DNA sequences from numerous individuals for population-level studies. To demonstrate that our procedures work across taxa, we have also extracted, amplified (via PCR), and sequenced 16S rDNA from multiple individuals of eight protobranch bivalves (*Deminucula atacellana*, *Ledella ultima*, *Malletia abyssorum*, *Tindaria callistiformis*, *Malletia estheri*, *Malletia obtusa*, *Nuculoma similis*, *Nuculoma granulosa*) and seven gastropod

species (*Frigidoalvania brychia*, *Sipho caelatus*, *Benthonella tenella*, *Benthomangelia antonia*, *Onoba pelagica*, *Pusillina harpa*, *Xyloskenea naticiformis*) collected more than 30 years ago from depths of 100 to 5000 m. For some of these species, two regions of the mtDNA, the large-subunit ribosomal RNA gene (16S rRNA) and cytochrome *b* (Cyt *b*), as well as 270 bp of the 28s nuclear gene have been successfully amplified and sequenced allowing us to explore multiple regions of the nuclear and cytoplasmic genome. However, working with nuclear DNA is more problematic and less consistent.

A fundamental question that has long perplexed marine ecologists is why species diversity in the deep sea is greater than in similar shallow-water habitats (Sanders 1968; Gage and Tyler 1991; Gage 1996). An equally perplexing and perhaps more fundamental question is how such a rich and highly endemic fauna evolved. This question is especially intriguing given the potential wide dispersal ability of most organisms and the lack of obvious isolating barriers. Molecular methods are powerful tools to elucidate basic features of population structure and the potential roles of dispersal, geography, and the selective regime in generating this structure. Genetic analyses of the kind presented here will enable us for the first time to formulate clear alternative hypotheses about evolutionary processes in the deep-sea macrofauna that focus on specific geographic regions and the physical and biotic features associated with these regions, and to test these hypotheses in a comparative way by using taxa with different life-history features and distributional patterns. Because of the remoteness of the deep sea and the extremely small size of most of its inhabitants, molecular genetics may provide the only tools to quantify the role of various evolutionary processes in population differentiation and speciation in this vast and complex ecosystem.

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