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Bathymetric and geographic population structure in the pan-Atlantic deep-sea bivalve *Deminucula atacellana* (Schenck, 1939)

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Abstract

The deep-sea soft-sediment environment hosts a diverse and highly endemic fauna of uncertain origin. We know little about how this fauna evolved because geographic patterns of genetic variation, the essential information for inferring patterns of population differentiation and speciation are poorly understood. Using formalin-fixed specimens from archival collections, we quantify patterns of genetic variation in the protobranch bivalve *Deminucula atacellana*, a species widespread throughout the Atlantic Ocean at bathyal and abyssal depths. Samples were taken from 18 localities in the North American, West European and Argentine basins. A hypervariable region of mitochondrial 16S rDNA was amplified by polymerase chain reaction (PCR) and sequenced from 130 individuals revealing 21 haplotypes. Except for several important exceptions, haplotypes are unique to each basin. Overall gene diversity is high ($h = 0.73$) with pronounced population structure ($\Phi_{ST} = 0.877$) and highly significant geographic associations ($P < 0.0001$). Sequences cluster into four major clades corresponding to differences in geography and depth. Genetic divergence was much greater among populations at different depths within the same basin, than among those at similar depths but separated by thousands of kilometres. Isolation by distance probably explains much of the interbasin variation. Depth-related divergence may reflect historical patterns of colonization or strong environmental selective gradients. Broadly distributed deep-sea organisms can possess highly genetically divergent populations, despite the lack of any morphological divergence.

Keywords: bathymetric patterns, deep sea, dispersal barrier, isolation by depth, mid-Atlantic Ridge, population genetic structure

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Introduction

The high species diversity of the deep-sea fauna (Hessler & Sanders 1967) must reflect the culmination of both ecological and evolutionary processes. Contemporary research in the deep sea centres primarily on the ecological mechanisms that shape geographic and bathymetric patterns of diversity (Etter & Mullineaux 2001; Levin *et al*. 2001; Snelgrove & Smith 2002; Rex *et al*. 2005). Virtually nothing is known about how this rich and highly endemic fauna evolved. For most of the deep ocean (below 200 m), we lack basic information on the spatial and temporal scales of population differentiation and the forces that might lead to population divergence and ultimately speciation. To elucidate how the deep-sea fauna evolved, we need to quantify geographic and bathymetric patterns of genetic variation across a variety of scales.

Genetic studies of deep-sea organisms have focused mostly on species living at patchy chemosynthetic habitats (e.g. Vrijenhoek 1997; Baco *et al*. 1999; Van Dover *et al*. 2002; Goffredi *et al*. 2003). Less is known about population structure of species inhabiting the continuous and far more extensive soft-sediment habitats of bathyal and abyssal depths. On regional scales, sediment-dwelling species tend to show more differentiation with depth than with horizontal separation. For example, genetic variation was low in a brittle star from the Rockall Trough (Hensley *et al*. 1995) and in a trochid gastropod (Siebenaller 1978) and brooding amphipod among five basins of the California Continental Borderland (France 1994). The latter study

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however, found that individuals from a deeper sixth basin differed substantially, possibly representing a cryptic species. Several other studies on various mollusks at bathyal depths within the North American basin (Chase *et al*. 1998a; Etter *et al*. 1999, 2005; Quattro *et al*. 2001) and on echinoderms in the North Atlantic (Doyle 1972; Howell *et al*. 2004), found strong genetic variation over surprisingly small distances, but this was usually associated with differences in depth.

Larger-scale studies of population structure in the deep sea remain rare, but suggest population differentiation may decrease with increasing depth (Etter *et al*. 2005). A global-scale analysis (Pacific, Atlantic and Arctic oceans) of the cosmopolitan amphipod (*Eurythenes gryllus*) found little divergence at abyssal depths, but considerable divergence at bathyal depths, varying enough among ocean basins perhaps to merit recognition as cryptic species (France & Kocher 1996). Similarly, two abyssal holothurians on either side of the Atlantic Ocean exhibited very low genetic variation (Bisol *et al*. 1984). In contrast, populations of the upper-bathyal (200–2000 m) benthopelagic fish *Helicolenus dactylopterus* on either side of the North Atlantic, and at different latitudes (15°N compared to 30–40°N) within the eastern North Atlantic were genetically distinct (Aboim *et al*. 2005).

Recent work quantifying genetic variation in several deep-sea organisms (France & Kocher 1996; Chase *et al*. 1998a; Etter *et al*. 1999, 2005; Quattro *et al*. 2001) suggests that many species defined by morphological criteria, may in fact be comprised of multiple cryptic species. This may be especially true for species with broad (e.g. pan-Atlantic) distributions. Geographic ranges of many deep-sea species encompass entire oceans and in some cases are found in multiple oceans. If these cosmopolitan species defined on morphological grounds are valid, an important question arises: How are they maintained? The geographic scales are enormous and the low fecundity (Gage & Tyler 1991), limited dispersal potential and relatively slow deep-ocean currents (Hogg 1983; Schmitz & McCartney 1993) make it difficult to envision how gene flow can be sufficient to retard population divergence.

To identify the scales of population structure and explore what forces might contribute to population differentiation, we quantify geographic and bathymetric patterns of genetic variation (mitochondrial 16S rDNA) in the widely distributed deep-sea protobranch bivalve, *Deminucula atacellana* (Schenck, 1939). This bivalve exhibits strong genetic divergence between North and South Atlantic populations, but little divergence between populations from the eastern and western North Atlantic. Populations at similar depths but separated by large geographic distances are genetically homogeneous, while those geographically proximal but from different depths are highly divergent.

Materials and methods

Source material and sequence generation

A major challenge in studying population genetics in the deep sea is to obtain material that can be used for genetic analysis. This is especially true for macrofauna that are extremely small, difficult to separate from sediment, and typically fixed in formalin, which degrades DNA. Because macrofaunal organisms comprise the majority of species in the deep sea and are difficult to collect fresh, we developed methods to extract, amplify and sequence DNA from formalin-fixed tissues (Chase *et al*. 1998a, b). This allowed us to use archival material collected several decades ago despite being fixed in formalin.

Preserved specimens of *Deminucula atacellana* were obtained from the Woods Hole Oceanographic Institution (Woods Hole, MA), the Minerals Management Service of the United States Department of Interior (Washington, D.C.) and the Dunstaffnage Marine Laboratory (Oban, Scotland). The material was collected by box core and epibenthic sled as early as 1964 (Sanders *et al*. 1965; Allen & Sanders 1996) and as recently as 1985 (Maciolek *et al*. 1987a, b). Samples were fixed in formalin immediately upon collection for 24 h and transferred to ethanol for storage. We extracted, amplified and sequenced DNA from 130 specimens collected at 18 localities in the Argentine, North American and West European basins (Fig. 1) at depths of 1102 to 3912 m.

DNA was extracted with extended proteinase-K digestion using the QIAamp Tissue Extraction Kit (QIAGEN) as described in Chase *et al*. (1998a, b). A pair of previously designed species-specific primers (Chase *et al*. 1998a; Boyle *et al*. 2004) were used to target a hypervariable region of the 16S gene for *D. atacellana* and a confamilial species, *Nucula proxima*. The forward primer, Demi16F (5′-GACGAGAAGACCCTATTGAGT-3′), was paired with the reverse, Demi16R (5′-GATTACGCTGTTATCCCTRTG-3′) to yield a ~200-bp fragment of the 16S gene. Each sample was amplified by polymerase chain reaction (PCR) in a microcentrifuge tube using a reaction mix of 10 μL of template DNA (undiluted from extraction), 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.2 μL each dNTP, 20 pM each primer, 1.0 U *Taq* (Promega), an equal volume of *Taq*Start Antibody (Clontech) and H_2O to a final volume of 50 μL. Reactions were layered with mineral oil and heated in a thermal cycler to 95 °C for 1 min followed by five cycles of 95 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min, then 35–40 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s. Negative and positive controls were included with each round of reactions. PCR products were verified for size conformity by gel electrophoresis (1.5% agarose) and purified using the QIAquick PCR Purification Kit (QIAGEN).

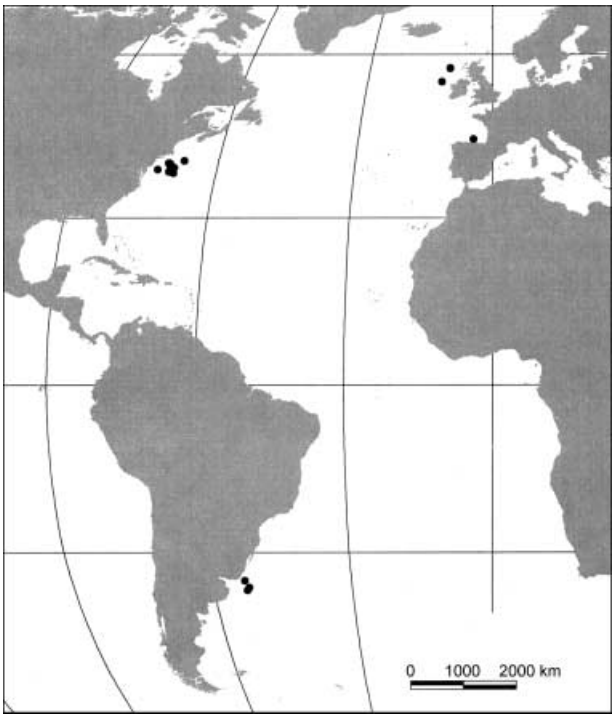

Fig. 1 Sampling localities (filled circles) for the bivalve *Deminucula atacellana* in the North and South Atlantic. See Boyle *et al*. (2004) for station coordinates, depth, and sampling date.

Purified products were sequenced with a *Taq* Dye Deoxy Termination cycle sequencing kit (PE Applied Biosystems), ethanol precipitated and run on an Applied Biosystems Model 373 automated DNA Sequencer. In almost all instances PCR products were directly sequenced but three specimens from the Argentine basin yielded multibanded or very weak product in PCR. The multiband products were separated by gel electrophoresis and bands of the appropriate size excised and gel purified using a QIAquick Gel Extraction Kit (QIAGEN). Gel purified and weak PCR products were then inserted into a bacterial vector for amplification through cloning using the pGEM-T Easy Vector System (Promega). The bacterial plaques generated were screened by PCR for inserts of the correct size and at least three clones for each sample, positive for the insert, were sequenced and aligned to verify the product. To reduce chances of accepting errors introduced through PCR and cloning, consensus sequences were generated for all samples through alignment of both sense and antisense products. Ambiguous sequences were discarded from the analysis. Sequences were aligned using SEQUENCHER™ version 3.1 (Gene Codes Corp.) and unique haplotypes were identified.

Sequence analysis

ARLEQUIN 2.0 (Schneider *et al*. 2000) was used to calculate haplotype (h) and nucleotide (π) diversity, and to estimate levels of population structure within species by analyses of molecular variance (AMOVA) (Excoffier *et al*. 1992). Levels of intraspecific divergence were estimated from pairwise genetic distances (Φ_{ST}) among samples. For the AMOVA, stations were grouped by depth (above or below 3000 m), basin (Argentine, N. American or W. European) or both. PAUP* 4.0b10 (Swofford 2000) was used to construct a neighbour-joining tree depicting relationships among samples based on pairwise genetic distances (Φ_{ST}) between stations. Because genetic divergence among deep-sea organisms can be influenced by both the geographic distance and depth separating samples, we used partial Mantel tests to estimate the relative strength of each in explaining spatial patterns in genetic variation. The partial Mantel is similar to a partial correlation, allowing one independent variable to be held constant while evaluating the explanatory power of the other. The relative importance of depth (depth differences among stations) and geographic distance (great-circle distance among stations) in explaining spatial patterns of genetic divergence were tested with PARTIAL MANTEL (R-PACKAGE, version 4.0, Legendre & Vaudor 1991). All haplotypes were identified among the samples and their phylogenetic relationships estimated via a maximum-parsimony network using the program TCS 1.13 (Clement *et al*. 2000). The association of genetic structure with geography was tested in a nested clade analysis using GEODIS version 2.2 (Posada *et al*. 2000) following methods of Templeton *et al*. (1995) and Templeton (1998, 2004).

Results

Sequences were obtained for 130 individuals of the bivalve *Deminucula atacellana*. Success in DNA amplification and sequence production varied among the samples by locality. In most instances, PCR product and sequence data were obtained either from all specimens from a given station or from none. Because sequences were obtained from localities at the extremes of the geographic range and at intermediate sampling sites, sampling techniques or storage conditions by lot probably account for this variation. Inadequate complimentarity of the oligonucleotide primers remains a possibility, but when samples failed, we also attempted amplification of smaller fragments with highly conserved 'Universal' primers. Because this also failed, we suspect the failures reflect collection or preservation deficiencies. For a detailed report of success and failure by station, see Boyle *et al*. (2004). Twenty-one haplotypes were found (GenBank Accession nos AF029093–AF029104 and DQ269458–DQ269466) generally clustering into the Northern and Southern Hemisphere or above and below 3300 m (Table 1). Genetic diversity measured across stations and basins (Table 2) revealed high overall gene diversity (h = 0.731) and nucleotide diversity (π = 0.022). We obtained the greatest number of sequences (n = 94) and haplotypes

Table 1 Geographic and genetic data for specimens of *Deminucula atacellana* from three basins of the Atlantic Ocean. The number of transitions and transversions for each haplotype relative to the most common haplotype (A) is given in the bottom row. Asterisks in the bottom row indicate haplotypes with a deletion relative to A

Basin	Station	Depth (m)	Individuals sequenced	Number of haplotypes	Haplotype frequency																				
					A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
N. American	87	1102	11	2	10											1									
	73	1400	6	2	5	1																			
	209	1500	14	4	10							2	1	1											
	103	2022	17	3	15	1	1																		
	115	2040	6	2	5	1																			
	MMSM3	2055	2	1	2																				
	MMSN8	2180	1	1	1																				
	62	2496	8	4	5			1	1						1										
	340	3310	4	1						4															
	77	3806	17	1						17															
	85	3834	8	2						7	1														
W. European	DS87	1913	1	1																					1
	ES289	2190	1	1	1																				
	ES197	2200	1	1	1																				
	ES283	2946	3	1	3																				
Argentine	245	2707	8	6													2	2	1	1	1	1			
	259	3310	4	4						1							1	1						1	
	256	3910	18	2													17						1		
Transitions/transversions					0/0	1/0	1/0	2/0	1/0	7/1	1/0	1/0	1/0	1/0	1/0	1/0	5/1	0/0*	1/0	1/1	5/1*	7/1	7/1	7/1	1/0

Table 2 Estimates of haplotype (*h*) and nucleotide (π) diversity overall and for each station and basin

Basin	Station	Depth (m)	*h*	π
N. American	87	1102	0.182	0.0009
	73	1400	0.333	0.0017
	209	1600	0.495	0.0028
	103	2022	0.228	0.0012
	115	2040	0.333	0.0017
	MMSM3	2055	0.000	0.0000
	MMSN8	2180	1.000	0.000
	62	2496	0.643	0.0051
	340	3310	0.000	0.0000
	77	3806	0.000	0.0000
	85	3834	0.250	0.0115
	basin average	2350	0.597	0.0186
W. European	DS87	1913	1.000	0.0000
	ES289	2190	1.000	0.0000
	ES197	2200	1.000	0.0000
	ES283	2906	0.000	0.0000
	basin average	2302	0.333	0.0017
Argentine	245	2707	0.929	0.0225
	259	3311	1.000	0.0325
	256	3912	0.111	0.0017
	basin average	3310	0.556	0.0134
Overall average		2499	0.731	0.0217

(n = 12) from the more thoroughly sampled North American basin. The West European basin, represented by the fewest sequences (n = 6) and sampled only above 3000 m, yielded the dominant North American haplotype, A, from three stations in the Rockall Trough and a unique haplotype, U, from a station in the Bay of Biscay. Haplotype U differs from haplotype A by only one substitution so there is little differentiation between the eastern and western North Atlantic. Thirty individuals from three stations in the Argentine basin were sequenced and revealed nine haplotypes. Most of these (M, Q, R, S and T) were considerably different from those found in the North Atlantic, although three others (N, O and P) were quite similar to the most frequent haplotype in the Northern Hemisphere. Surprisingly, a single individual in the Argentine basin at 3311 m corresponded to haplotype F, the most common haplotype below 3000 m in the North American basin. No haplotype was ubiquitous among the basins and most were common to only one, but haplotype A, the most common haplotype above 3000 m in the North American basin was the most widely distributed. It was found across the mid-Atlantic ridge in the Rockall Trough of the West European basin, and closely related haplotypes (separated by one mutation) were found in the Argentine basin.

Uncorrected pairwise genetic distances among the haplotypes ranged from 0 (due to a single deletion in haplotype N relative to A) to 0.072. Genetic distance among haplotypes was greater below 3000 m than above (0.033 vs. 0.022), and divergence among southern (Argentine basin) haplotypes was more than twice that of northern (N. American basin and W. European basin) haplotypes (0.036 vs. 0.015).

AMOVA revealed strong population structure whether stations were grouped by basin, depth or a combination of both (Table 3). The strongest population divergence emerged when stations were grouped by depth within basin or by depth independent of basin.

Clustering of pairwise genetic distance (Φ_{ST}) among stations, resulted in four main clades (Fig. 2): (i) North American and Rockall Trough stations above 3300 m, (ii) Bay of Biscay and Argentine basin stations 3311 m and

Table 3 AMOVA results comparing genetic variation in *Deminucula atacellana* collected from 18 stations among three hydrographic basins of the Atlantic Ocean at depths ranging from 1102 to 3910 m

Grouping	Source of variation	d.f.	Sum of squares	Variance component	Percent of total	F_{CT}	F_{SC}	F_{ST}
Depth	Between depths	1	146.88	2.219	66.40	0.664***		
(above/below 3000 m)	Among stations w/in depth	16	81.81	0.712	21.30		0.634***	
	Within stations	112	46.04	0.147	12.30			0.877***
	Total	129	274.73	0.411	100.00			
Hydrographic basin	Between basins	2	67.40	0.786	29.01	0.290*		
(N. American,	Among stations w/in basins	15	161.29	1.511	55.80		0.786***	
Argentine and	Within stations	112	46.04	0.411	15.18			0.848***
W. European)	Total	129	274.73	2.708	100.00			
Depth and basin	Between groups	4	222.37	2.545	85.74	0.858***		
	Among stations w/in groups	13	6.31	0.012	0.41		0.029**	
	Within stations	112	46.04	0.411	13.85			0.862***
	Total	129	274.73	2.969	100.00			

Statistical probabilities were derived from 1023 permutations; *P < 0.10, **P < 0.05, and ***P << 0.0001.

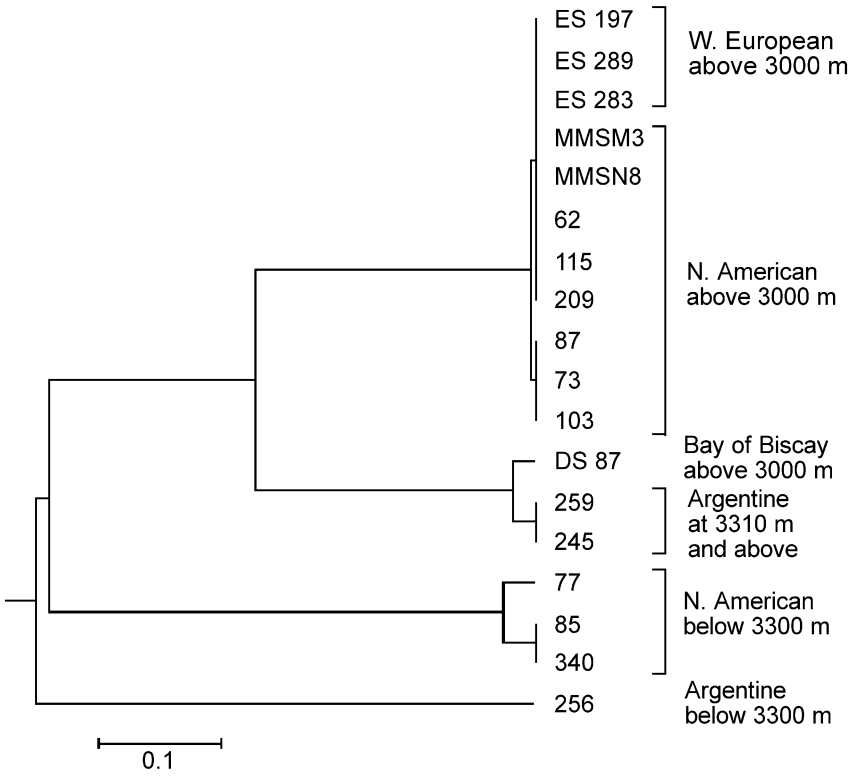

Fig. 2 Neighbour-joining tree comparing genetic distance among stations using Φ_{ST} values. Station depths are given in Table 1.

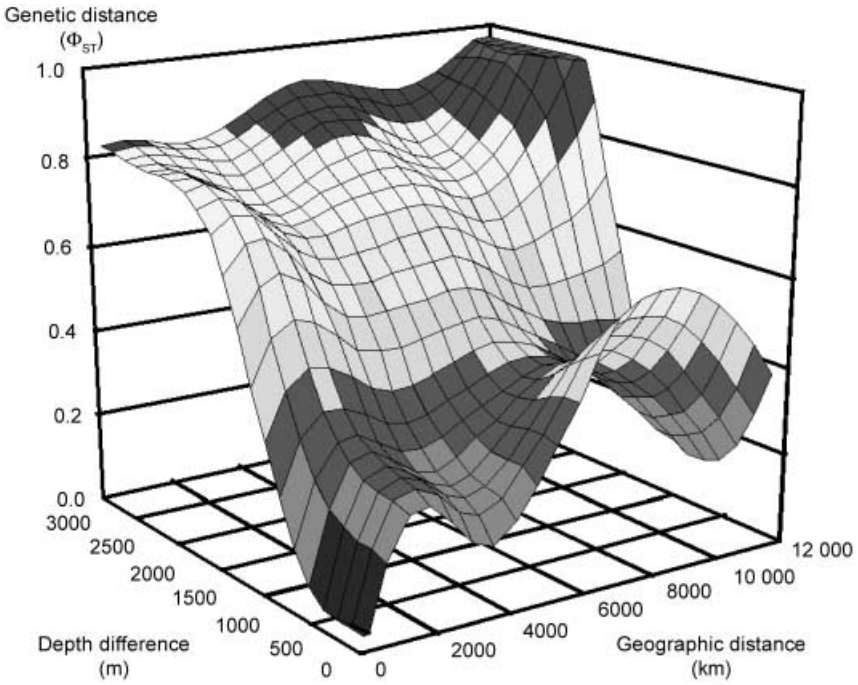

Fig. 3 Surface plot showing the relationship of genetic distance, geographic distance, and depth difference for pairwise comparisons among all sampling stations (smoothing by linear interpolation).

above, (iii) North American basin stations deeper than 3300 m and (iv) a single Argentine basin station at 3912 m. The clustering indicates that stations shallower than approximately 3300 m, regardless of basin, are more similar to each other than to their deeper counterparts and that the deep Argentine and deep North American stations are widely divergent.

Genetic distance varies considerably more with depth than with geographic distance (Fig. 3). Levels of genetic divergence for a 1200 m depth separation are nearly

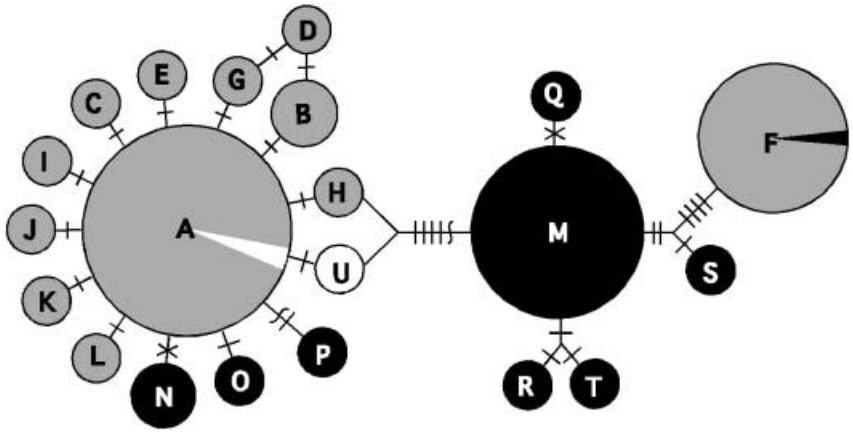

Fig. 4 Unrooted maximum-parsimony network for 21 haplotypes of the bivalve *Deminucula atacellana* with all symmetries left unbroken. Haplotypes are indicated by letter codes and circle size represents the relative abundance of each haplotype. Shading corresponds to ocean basin: black, Argentine basin; grey, North American basin; and white, West European basin. Cross bars designate the number of nucleotide differences between haplotypes: slash, transition; sigma, transversion; and cross, deletion.

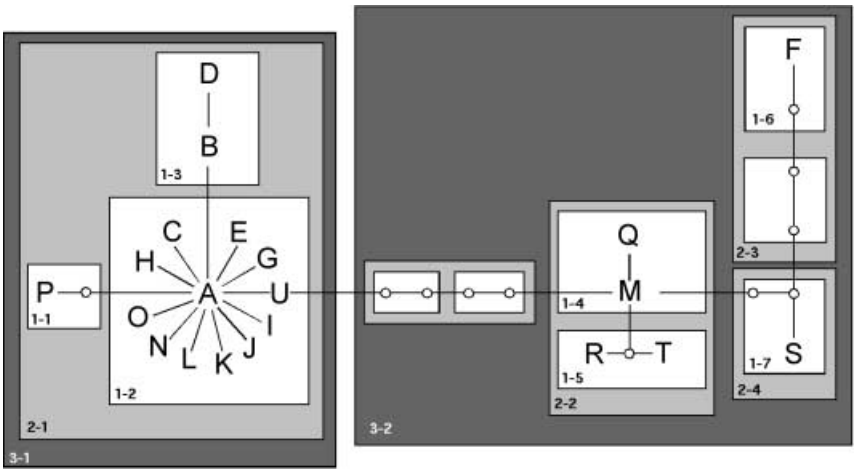

Fig. 5 Schematic of nesting clades for 21 haplotypes of the bivalve *Deminucula atacellana* derived from the parsimony network (Fig. 4) showing hierarchical grouping of haplotypes by number of base pair differences. Shading corresponds to levels of nesting: white, first order; light grey, second order; and dark grey, third order clades.

equivalent to 12 000 km geographic distance at similar depths. Stations separated by approximately 2500 m in depth were highly divergent over all geographic distances, whereas stations separated by less than 1500 m depth are much more similar genetically. When samples are at similar depths, Φ_{ST}'s increase slowly with geographic distance. The peak of higher genetic divergence at distances of approximately 6000–8000 km corresponds to pairwise comparisons between the North American basin samples with both the West European and Argentine samples. The drop in divergence with large and small distances corresponds to comparisons between the Argentine and West European basin (with the smallest depth differences). Partial mantel tests also support a relationship of greater genetic divergence with depth (Mantel $r = 0.4975$, $P = 0.0004$) than with distance (Mantel $r = 0.0712$, $P = 0.2922$).

An unrooted maximum-parsimony network shows that the dominant haplotype in North American basin stations below 3300 m (F) is phylogenetically closer to Argentine haplotypes below this depth (M, R, S and T) than to North American haplotypes above this depth (Fig. 4). The nested clade network (Fig. 5) has four nesting levels (i.e. stepped clades): one centred around interior haplotype A, another around interior haplotype M and a third and fourth encompassing tip haplotypes F and Q, respectively. The A clade includes individuals from all three basins but only from stations above 3300 m, except for haplotype G that was encountered once at 3800 m in the North American basin. The F clade includes individuals from the deep North American basin and a single individual from the deep Argentine. The M clade includes individuals only from the Argentine basin but from all depths sampled and the Q clade from the Argentine basin above 3000 m. The greatest number of haplotypes were found in the North American basin but average pairwise genetic distances were largest in the Argentine basin ($d = 0.033$), the only basin with representatives from each of the four major clades, followed by the West European basin ($d = 0.029$) and lastly the North American basin ($d = 0.011$). The interior location of haplotypes A and M in the network indicates their probable phylogenetically ancestral position among the sampled haplotypes (Templeton *et al*. 1995).

Table 4 Significant chi-squared tests of geographic association of clades (Fig. 5) and biological inference chain for a nested clade analysis of 21 haplotypes of the bivalve *Deminucula atacellana*. Inferences were derived from the key cited in Templeton (2004)

Clades	χ^2	*P*	Inference chain	Inference
Clade 1-2	268.58	0.032	1-2-3-5-6-13-14 yes	Cannot discriminate among CRE, LDC and PF
Clade 3-2	50.85	0.000	1-2-3-5-6-7-8 no	Cannot discriminate between IBD and LDD
Total clades	114.99	0.000	1-2	inconclusive

Abbreviations: CRE, continuous range expansion; IBD, isolation by distance; LDC, long-distance colonization; LDD, long-distance dispersal; PF, past fragmentation.

Significant population subdivision was also identified by the nested clade analysis. In three of the four levels of nesting, the null hypothesis of no geographic associations within the nesting clade was rejected (Table 4). However, inferences on how population subdivision has occurred were limited due to a paucity of geographically intermediate samples. At the first level of nesting, the analysis could not distinguish between mechanisms of isolation by distance and long-distance dispersal for haplotypes within clade 1-2 which encompasses 61 individuals from the North American basin above 3000 m, six individuals from the West European basin above 3000 m, one individual from below 3000 m in the North American basin and three individuals from the Argentine basin above and below 3000 m. At the third level of nesting, discrimination was not possible among contiguous range expansion, long-distance colonization and past fragmentation as possible mechanisms of present-day patterns for clade 3-2, which includes 25 individuals from all depths in the Argentine basin and 28 individuals from the North American basin below 3000 m. At the fourth level of nesting, contiguous range expansion is inferred over the entire data set.

Discussion

Little is known about the geographic or bathymetric scales of population differentiation for organisms inhabiting the continuous soft-sediment habitats of the deep ocean, especially on the ocean-wide scales that encompass the geographic distribution of many deep-sea species (Etter & Rex 1990; Allen & Sanders 1996). A pan-Atlantic analysis of genetic variation of 16S mtDNA in *Deminucula atacellana* revealed strong population structure associated with both ocean basin and depth regime. Population differentiation was much stronger with depth than distance; individuals separated by hundreds of metres in depth were considerably more divergent than those at similar depths but separated by thousands of kilometres in distance. In fact, for the North Atlantic there appears to be quite distinct upper bathyal (*c*. 3300 m and above) and lower bathyal (*c*. 3300 m and below) populations. This is true in the South Atlantic as well where genetic similarity was high between two stations at about 2700 and 3300 m (pairwise $\Phi_{ST} = -0.09747$) but markedly divergent with a third at approximately 3900 m (pairwise $\Phi_{ST} = 0.43908$). Lacking samples below 3000 m, we were unable to test this hypothesis in the eastern North Atlantic. When comparisons were made at similar depths throughout the Atlantic, eastern and western North Atlantic populations were genetically homogeneous, but considerable divergence emerged between the North and South Atlantic. What accounts for these geographic and bathymetric patterns?

Geographic divergence

The mid-Atlantic ridge, the largest and most distinctive topographic feature in the Atlantic, does not appear to be a strong barrier separating eastern and western North Atlantic populations of *D. atacellana*. The lack of divergence suggests a very recent colonization of the West European Basin, or the dispersal of larvae above or through the ridge. In the North Atlantic, the ridge is topographically complex and often rises to depths above 1000 m; however, there are numerous deep-water corridors that may allow demersal larvae of bathyal organisms to move between eastern and western basins. Deep-water currents in the North Atlantic were originally thought to spread primarily southward (Pickart 1992), but recent empirical estimates indicate highly complex circulation patterns with basin-wide recirculation and flow across the ridge (Bower & Hunt 2000; Lavender *et al*. 2000; Bower *et al*. 2002; Ollitrault & de Verdière 2002). Subsurface drifters dispersing at bathyal depths (400–1750 m) typically moved north or south along the ridge axis, but several were swept across the ridge through deep-water corridors (Bower *et al*. 2002; Sparrow *et al*. 2002; Lavender *et al*. 2005). These corridors may provide important conduits for the movement of demersal larvae between eastern and western basins of the North Atlantic (Mullineaux *et al*. 2002).

Although the ridge may not represent an impenetrable barrier for upper-bathyal organisms with demersal larvae, it may prove more of an obstacle between eastern and western populations of these animals at lower-bathyal and abyssal depths. Both the abyssal protobranch *Ledella ultima* (Etter *et al*. in prep) and prosobranch gastropod *Benthenella tenella* (Boyle *et al*. in prep) exhibit strong divergence between eastern and western Atlantic conspecifics. The efficacy of the ridge as an isolating barrier will likely depend on the nature, duration and depth of dispersal, as well as the reproductive biology (e.g. number of propagules, timing and frequency of reproduction) of each species. The evolutionary implications relative to submarine ridges may be especially important for taxa that are more abundant or speciose at lower bathyal-abyssal depths, such as protobranch bivalves (Zardus 2002).

The forces creating the pronounced genetic divergence between the North and South Atlantic are poorly understood. The limited sampling at intermediate localities precludes discriminating among isolation by distance, long-distance colonization, long-distance dispersal or past fragmentation. *Deminscula atacellana* presumably develops via a lecithotrophic, free-swimming pericalymma larva typical of protobranch bivalves (Zardus & Martel 2002), which in shallow-water species develop in the plankton for a few days and probably stay near the substratum (Zardus & Morse 1998). The deep-water currents at bathyal depths are typically quite slow (1–40 cm/s) (Hogg 1983; Saunders 1983; Schmitz & McCartney 1993; Bower & Hunt 2000), suggesting larvae would disperse passively about 17 km/d (based on a median current velocity of 20 cm/s, which is much greater than the mean; Bower & Hunt 2000). If larvae spend a few days drifting in the currents, single-generation dispersal ranges would be less than 100 km, and probably much less if shallow-water estimates for planktonic dispersal ranges provide any indication (Palumbi 2004). The distances between the North and South Atlantic populations are great (> 8000 km) indicating little gene flow should occur at these scales for small sedentary organisms with low fecundity dispersing in such slow currents. In the vast uninterrupted habitat of the deep sea, distance is likely a frequent isolating mechanism.

The higher level of genetic divergence between North and South Atlantic populations at lower-bathyal depths (Fig. 2), compared to upper-bathyal, is surprising and contrasts with expectations based on environmental heterogeneity, biogeography and previous genetic work. The upper bathyal zone is topographically complex (Mellor & Paull 1994) with more heterogeneous sediments (Cooper *et al*. 1987; Etter & Grassle 1992) and can be impacted by oxygen minimum zones potentially isolating gene pools and facilitating population differentiation and speciation (White 1987; Wilson & Hessler 1987; Wilson 1998; Rogers 2000; Helly & Levin 2004). Species diversity (Rex 1981; Etter & Grassle 1992), intraspecific morphological divergence (Etter & Rex 1990) and zonation (Grassle *et al*. 1979; Rex 1981; Carney *et al*. 1983; Etter & Rex 1990) all peak at upper-bathyal depths suggesting communities are biologically more heterogeneous. Geographic distributions tend to increase with depth (Etter & Rex 1990; Pineda 1993; Allen & Sanders 1996) also suggesting more uniform selective regimes at greater depths. The strong gradients and greater biotic and abiotic heterogeneity at upper-bathyal depths should be more conducive to population differentiation. Consistent with these notions, genetic divergence tends to be greater at upper-bathyal depths compared to the abyss (France & Kocher 1996; Etter *et al*. 2005). Yet, at oceanwide scales, *D. atacellana* exhibits the opposite pattern.

Despite a generally more uniform environment at lower-bathyal depths, gene flow at these scales might be considerably less. Both the biomass and abundance of benthic organisms decrease exponentially with depth (Etter & Mullineaux 2001; Rex *et al*. 2005). Because these aspects of community structure represent the best measure of integrated energy flux to the benthos, food resources probably also decline exponentially with depth, severely limiting resources available for growth and reproduction. Deep-water current velocities also tend to decline with depth (Hogg 1983; Saunders 1983; Schmitz & McCartney 1993). The small size, low fecundity and slow currents may all conspire to reduce connectivity among populations at lower-bathyal depths.

Bathymetric divergence

In both the North and South Atlantic, populations above and below approximately 3300 m were genetically distinct with Φ_{ST}'s nearly one, while within these depth regimes, populations showed little divergence. The geographic separation between the upper and lower bathyal populations in both regions is quite small (< 100 km), suggesting the divergence is not related to the distances separating nearby samples. France & Kocher (1996) documented a similar pronounced genetic divergence between bathyal (< 3500 m) and abyssal (> 3500 m) populations of the cosmopolitan amphipod *Eurythenes gryllus*. Genetically distinct bathyal and abyssal populations were found in both the Pacific and Atlantic. Remarkably, the depth of the break is quite similar to what we found for *D. atacellana*. Because these two species have extremely different lifestyles, natural histories and phylogenetic affinities, yet exhibit congruent genetic breaks at 3300 m, and this occurs consistently in vastly different regions of the world oceans, 3300 m may represent a ubiquitous unrecognized phylogeographic barrier isolating organisms inhabiting different depth regimes.

No obvious mechanisms separate upper and lower-bathyal populations, but changes in depth are attended by

strong environmental gradients that might create sufficiently different selective regimes to isolate conspecifics (Doebeli & Dieckmann 2003). One key factor potentially playing an important role in isolating populations along depth gradients is pressure (Chase *et al*. 1998a). Most biochemical reactions involve changes in volume that are little affected by atmospheric pressure, but for some classes of proteins, pressure changes of 51 atm or more may significantly inhibit reaction function and influence a species' bathymetric distribution (Somero 1990). Hydrostatic pressure may influence larval dispersal, and thus, gene flow in many deep-sea organisms. For example, some echinoid larvae develop only at particular ranges of hydrostatic pressure and temperature (Tyler & Young 1998), and the bathymetric range of the seastar *Plutonaster bifrons* corresponds to the upper and lower pressure limits of its developing larvae (Young *et al*. 1996). The pressure gradient *D. atacellana* experiences over its range varies from 110 to 391 atm, well within the range for a barophysiological affect. Water viscosity also varies with pressure and temperature (Macdonald 1975) and can influence the mechanics of ciliary motion in larvae (Podolsky 1994). Thus, mechanical or physiological capabilities of *D. atacellana* larvae may be adapted to specific depth regimes. Isolation by depth may represent a novel structuring mechanism operating exclusively within the deep-sea ecosystem.

Cryptic species?

Although there is no evidence of morphological variability associated with depth or distance throughout *D. atacellana*'s range in the Atlantic (Rhind & Allen 1992), the highly divergent haplotypes between depth regimes might represent cryptic species or incipient speciation. The pairwise genetic difference between the predominant haplotypes of the two depth regimes (A vs. F, 0.041) is in the lower range of what has been found for 16S among congeners in other bivalves (e.g. Etter *et al*. 1999, 2005; *Tridacna*, Schneider & Ó Foighil 1999; *Lasea*, Jozefowicz & Ó Foighil 1998; *Crassostrea*, Lapegue *et al*. 2002; *Dreissena*, Therriault *et al*. 2004). This comparison needs to be interpreted cautiously though because estimates of interspecific divergence in the shallow-water bivalves were based on a much larger fragment of the 16S gene. Our fragment is smaller and centred in a highly variable region of the gene, which may inflate estimates of haplotype distance.

More importantly though, we lack a basic understanding of intraspecific genetic variation in deep-sea organisms, and it may be naive to assume that they will mirror shallow-water analogues. There are at least two reasons why levels of intraspecific variation might be higher in *D. atacellana*. First, it may have an enormous population — it has a pan-Atlantic distribution and is numerically abundant across a wide bathymetric range (Allen & Sanders 1996). Given the areal extent of the deep Atlantic, the continuity of soft-sediment habitats and the relatively high abundance of this species, population size is likely to be much larger than is typical for shallow-water analogues. Second, because the deep sea is generally more stable than shallow water, populations may be more stable, limiting the loss of genetic diversity due to population fluctuations and environmentally induced bottlenecks. Both the large population size and the greater population stability would allow organisms to retain higher levels of ancestral polymorphism.

Genetic distances between sibling species can vary greatly (Burton & Feldman 1982; Burton 1983; Knowlton 2000) yet high genetic variability is not a prerequisite for species divergence nor for reproductive isolation. For instance, sibling species of sea urchins differ little genetically or morphologically, but cannot cross-fertilize due to incompatibility between their gamete recognition proteins (Palumbi & Metz 1991; Metz & Palumbi 1996). On the other hand, extreme intraspecific genetic divergence has been found in mollusks (Thomaz *et al*. 1996; Quattro *et al*. 2001; Pfenninger & Posada 2002; Lee & Ó Foighil 2004) as well as other invertebrates (Burton & Lee 1994; Romano & Palumbi 1997; Riginos & Nachman 2001; Vollmer & Palumbi 2004). Unfortunately, we lack the necessary phylogenetic framework to arbitrate clearly between high levels of intraspecific polymorphism or the presence of cryptic species in *D. atacellana*.

Population structure reflects the interplay of physical, ecological and physiological isolating mechanisms. Despite the lack of obvious isolating barriers, deep-sea organisms can possess strong genetic structure on a variety of scales. Divergence across depth contours is greater than along them, paralleling the intensity of environmental gradients. Environmental changes that attend changes in depth may play a key role in the evolution of the deep-sea fauna, precipitating population differentiation and ultimately leading to speciation.

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Each of the authors is interested in understanding evolution in the immense, remote and diverse ecosystem of the deep sea. Using techniques in population genetics, morphometrics, and ecology, they seek to identify the patterns and processes controlling biogeographic variation in biodiversity.
