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Phylogeography of a pan-Atlantic abyssal protobranch bivalve: implications for evolution in the Deep Atlantic

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Abstract

The deep sea is a vast and essentially continuous environment with few obvious barriers to gene flow. How populations diverge and new species form in this remote ecosystem is poorly understood. Phylogeographical analyses have begun to provide some insight into evolutionary processes at bathyal depths (<3000 m), but much less is known about evolution in the more extensive abyssal regions (>3000 m). Here, we quantify geographical and bathymetric patterns of genetic variation (16S rRNA mitochondrial gene) in the protobranch bivalve *Ledella ultima*, which is one of the most abundant abyssal protobranchs in the Atlantic with a broad bathymetric and geographical distribution. We found virtually no genetic divergence within basins and only modest divergence among eight Atlantic basins. Levels of population divergence among basins were related to geographical distance and were greater in the South Atlantic than in the North Atlantic. Ocean-wide patterns of genetic variation indicate basin-wide divergence that exceeds what others have found for abyssal organisms, but considerably less than bathyal protobranchs across similar geographical scales. Populations on either side of the Mid-Atlantic Ridge in the North Atlantic differed, suggesting the Ridge might impede gene flow at abyssal depths. Our results indicate that abyssal populations might be quite large (cosmopolitan), exhibit only modest genetic structure and probably provide little potential for the formation of new species.

Keywords: ecological genetics, molecular evolution, Molluscs, phylogeography

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Introduction

The deep sea represents the largest ecosystem on the planet and supports a diverse and mostly endemic fauna (Hessler & Sanders 1967) that plays a critical role in global biogeochemical cycling and important ecosystem processes (Jahnke & Jackson 1992; Archer & Maier-Reimer 1994, Danovaro *et al.* 2008). We know very little about how this remarkable fauna evolved. Even basic questions about the scales and geography of population divergence are poorly understood, especially for abyssal organisms. This nescience limits our ability to understand or predict how abyssal ecosystems might respond to regional or global shifts in climate, productivity or circulation.

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Recent work has begun to provide some insight into the geographical and bathymetric patterns of genetic variation for bathyal organisms. Divergence among conspecifics can occur on a wide variety of scales and appears to be associated with oceanic basins, topography, currents, depth, oxygen levels and vicariance (e.g. Creasey *et al.* 1997; Rogers 2000; Roques *et al.* 2002; Weinberg *et al.* 2003; Howell *et al.* 2004; Le Goff-Vitry *et al.* 2004; Aboim *et al.* 2005; Roy *et al.* 2007). Two interesting patterns have emerged from this early work. First, vertical separation appears to be more important than horizontal in promoting population differentiation—genetic divergence is much greater between populations at different depths than those separated geographically at the same depth (Bucklin *et al.* 1987; France & Kocher 1996; Etter *et al.* 2005; Zardus *et al.* 2006; Raupach *et al.* 2007). Second, levels of population

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divergence vary with depth such that bathyal organisms appear to exhibit much greater population structure than do those from abyssal depths (France & Kocher 1996; Etter *et al.* 2005; Zardus *et al.* 2006). The latter pattern is referred to as the Depth-Differentiation Hypothesis (DDH) and suggests that the continental margins may be the primary site of adaptive radiation in the deep sea (Etter *et al.* 2005).

Although we have made some progress in understanding evolutionary processes at bathyal depths, much less is known about geographical or bathymetric patterns of genetic variation at abyssal depths. Few studies have examined population structure for abyssal organisms, in large part because they are generally extremely rare, minute and were fixed in formalin when collected, which degrades the DNA. For those taxa that have been analysed, the results so far are consistent—abyssal populations exhibit little divergence and appear to be panmictic over enormous scales. In fact, for two very different abyssal organisms (foraminifera—Pawlowski *et al.* 2007, 2008, Lecroq *et al.* 2009 and amphipods—France & Kocher 1996), Pacific and Atlantic conspecifc populations exhibited little genetic divergence.

While low genetic divergence of abyssal organisms is consistent with the DDH, it is surprising that populations would remain essentially genetically homogeneous over such vast scales. Because of the extremely slow currents at abyssal depths (often < 1 cm/sec, Richardson 1993; Hogg & Owens 1999) and the likely low fecundity of minute organisms feeding on a meagre food supply, isolation by distance should result in

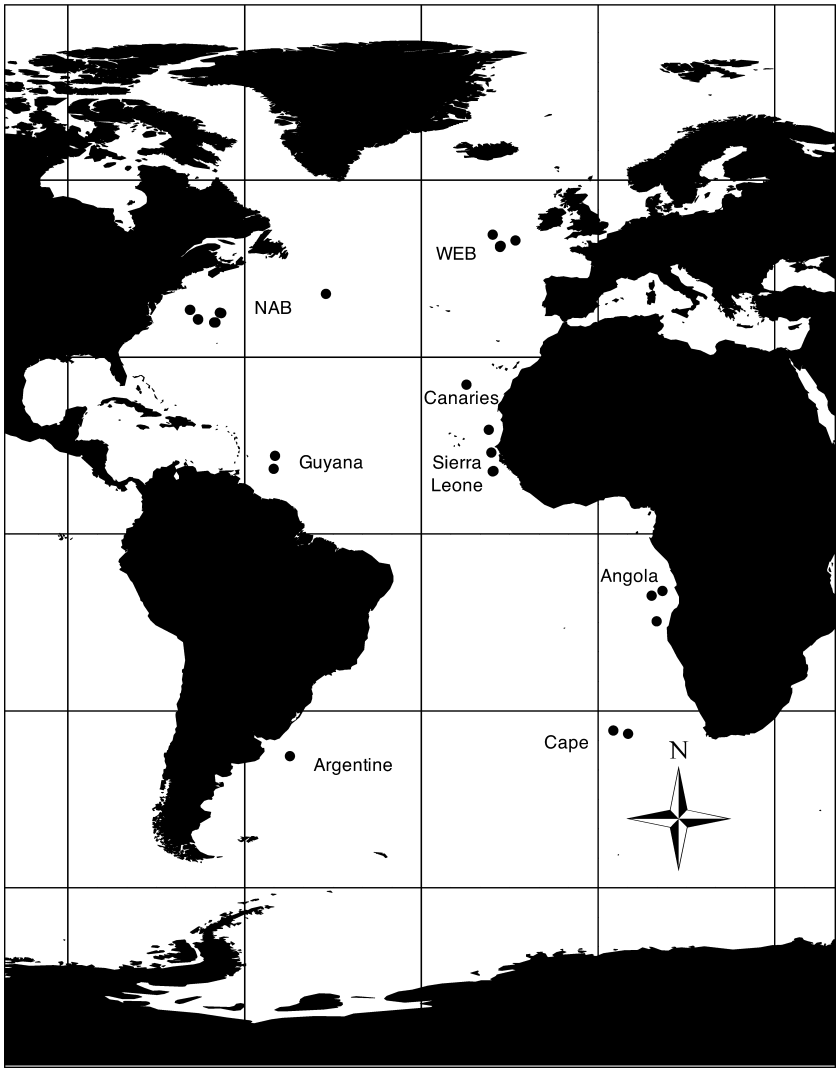

Fig. 1 The sampling localities used in our analysis of the protobranch bivalve *Ledella ultima*. Not all samples are shown in the NAB and WEB basins because some symbols overlap.

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genetic differences on ocean-wide scales. How is population connectivity maintained across such enormous scales and what does this imply about evolutionary processes over most of the Earth's surface? Quantifying these dynamics will have important implications for understanding how abyssal populations might respond to the accelerating pace of climate change and how this might impact key ecosystem processes (Danovaro *et al.* 2008, 2009; Smith *et al.* 2008; Glover *et al.* 2010).

To better understand the role of the extensive abyssal regions in the evolution of the deep-sea fauna, we need to know how populations diverge over large scales at these depths. Here, we quantify the geographical and bathymetric patterns of genetic variation in the protobranch bivalve *L. ultima*, which is one of the most abundant abyssal protobranchs in the Atlantic with a broad bathymetric and geographical distribution (Allen & Sanders 1996; Allen 2008). It is an infaunal deposit feeder with a nonfeeding pericalymma larva that disperses demersally (Zardus 2002). Ocean-wide patterns of genetic variation indicate basin-wide divergence that is greater than what others have found for abyssal organisms, but much less when compared to bathyal protobranchs across similar scales.

Table 1 Station Data. Number of sequences (N) and haplotypes, haplotype (h) and nucleotide (π) diversity, Tajima's D and Fu's F_s for each sample and for pooled samples within basins

Station	Depth	Latitude	Longitude	Basin	N	Haplotypes	h	π	D	F_s
East										
50603	4000	49°45.3′N	14°01.0′W	WEB	3	3	1	0.0141	0	**1.44**
330	4632	50°43.5′N	17°51.7′W	WEB	4	4	1	0.0300	16.5	**0.56**
52701	4846	48°51.5′N	16°32.5′W	WEB	2	2				
52602	4850	48°49.86′N	16°34.09′W	WEB	1	1				
11908	4865	48°41.1′N	16°39.4′W	WEB	4	4	1	0.0202	0.37	**−1.01**
52216	4900	48°47.77′N	16°38.61′W	WEB	5	2	0.4	0.0028	−0.82	0.09
8532	2954	13°47.8′N	18°7.25′W	Canaries	1	1				
8528	3155	17°38.5′N	18°35.4′W	Canaries	5	3	0.7	0.0127	20.1	3.35
10148	4844	25°17.1′N	22°23.1′W	Canaries	5	5	1	0.0141	0.27	**−2.24**
146	2866	10°39.5′N	17°44.5′W	Sierra Leone	2	2				
147	2934	10°38.0′N	17°52.0′W	Sierra Leone	2	2				
200	2699	9°43.5′S	10°57.0′E	Angola	3	2	0.667	0.0047	0	0.2
197	4596	10°29.0′S	9°4.0′E	Angola	5	3	0.7	0.0083	25.16	3.29
195	3797	14°49.0′S	9°56.0′E	Angola	2	2				
DS02	5280	33°54.7′S	5°7.3′E	Cape	10	7	0.911	0.0161	**−0.86**	**−2.05**
DS05	4560	33°20.5′S	2°34.9′E	Cape	9	7	0.917	0.0137	12.65	**0.27**
West										
77	3806	38°00.7′N	69°16.0′W	NAB	13	6	0.769	0.0086	5.93	**0.66**
78	3828	38°00.8′N	69°18.7′W	NAB	8	5	0.857	0.0139	**6.85**	1.28
334	4400	40°42.6′N	46°13.8′W	NAB	8	5	0.857	0.0093	13.92	1.03
70	4680	36°23.0′N	67°58.0′W	NAB	11	3	0.564	0.0085	0.58	1.76
92	4694	36°20.0′N	67°56.0′W	NAB	4	3	0.833	0.0106	**−0.75**	**−0.29**
84	4749	36°24.4′N	67°56.0′W	NAB	15	5	0.629	0.0097	0.37	**0.10**
121	4800	35°50.0′N	65°11.0′W	NAB	16	8	0.867	0.0102	3.96	**−0.83**
122	4833	35 50.0′N	64 57.5′W	NAB	1	1				
123	4853	37°29.0′N	64°14.0′W	NAB	3	2	0.667	0.0094	0	1.61
124	4862	37°26.0′N	63°59.5′W	NAB	3	3	1	0.0189	0	**−0.08**
288	4423	11°02.2′N	55°05.5′W	Guyana	9	6	0.833	0.0106	**5.19**	**0.14**
287	4957	13°16.0′N	54°52.2′W	Guyana	13	4	0.718	0.0114	12	4.99
256	3912	37°40.9′S	52°19.3′W	Argentine	9	5	0.722	0.0094	**4.85**	**1.04**
Pooled regions										
WEB					19	10	0.825	0.0164	**2.16**	**−0.76**
Canaries					11	9	0.945	0.0122	**4.68**	**−3.15**
Sierra Leone					4	3	0.833	0.0082	0.59	**−0.66**
Angola					10	8	0.643	0.0070	9.06	2.32
Cape					19	10	0.895	0.0144	**3.54**	**−0.36**
NAB					82	21	0.752	0.0101	**0.55**	**−6.1**
Guyana					22	8	0.758	0.0117	**5.11**	**1.46**

Bold values are significant $P < 0.05$.

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Methods

Preserved specimens of *L. ultima* were obtained from Woods Hole Oceanographic Institution (Woods Hole, MA) and the Southampton Oceanography Centre. The material was collected by box core and epibenthic sled as early as 1964 (Sanders *et al.* 1965; Sanders 1977) and as recently as 1991 (Rice *et al.* 1991; Billett *et al.* 2001). Samples were fixed in formalin immediately upon collection for 24–48 h and transferred to ethanol for storage. We extracted, amplified and sequenced DNA from 176 specimens collected at 29 localities in the North American (NAB), West European (WEB), Guyana (Guy), Canaries (Can), Sierra Leone (SL), Angola (Ang), Cape and Argentine (Arg) basins (Fig. 1). The samples ranged in depth from 2699 to 4957 m (Table 1).

Like most deep-sea samples, these were fixed in formalin, which degrades the DNA, making it difficult to quantify genetic variation. We developed protocols to extract and amplify (with PCR) mtDNA from small macrofaunal metazoans that were fixed in formalin (described in Chase *et al.* 1998a) and used these techniques to sequence a 198-bp fragment of the variable region of the 16S rRNA mitochondrial gene. The 16S gene has both highly conserved regions and more variable regions (Hillis & Dixon 1991). We targeted a variable region to maximize intraspecific variation. The formalin fixation precludes the amplification of nuclear loci in a consistent manner for population-level studies, probably because the low copy number of nuclear loci and degradation by the formalin leave few amplifiable fragments. Even multicopy nuclear regions [e.g. Internal Transcribed Spacer (ITS)] have not amplified consistently. Consequently, our analyses are restricted to a single locus. While we recognize the limitations of evolutionary inferences based on a single locus (e.g. Pritchard *et al.* 2000; Avise 2004; Felsenstein 2006; Knowles & Carstens 2007; Knowles 2009a,b; Nielsen & Beaumont 2009), the results provide a novel and critical window into evolution in this vast and remote ecosystem where fresh material would be prohibitively expensive to collect across these scales.

DNA was extracted as described by Etter *et al.* (2005). Species-specific primers were developed using the general procedures described previously (Chase *et al.* 1998a; Boyle *et al.* 2004). A forward primer, LMY16F (5′-GAC GAR AAG ACC CYR TCA AAC-3′), was paired with the reverse primer, Lu16R4 (5′-GCT GTT ATC CCT CCA GTA ACT-3′), to yield a 198-bp fragment of the 16S gene. Each sample was amplified by the polymerase chain reaction (PCR) 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 unit *Taq* (Promega, Madison, WI, USA), an equal volume of *Taq*Start Antibody (Clontech, Palo Alto, CA, USA) 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, Chatsworth, CA, USA).

Purified products were sequenced with a *Taq* Dye Deoxy Termination cycle sequencing kit (PE Applied Biosystems, Foster City, CA, USA), ethanol-precipitated and run on an Applied Biosystems Model 373 automated DNA sequencer. In almost all instances, PCR products were directly sequenced but a few (4/176) yielded multibanded or very weak PCR products. The multiband products were separated by gel electrophoresis, and bands of the appropriate size were 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 the chances of accepting errors introduced through PCR and cloning, consensus sequences were generated for all samples through alignment of both sense and antisense products. If the two strands were not complimentary, sequences were discarded from the analysis. Sequences were assembled and edited in Sequencher™ version 4.8 (Gene Codes Corp., Ann Arbor, MI, USA) and aligned by eye using MacClade (Maddison & Maddison 2003).

Phylogeographical analyses

Phylogenetic relationships among the haplotypes were inferred using statistical parsimony (Templeton *et al.* 1992) as implemented in TCS (Clement *et al.* 2000). Ambiguities in the networks were resolved using predictions from coalescent theory (Crandall & Templeton 1993) as summarized in Pfenninger & Posada (2002).

Analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was used to quantify the levels of population structure within and among regions. Stations were grouped by basin and depth. An AMOVA was also used to test specifically for divergence across the Mid-Atlantic Ridge (MAR) in the North Atlantic where sampling was greatest. For the MAR test, stations were grouped

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as eastern or western North Atlantic. For the basin analyses, we also pooled samples into demes to increase our statistical power and to avoid potential artefacts introduced by small sample sizes at some stations (Crandall *et al.* 2008), although clearly this can reduce power in other ways (Fitzpatrick 2009). Sampling stations within basins were pooled into demes if they had pairwise Φ_{ST} that were not significantly different from 0 based on 10 000 random replicates with standard Bonferroni corrections for multiple tests. ARLEQUIN 3.11 (Excoffier *et al.* 2005) was used to calculate AMOVAS, neutrality tests (Tajima's *D* and Fu's F_s), haplotype (*h*) and nucleotide (π) diversity. Samples with <3 individuals were excluded from all analyses (e.g. AMOVAS, genetic diversity), except those involving pooled stations.

We used nonmetric multidimensional scaling (r-project, http://www.r-project.org/) to depict the relationships among samples based on pairwise genetic distances (Slatkin's linearized Φ_{ST}) between stations. The MDS plot was used to visualize the relative genetic divergence among all samples. It provides a two-dimensional representation of the multidimensional genetic distances among all pairwise comparisons.

Because genetic divergence among deep-sea organisms can be influenced by both the geographical distance and depth separating samples (Etter *et al.* 2005), 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 geographical distance (great-circle distance among stations) in explaining spatial patterns of genetic divergence was tested with Partial Mantel (R-Package, version 4.0, Legendre & Vauder 1991).

Demographic analyses

Demographic history was inferred from Bayesian skyline plots (Drummond *et al.* 2005). A coalescent model of isolation with migration (IMa—Hey & Nielsen 2007) was used to estimate the size of the populations in each region (θ), the migration between them (*m*), the size of the ancestral population (θ_A) and the time since they split (*t*). The results of these analyses should be interpreted with caution because they are dependent on a single locus and we lack a good estimate of the mutation rate or generation time for abyssal organisms. The details of the demographic analyses, including an information theoretic evaluation of the nested models in IMa (Carstens *et al.* 2009), are included in Data S1.

Results

We identified 49 haplotypes from 176 individuals in eight Atlantic deep-ocean basins (Fig. 2). The most abundant haplotypes (L1 and L3) were widely distributed occurring in most basins, although L1 tended to have a higher frequency in the South Atlantic. It reached its highest frequency in the Argentine and Cape basins, the most southern samples. Interestingly, it was completely absent from the Angola basin, probably due to the relatively small sample size. Other common haplotypes were less widely distributed, often restricted to either the western North Atlantic (L10) or primarily the eastern North Atlantic (L13). The vast majority of haplotypes (38/49) were singletons. Genetic diversity measured as haplotype diversity (*h*) or nucleotide diversity (π) within stations or when pooled within regions (Table 1) showed no clear geographical or bathymetric variation (ANOVA all $P > 0.1$). Although the eastern Atlantic tended to have greater genetic diversity than the western Atlantic for both pooled and individual stations, the differences were not significant (ANOVA, all $P > 0.25$). Both Tajima's *D* (Tajima 1989) and Fu's F_s (Fu 1997) indicated departures from drift–mutation equilibrium for pooled regional samples (Table 1). The only exceptions were for the pooled regions with the least sampling, Sierra Leone and Angola, where the results were mixed. The results were also highly mixed for individual samples, with F_s indicating departures from neutrality more frequently. Departures were primarily positive for *D* and negative for F_s.

Phylogeographical analyses

The haplotype network indicated that most singleton haplotypes were closely related to one of the two most abundant and widely distributed haplotypes (L1 or L3) with no clear separation among the basins (Fig. 2). Consistent with this impression, pairwise Φ_{ST} indicated few significant differences among samples (Table 2). The Argentine sample was significantly different from most other samples, except those from the nearby Cape basin. Both of these Southern basins had a much higher frequency of L1-type haplotypes than did the more northern basins. The pairwise genetic differences among stations were not related in any consistent way to the depth separating samples (Partial Mantel, $r = 0.0696$, $P = 0.2$) but were influenced by geographical distance (Partial Mantel, $r = 0.2653$, $P = 0.009$) indicating isolation by distance (IBD). The correlation with distance is largely a consequence of the strong difference between the Argentine basin and most other samples (Table 2). When the Argentine

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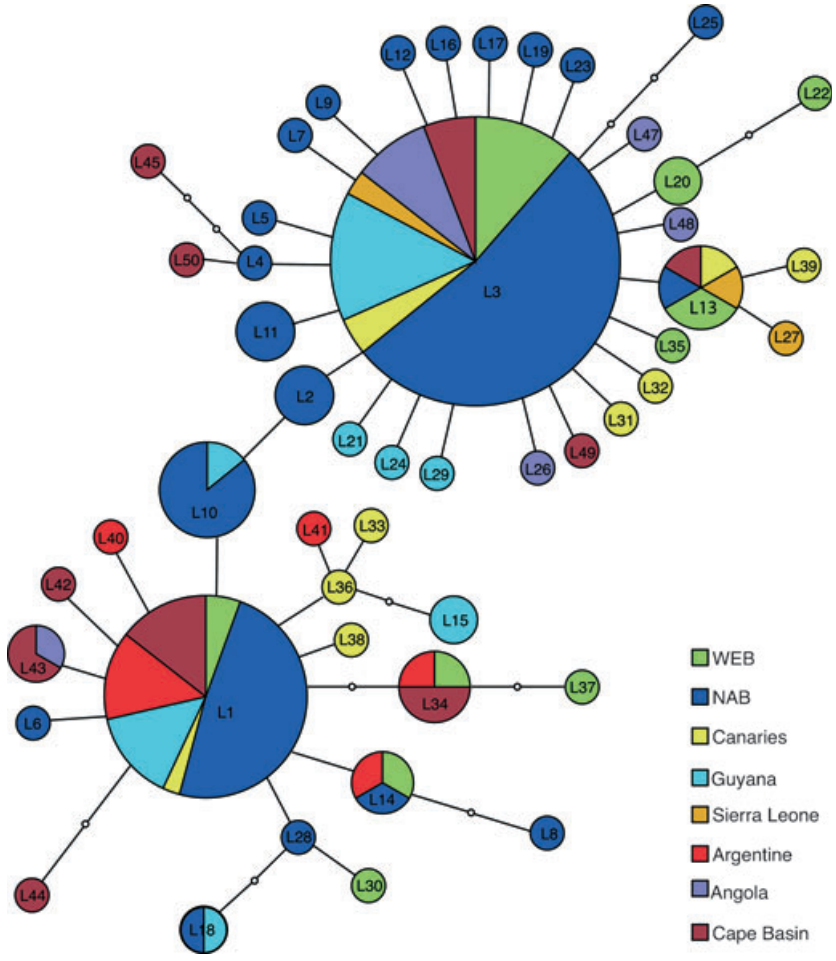

Fig. 2 A maximum parsimony haplotype network based on the 16S rRNA sequences from *Ledella ultima*. Each haplotype is represented by a circle with the area proportional to its relative abundance and colour indicating geographical distribution. Small open circles indicate missing intermediate haplotypes. Lines connecting haplotypes indicate a single nucleotide difference. GenBank accession numbers are HQ452629–HQ452677.

basin samples were excluded, the relationship with geographical distance becomes marginally nonsignificant (Partial Mantel, $r = 0.1678$, $P = 0.054$). Levels of genetic divergence were considerably lower for *L. ultima* (Fig. 3) compared to a bathyal pan-Atlantic protobranch analysed across similar geographical scales (Zardus *et al.* 2006). For both species, bathymetric divergence occurred on much smaller scales than geographical (Fig. 3). Genetic divergence among populations separated by 3 km in depth was similar to populations at the same depth but separated by 12 000 km in distance. While the contrast between bathymetric and geographical divergence was consistent for both species, the level of divergence was substantially less at abyssal depths.

Samples from different geographical regions tended to overlap within the nMDS space defined by pairwise Φ_{ST} suggesting modest genetic divergence (Fig. 4). The most divergent basins were those from the South Atlantic, but the limited sampling within these basins makes it difficult to statistically test for basin level divergence. The North Atlantic basins also indicated some divergence, and sample sizes were sufficient for statistical analysis. An envelope surrounding samples from the NAB overlaps little with one encompassing the WEB samples, suggesting these two regions might be genetically different (Fig. 4). An AMOVA comparing NAB and WEB samples indicated a slight difference among groups, but most of the variation was within populations (Table 3). In general, the AMOVAs among basins, between the eastern and western North Atlantic and between the North and South Atlantic were all significant, indicating abyssal populations exhibit modest divergence at these ocean-wide scales. However, in each case, nearly 100% of the genetic variation existed within stations (Table 3).

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Table 2 Pairwise Φ_{ST} (Slatkin's linearized) among stations

	North American Basin									Guyana		West European Basin				Canaries		Arg	Angola		Cape	
Stations	77	78	334	70	92	84	121	123	124	288	287	330	52216	50603	11908	10148	8528	256	197	200	DS02	DS05
77	0																					
78	0	0																				
334	0	0	0																			
70	0	0	0	0																		
92	0	0	0	0	0																	
84	0	0	0	0	0	0																
121	0	0	0	0	0	0	0															
123	0.012	0	0	0	0	0	0	0														
124	0	0	0	0	0	0	0	0	0													
288	0	0	0	0.006	0	0.020	0.112	0.158	0	0												
287	0.007	0.012	0	0	0	0	0	0	0	0.103	0											
330	0.152	0.055	0.087	0.141	0	0.168	0.209	0.003	0	0.101	0.169	0										
52216	0	0	0.027	0.027	0	0.040	0.156	0.667	0.033	0	0.144	0.076	0									
50603	0.051	0	0	0.013	0	0.006	0	0	0	0.036	0.019	0	0.214	0								
11908	0.013	0	0	0	0	0	0	0	0	0.071	0	0	0.137	0	0							
10148	0	0	0	0	0	0	0	0	0	0	0	0.007	0.030	0	0	0						
8528	0	0	0	0	0	0	0	0	0	0	0	0.009	0	0	0	0	0					
256	**0.597**	**0.471**	**0.433**	**0.495**	**0.575**	**0.443**	**0.243**	0	**0.426**	**0.845**	**0.269**	**0.536**	**1.345**	**0.263**	0.128	**0.326**	**0.501**	0				
197	0	0	0	0	0	0	0.036	0.119	0	0	0.018	0.015	0	0.032	0	0	0	**0.793**	0			
200	0.068	0	0.121	0.136	0	0.118	0.266	0.675	0	0	0.200	0	0.044	0.206	0.115	0.061	0.011	**1.420**	0	0		
DS02	0.047	0.027	0	0.009	0.002	0.012	0	0	0	0.153	0	0.113	0.189	0	0	0	0.009	0.057	0.066	0.234	0	
DS05	0.141	0.096	0.051	0.086	0.105	0.083	0	0	0.038	**0.279**	0.043	0.184	**0.389**	0	0	0.006	0.087	0.002	0.194	**0.456**	0	0

Bold values are significant $P < 0.05$.
Arg, Argentine.

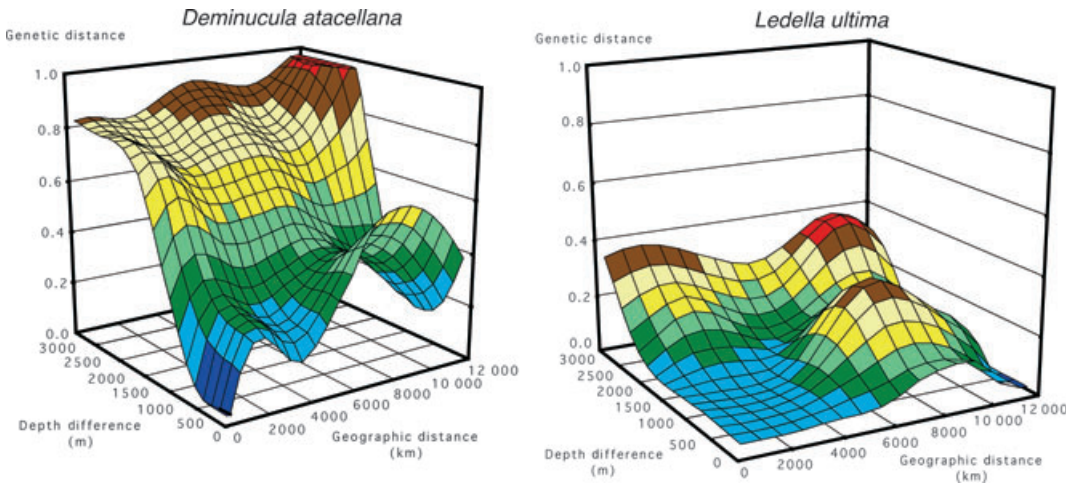

Fig. 3 Genetic distance (pairwise Φ_{ST}) as a function of the geographical distance (km) and depth difference (m) separating all samples for a bathyal protobranch bivalve *Deminucula atacellana* (from Zardus *et al.* 2006) and for the abyssal protobranch bivalve *Ledella ultima*. Both species have pan-Atlantic distributions and were sampled across similar scales. Distance-weighted least squares was used to smooth the surface. Only samples with greater than three individuals were included.

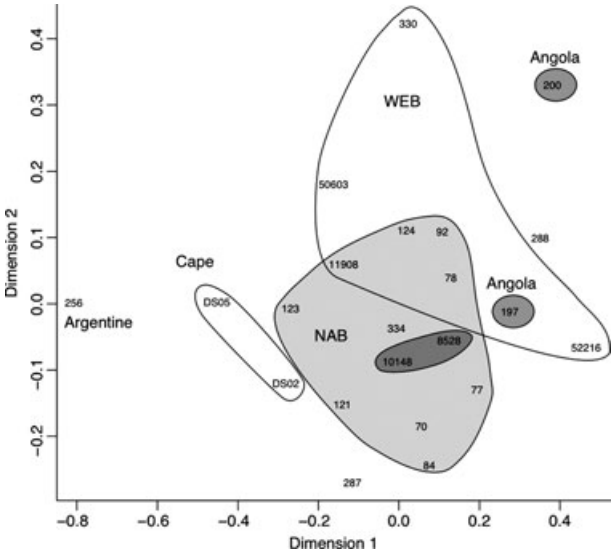

Fig. 4 Nonmetric multidimensional scaling of the pariwise genetic distances (Slatkin's linearized Φ_{ST}) among samples of *Ledella ultima*. Envelopes encircle the samples from the Cape, North American and Western European Basins. The samples from the Canaries are circled within the NAB envelope. Only samples with greater than three individuals were included.

Discussion

Abyssal populations of *L. ultima* exhibited virtually no genetic divergence within basins and only modest divergence among Atlantic basins. Levels of genetic divergence were related to the geographical distance separating samples with the most divergent sample from the Argentine basin, our most southern site. Very few other studies have examined population structure of abyssal organisms, but those that have generally found extremely low divergence. Atlantic and Pacific populations of the amphipod *Eurythenes gryllus* were genetically homogeneous at abyssal depths for the 16S ribosomal gene (France & Kocher 1996), while Arctic and Antarctic populations of three foraminifera species were essentially genetically identical based on a nuclear ribosomal RNA gene (Pawlowski *et al.* 2007; Lecroq *et al.* 2009). A Southern Ocean abyssal munnopsid isopod exhibited considerable variation at the 16S ribosomal gene indicative of cryptic species, but little variation within putative species (haplogroups) (Raupach *et al.* 2007). Evidence to date suggests abyssal populations appear to be genetically similar over unusually large distances (although see Scheckenbach *et al.* 2010 on abyssal microbial diversity), but much more work will be required to know whether this pattern is ubiquitous.

Low genetic divergence over enormous geographical scales is not unique to abyssal organisms. Shallow-water marine species sometimes exhibit little population structure over huge areas (e.g. Lessios *et al.* 1998, 2003; Lessios & Robertson 2006; Craig *et al.* 2007; Horne *et al.* 2008), but this is typically for species that have planktotrophic development, long pelagic periods and that disperse in much faster surface currents. For example, dispersal in the surface currents across the East Pacific Barrier (7000 km) would take 100–155 days (Craig *et al.* 2007). Larvae dispersing in abyssal currents (assuming an average velocity of 1 cm/sec) would take more than 20 years to cover the same distance. Consequently, gene flow should be considerably less and

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Table 3 AMOVA results for *Ledella ultima* from eight basins within the Atlantic. The degree of population structure among basins was analysed for both pooled (within basins) and individual samples

Grouping	Source of variation	d.f.	Sum of squares	Variance component	Per cent of total	*P*
Basin (individual samples)	Total	164	145.83	0.91		
	Among basins	6	13.32	0.08	8.60	**<0.001**
	Among stations within Basins	15	10.42	−0.02	−2.38	0.866
	Within stations	143	122.1	0.85	93.78	0.059
Depth (individual samples)	Total	168	149.38	0.88		
	Among Depths	3	2.24	−0.01	−1.75	0.728
	Among stations within Depths	19	23.19	0.05	5.79	**0.017**
	Within stations	146	123.94	0.85	95.96	**0.037**
Basin (pooled samples)	Total	173	155.58	0.92		
	Among basins	7	13.79	0.06	6.73	**<0.001**
	Within basins	166	141.79	0.85	93.27	
NAB vs. WEB (individual samples)	Total	96	183.21	1.94		
	Between groups	1	3.04	0. 04	2.34	**0.006**
	Among stations within groups	11	19.69	−0.02	−0.84	0.779
	Within stations	84	160.48	1.91	98.5	0.472
North vs. South (individual samples)	Total	164	145.83	0.93		
	Between groups	1	4.12	0.05	5.98	**0.016**
	Among stations within groups	20	19.61	0.02	1.87	0.313
	Within stations	143	122.1	0.85	92.16	0.06

Statistical probabilities were derived from 1023 permutations; significant values are in bold.

population structure much greater across similar distances for abyssal organisms dispersing in substantially slower currents. Yet surprisingly, we find populations exhibit only modest divergence with shared haplotypes between the North and South Atlantic.

The modest divergence at abyssal depths contrasted strongly with patterns at bathyal depths. Pan-Atlantic and Trans-Atlantic analyses of a wide variety of bathyal organisms have identified significant population structure that appears to be associated with various geographical, topographical, hydrographic or environmental features (e.g. Creasey *et al.* 1997; Chase *et al.* 1998a; Rogers 2000; Stepien *et al.* 2000; Roques *et al.* 2002; Weinberg *et al.* 2003; Howell *et al.* 2004; Le Goff-Vitry *et al.* 2004; Aboim *et al.* 2005; Etter *et al.* 2005; Zardus *et al.* 2006; Pawlowski *et al.* 2007, Roy *et al.* 2007). Divergence is often sufficient to suggest cryptic species, especially for populations separated by large distances or at different depths (France & Kocher 1996; Chase *et al.* 1998a; Etter *et al.* 1999, 2005; Kojima *et al.* 2001; Quattro *et al.* 2001; Zardus *et al.* 2006; Raupach *et al.* 2007), although this has yet to be unequivocally established because most analyses involve only a single locus. Multiple loci will be essential to distinguish speciation from extreme population divergence within a single lineage (Maddison & Knowles 2006; Knowles & Carstens 2007; Knowles 2009a,b) because of the stochasticity inherent in mutagenesis and coalescence. Vicariant events have also been implicated in promoting divergence at bathyal depths and the radiation of the deep-water fauna (Wilson 1998; Kojima *et al.* 2001; Aboim *et al.* 2005, Iguchi *et al.* 2007a,b; Stefanni & Knutsen 2007). The obvious contrast between bathyal and abyssal patterns suggests that the ecological and evolutionary forces that promote population differentiation decrease with depth. This pattern is consistent with the DDH and appears to be scale invariant, operating within and between deep-ocean basins.

Why should divergence be lower at abyssal depths? Although not homogeneous, the abyss is considerably less variable than the bathyal environments of the continental margins. The intensity of environmental gradients (temperature, pressure, nutrient flux, disturbance, etc.) parallels changes in depth, with the steepest changes at upper bathyal depths. The continental margins are topographically complex, creating pronounced spatial variability in currents, sedimentation, nutrient flux and sediment geochemistry that, in turn, creates strong heterogeneity in the composition, structure and function of bathyal communities (e.g. Danovaro *et al.* 2008; Gooday *et al.* 2010; Levin *et al.* 2010; Vanreusel *et al.* 2010). In contrast, the abyss is a vast continuous plain with gently rolling hills and gradual changes in depth. Currents are much weaker and vary on larger spatial scales and longer timescales (Richardson 1993; Hogg & Owens 1999; Speer *et al.* 2003). Temperature changes little at abyssal depths with the temperatures at the poles within a couple of degrees of those at the

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equator. Temporal variation is also greater at bathyal depths, which have been impacted by fluctuating global temperature (Kurihara & Kennett 1988; Cronin & Raymo 1997), sea level regressions associated with Pleistocene glaciation (Slowey & Curry 1995; Lambeck & Chappell 2001), gravity-driven sediment slumps (Masson *et al.* 1994, 1996; Levin *et al.* 2001) and oxygen minimum zones (Rogers 2000; Helly & Levin 2004; Gooday *et al.* 2010). The pronounced environmental heterogeneity on the continental margins plays an important role in the maintenance of the high diversity at bathyal depths (Levin & Dayton 2009; Gooday *et al.* 2010; Levin *et al.* 2010; Vanreusel *et al.* 2010) and very likely plays an equally important role in promoting population divergence and ultimately the formation of new species. Our work indicates that bathymetric patterns of population divergence are correlated with patterns of species diversity and that these two metrics of biodiversity may be interdependent and jointly influenced by depth-related variation in environmental heterogeneity. The interdependence of genetic and species diversity is common in other systems (e.g. Vellend 2005; Vellend & Geber 2005), suggesting ecological and evolutionary processes are intimately linked. Ultimately, the weaker environmental gradients, reduced environmental heterogeneity and lower diversity at abyssal depths may limit population differentiation.

Another possible explanation for reduced divergence at abyssal depths, which is not mutually exclusive, might be extensive gene flow among distant populations. Initially, this hypothesis seems counterintuitive. As with most abyssal macrofauna, *L. ultima* is small (generally <2 mm), usually occurs at low density and persists on an extremely low food supply. Fecundity must be highly constrained by the meagre food supply and minute size (e.g. Tyler *et al.* 1992, 1994). In addition, abyssal currents are generally quite slow and extremely convoluted (Richardson 1993, Hogg & Owens 1999, Bower & Hunt 2000), implying it would take decades for larvae to disperse among basins (as noted previously). To a first approximation then, we might expect connectivity among basins to be limited at abyssal depths. However, several factors might circumvent these constraints and enhance gene flow.

The abyssal regions of the World Oceans represent a vast essentially continuous soft sediment habitat (Smith *et al.* 2008). *Ledella ultima* is widely distributed throughout the Atlantic and appears to be abundant, occurring in 72% of the 121 samples taken below 3000 m in the Atlantic (Allen 2008). On average, there were 122 individuals per sample with some samples exceeding 1000 individuals. Estimates of effective population size based on coalescence (Data S1) exceed 10 million just within the NAB (assuming a heuristic generation time of 1 year). Given the continuity of the abyssal regions and the frequency and abundance of *L. ultima* in samples, gene flow might be mediated over vast distances by stepping-stone dispersal among continuously distributed populations (Excoffier *et al.* 2009; Hellberg 2009). The observed IBD is consistent with stepping-stone dispersal effecting gene flow among populations. The distance larvae disperse in the deep sea is unknown, but the frigid temperatures should reduce metabolic demands and perhaps allow them to disperse for extended periods of time (Manahan 1990; Shilling & Manahan 1991; Welborn & Manahan 1991; Marsh *et al.* 1999; Kelly & Eernisse 2007, Peck *et al.* 2007). Larval durations and dispersal distances might be further enhanced if the larvae can absorb dissolved organic material as found for some shallow-water invertebrates (Jaekle & Manahan 1989, Shilling & Manahan 1991; Moran & Manahan 2004). Despite the extremely slow abyssal currents, stepping-stone dispersal and increased larval durations should increase connectivity among regions and reduce divergence.

Patterns of genetic variation within and between populations of *L. ultima* are consistent with large relatively stable populations with high connectivity. Over 90% of the genetic variation found throughout the Atlantic was within populations (basins) (Table 3). This contrasts sharply with bathyal organisms where much of the genetic variation is between populations (Creasey *et al.* 1997; Chase *et al.* 1998b, Rogers 2000; Stepien *et al.* 2000; Roques *et al.* 2002; Weinberg *et al.* 2003; Le Goff-Vitry *et al.* 2004; Howell *et al.* 2004; Aboim *et al.* 2005; Etter *et al.* 2005; Zardus *et al.* 2006). While each basin had a large number of endemic haplotypes for *L. ultima*, the two main haplotypes were found in most regions suggesting extensive gene flow or retention of ancestral polymorphisms. Large stable populations would also lose haplotypes very slowly, suggesting that the lack of strong geographical patterns might also reflect, to some extent, the retention of ancestral polymorphism. The low growth rates, extreme longevity (Turekian *et al.* 1975), continuous distribution, enormous population size and connectivity across vast distances mediated by stepping-stone dispersal would all conspire to reduce population divergence and slow evolution at abyssal depths.

Recent colonization

Other possible explanations for limited divergence at abyssal depths include a recent pan-Atlantic range expansion or a selective sweep followed by a population expansion. There is little evidence for a selective sweep, although mismatch analyses (not shown) were consistent with a recent bottleneck followed by an

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expansion. However, there were several lines of evidence for range expansions. Bayesian Skyline Plots (Fig. S1) indicate that populations of *L. ultima* began a slow steady expansion more than 1 Ma in many deep-water basins with the greatest expansions in the northern basins (NAB and WEB). An IMa analysis (Table S1) indicates that the NAB and WEB split from a smaller ancestral population around the same time as these expansions. In addition, the marginally nonsignificant IBD over much of the Atlantic (especially the northern basins) suggests a population that has been slightly displaced from gene flow–drift equilibrium, which might also reflect a range expansion.

Mid-Atlantic Ridge

Topographical and hydrographic features may play an important role in the evolution of deep-sea organisms because they can disrupt the continuity of the abyss and have the potential to impede gene flow among populations. The most distinctive topographical feature in the Atlantic is the Mid-Atlantic Ridge (MAR), an enormous mountain chain that separates the eastern and western Atlantic corridors. The MAR might isolate populations on either side if the changes in hydrostatic pressure required to pass over the ridge axis exceed their physiological tolerance. This might be especially true for *L. ultima*, which is rarely found above 3000 m. Some deep-sea organisms appear to evolve in response to differences in pressure (e.g. Siebenaller & Somero 1978, 1979, 1982; Siebenaller 1984; Somero 1990, 1992; Morita 2008) and are often restricted to specific windows of hydrostatic pressure (Young & Tyler 1993; Young *et al.* 1996a,b; Tyler & Young 1998). In addition, topographical steering of deep-water currents might hamper cross-axis larval transport and limit connectivity between eastern and western corridors (Mullineaux *et al.* 2002; Speer *et al.* 2003; Young *et al.* 2008).

Although *L. ultima* exhibits generally low levels of divergence throughout the Atlantic, the genetic divergence between NAB and WEB (Table 3) is consistent with the MAR disrupting gene flow between eastern and western populations. Populations separated by similar distances within the western North Atlantic corridor were not different (AMOVA comparing NAB to Guyana, $P = 0.076$), suggesting the differences between NAB and WEB were not just a function of distance. Although the NAB and WEB were genetically different, the IMa analysis suggested the divergence occurred despite some continual low level of gene flow (Tables S1–S3). Divergence also occurred across the MAR in the South Atlantic between the Argentine and Angola basins, although we lack sufficient replication in the south to test statistically.

Of course, many other mechanisms might mediate divergence across such vast distances (e.g. Palumbi 1994). Elucidating the role of specific oceanographic or topographical features in the evolution of the deep-water fauna will require more detailed studies focused on putative mechanisms. These early studies are meant to narrow the possibilities and identify the scales and geography of population structure in this immense and remote ecosystem.

Our knowledge of the evolutionary processes within the vast deep-sea theatre is extremely limited and highly fragmented, especially at abyssal depths. The results so far indicate that population divergence is modest over surprisingly large distances, suggesting diversification might be significantly lower at these depths. The low divergence at abyssal depths is consistent with the DDH and may, in part, explain why species diversity peaks at intermediate depths and declines out on the abyssal plains. The increase in cosmopolitan species with depth (Allen & Sanders 1996) might also reflect low levels of divergence at abyssal depths. Because the deep sea is intimately connected to surface processes (Gooday 1988; Smith & Kaufmann 1999; Billett *et al.* 2001, Ruhl & Smith 2004; Ruhl 2007; Glover *et al.* 2010), the reduced divergence at abyssal depths has important implications for how abyssal organisms might respond to rapid climate change at the surface, which might profoundly alter the structure, composition and function of abyssal ecosystems.

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The coauthors are members of the Etter lab group who have an interest in using molecular genetics to understand evolutionary processes in the deep sea and the origins of the deep-sea fauna. They address questions on the mechanisms of population differentiation, the geographic and bathymetric scales of population structure, phylogeography and speciation. Their work focuses on the evolution of mollusks in the deep Atlantic.

Supporting information

Additional Supporting Information may be found in the online version of this article.

Data S1 Demographic analyses

Fig. S1 Bayesian skyline plots showing changes in the estimated size of N_e (assuming 1-year generation time) for *L. ultima* from six Atlantic basins with sufficient sampling. Years indicate time before present. Coloured region represents the 95% CL for N_e.

Table S1 Estimates from IMa of θ (q) for the NAB and WEB, migration rates between basins and splitting time. Lo and Hi90 are the lower and upper bounds of the estimated 90% highest posterior density. Estimates of N_e assume a 1-year generation time. WEB -> NAB implies migration from WEB to NAB

Table S2 Results from an analysis of nested models in IMa. The high point values for each parameter, the Log(*P*) which is proportional to the marginal likelihoods of the models, the degrees of freedom (d.f.) and the likelihood ratio test (2LLR) are shown

Table S3 Information theoretic statistics for each of the nested models and the full model from IMa (Table S2). The nested models from IMa, the number of parameters for each model (k), the AIC score, AIC differences (Δ_i), model probabilities (ω_i) and the evidence ratio are given. All values are calculated as described in Carstens *et al.* (2009)

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