



Unexpected relationship between dispersal strategies and speciation within the association *Bathymodiolus* (Bivalvia) - *Branchipolynoe* (Polychaeta) inferred from the rDNA neutral ITS2 marker

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Introduction

A significant number of marine invertebrate species inhabiting the deep-sea hydrothermal vent environment produce planktonic larvae with varying life-spans in the water column. An obvious benefit derived from long-term dispersal abilities is that species may colonize new areas over a wide geographical range, and therefore maintain gene flow between their fragmented populations. Deep-sea hydrothermal-vent mytilid bivalves and their commensal polychaetes *Branchipolynoe* form large associations in many venting areas occurring along ridges of the eastern and western Pacific and the Atlantic. Hydrothermal-vent mytilids produce numerous pelagic larvae which can be carried away by water currents over great distances (Lutz, 1988). Such dispersal abilities should favour a low genetic variation between populations over a wide range of geographical distances and a low number of distinct allopatric species. However, allozymes indicated the existence of cryptic species in the Atlantic but no genetic differentiation between populations of the eastern Pacific species, *Bathymodiolus thermophilus* Kenk & Wilson, 1985 (see Craddock et al., 1995a, 1995b). Similarly, morphological studies allowed the description of numerous *Bathymodiolus* species in the western Pacific back-arc basins and along the mid-Atlantic Ridge (Cosel et al., 1994; Cosel et al., in press). The life cycle of the commensal polynoid polychaetes is not very well known and their reproductive biology still needs to be assessed. *Branchipolynoe* lives inside the mantle cavity of the vent mussels and might feed upon its host since it displays $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values similar to those found in the host. Only

three distinct species of this polynoid genus have been described, each one being associated with a different biogeographic area, namely the eastern Pacific (*B. symmytilida* Pettibone, 1984), the Atlantic (*B. seepensis* Pettibone, 1986, but see Chevaldonné et al., this volume) and the western Pacific (*B. pettiboneae* Miura & Hashimoto, 1991). Parasites and commensals are generally known to evolve more rapidly than their hosts and are thus often equally or more diversified. The unexpected imbalance between the high number of species in *Bathymodiolus* and the low number of species in *Branchipolynoe* raises questions about (i) the coevolutionary processes affecting these two taxa and (ii) the role of larval dispersal on genetic differentiation and speciation in such a patchy and unstable environment.

To examine these questions, several of the above-mentioned species were investigated using molecular techniques, applied both to the bivalves and their commensal polychaetes. The internal transcribed spacer ITS2 of the ribosomal DNA was chosen for its high substitution rate and as it is also a good species marker (Schlötterer et al., 1994). It was therefore used to estimate the level of genetic divergence between Pacific and Atlantic species within both genera of the association.

Materials and methods

I. Specimen collection

The Pacific mussel *Bathymodiolus thermophilus* was collected at 9°50'N, 13°N and 17°S on the East Pacific Rise during the HOT'96 cruise. The Atlantic mussels *Bathymodiolus puteoserpentis* Cosel, Métivier &

Hashimoto, 1994 and *Bathymodiolus* sp. were collected from vent sites at Lucky Strike (37°17'N), Menez Gwen (37°50'N), Broken Spur (29°N), Snake Pit (23°N) and Logatchev (14°45'N) during the DIVA 1994 and the 1995 MicroSmoke cruises. Mussels were collected with the submersible Nautilie and brought back to the surface inside an insulated box. The *Branchipolynoe* worms were collected alive from the pallial cavity of the infested mussels when present. The anterior part of the worm's body and the mussel's adductor muscle were dissected and individually preserved on board in BLB buffer (50 mM Tris-HCl pH 8.0, 250 mM EDTA, 5% SDS) at 4°C.

II. DNA extraction, PCR amplification and sequencing

BLB-preserved tissues (1 g) were digested overnight with proteinase K at 55°C in 0.5 ml PK-SDS lysis buffer (Tris-HCl 50mM pH 8.0, NaCl 100mM, 10 mM EDTA, 1% SDS). Genomic DNA was then purified using standard phenol/chloroform extractions and stored at 4°C in TE (10 mM Tris, 1 mM EDTA pH 8.0). The non coding transcribed spacer (ITS2) separating the 5.8S and 28S ribosomal genes was amplified using a set of "universally" applicable primers designed by Holland and associates from a wide range of metazoan sequences:

PH19 5'-CATC GACA CTTT/C GAAC GCA-3'
ITS2 5'-AATC CTGG TTAG TTTC TTTT CCTC CGCT- 3'

Amplification reactions were performed on a Stratagene Robot-Cycler. The optimal PCR cycling parameters were 1 cycle: 3 mn/96°C; 35 cycles: 1.15 mn/50°C, 1 mn /72°C, 1 mn/96°C; 1 cycle: 2 mn/50°C, 10 mn/72°C. PCR products were visualised on 2% agarose gels containing ethidium bromide under UV-light.

PCR-products of Atlantic and Pacific species of *Bathymodiolus* and their commensal polychaete *Branchipolynoe* were then purified and both strands were sequenced for at least two individuals per vent sector. PCR-products were first T/A-end ligated into a BlueScript™ T-vector plasmid at 16°C overnight and, cloned in to DH5α competent cells. Positive clones were screened using PCR techniques. Both DNA strands were sequenced using a 725 VISTRA™ DNA sequencer and either T3 or T7 TexasRed primers. Fragments were subjected to electrophoresis on to a 5% Hydrolink Long Ranger™ gel. Multiple sequence alignments were performed with the GCG package using the progressive pairwise alignment method (pileup). Deletions and polymorphic restriction sites were identified from the set of aligned ITS2 sequences. Unrooted Neighbor-Joining trees were obtained from the PHYLIP package. The number of substitutions between two non-coding sequences was calculated according to Kimura's two-parameter model.

Results

I. Genetic divergence in the MAR bivalves of the genus *Bathymodiolus*

A few *Bathymodiolus* sequences were aligned to perform a preliminary unrooted neighbor-joining tree (Fig. 1). Mussels sampled along the Mid-Atlantic Ridge display two distinct ITS2 sequences which differ from each other by 6 transitions, 4 transversions and 4 small deletion/insertions. These sequences distinguish mussels of Lucky Strike and Menez Gwen vent sectors (37°N) from that of the Snake Pit (23°N) area. However, the ITS2 sequences of the two individuals sampled at Broken Spur (29°N) greatly differ and indicate that populations of two distinct types (the Lucky Strike-type and the Snake Pit-type) are mixed in this sector (Fig. 1). The level of variation between the Snake Pit-type mussels is greater than both that found between the Lucky Strike-type mussels, and that of individuals of *B. thermophilus* along the East Pacific Rise (i.e. 9°50'N vs. 13°N), and corresponds to, at most, 5 substitutions (4 transitions and 1 transversion).

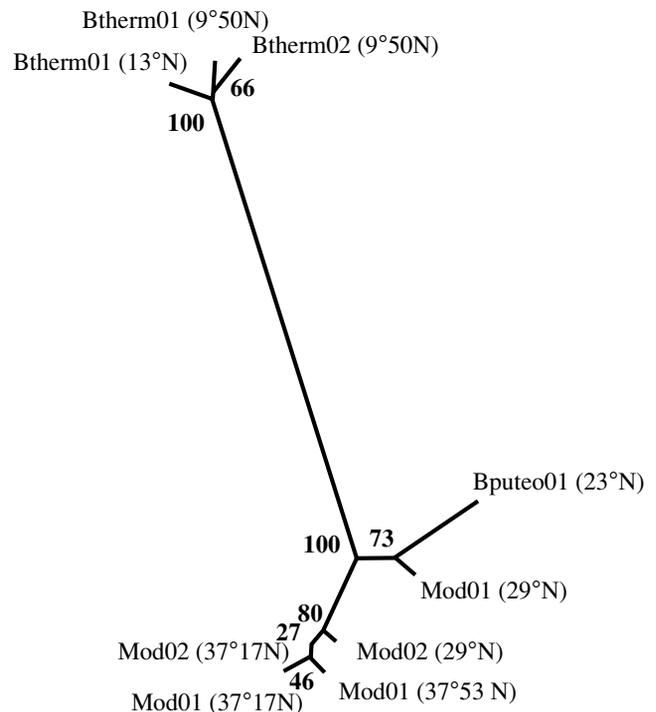


Figure 1. Unrooted neighbor-joining tree obtained from nine ITS2 430-452 bp. sequences of Pacific and Atlantic vent mussels. Distances are calculated from 16 informative sites according to the Kimura's two parameter model and bootstrap values are estimated from 100 replicates.

II. Genetic divergence in the MAR *Branchipolynoe*

In a similar way, a few *Branchipolynoe* ITS2 sequences were used to produce a second unrooted neighbor-joining tree in order to parallel the spatial evolution of deep-sea vent mussels and their commensal (Fig. 2). In contrast to the mussels, the level of divergence obtained between these polynoids along the Mid-Atlantic Ridge is low and not sufficient to reveal any cryptic species. Percentages of sequence homology vary from 98.6 to 99.8% and rely upon the occurrence of 6 deletion/insertions (due to intra-individual microsatellite length polymorphisms) and erratic substitutions, from which only one polymorphic transversion was detected. However, one transition and two GC insertions can be used to distinguish between individuals from the Logatchev area and those from the Snake Pit and the Lucky Strike vent sectors.

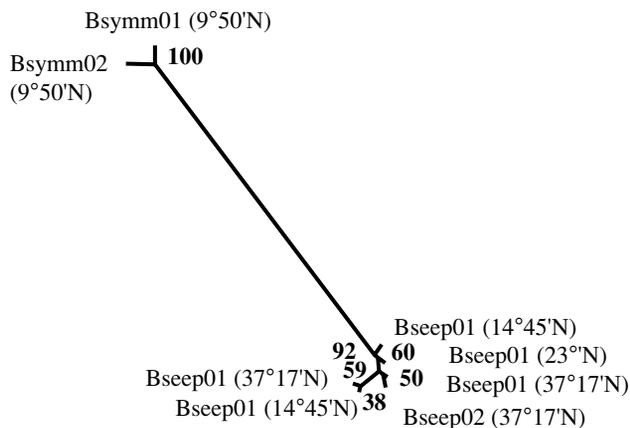


Figure 2. Unrooted neighbor-joining tree obtained from ten ITS2 455-465 bp. sequences of Pacific and Atlantic vent commensal polynoids (*Branchipolynoe* spp.). Distances are calculated from 47 informative sites according to the Kimura's two parameter model and bootstrap values are estimated from 100 replicates. *: in some individuals, ITS2 sequences slightly differ from one clone to another, indicating the occurrence of an intra-individual polymorphism.

III. Genetic divergence between Pacific and Atlantic species

Sequence alignments between Atlantic and Pacific bivalves indicate that *Bathymodiolus puteoserpentis* is equally related to *B. thermophilus* and *Bathymodiolus* sp. from Lucky Strike. The number of substitutions per nucleotide K between the two former species is equal to 0.0380 and corresponds to 15 substitutions (6 transitions and 9 transversions), whereas that obtained between the two latter species is about 0.0330 and corresponds to 14 substitutions (6 transitions and 8 transversions), both sequence alignments

showing 10 deletion/insertions. In contrast, K is equal to 0.1104 between the Pacific and Atlantic ITS2 consensus sequences of *Branchipolynoe*, which gives a theoretical number of 50 substitutions (based on 25 transitions and 22 transversions). This high level of divergence is also correlated to the occurrence of 20 deletion/insertions. These calculations show that the commensal *Branchipolynoe* diverged more rapidly than its host *Bathymodiolus*.

Discussion

The sequence alignment in *Bathymodiolus* spp. does support the occurrence of, at least, two distinct species along the Mid-Atlantic Ridge with a contact-zone at Broken Spur (29°N). Previous works (Craddock et al., 1995a) showed genetic differences between well-separated populations of various species of *Bathymodiolus*, which confirmed that deep-sea vent mussels are greatly diversified at a global scale. Larval retention due to the hydrothermal convection and the spatial isolation of the Atlantic vent fields may explain why two mussel species co-occur along the Mid-Atlantic Ridge. However, Craddock et al. (1995b) documented an extensive gene flow between populations of *Bathymodiolus thermophilus* along the East Pacific Rise and the Galapagos Rift which is in agreement with the supposed long-term persistence of the vent mussel larvae in the water column (Lutz, 1988). Conversely, the phylogeographical analysis of the ITS2 sequences of *Branchipolynoe* indicates that no cryptic species occurs along the Mid-Atlantic Ridge despite geological discontinuities (see also Chevaldonné et al. this volume). Such findings seem paradoxical and do not fit the dispersal expectations for both genera. In contrast to deep-sea mussels, the commensal polynoids seem to have a lecithotrophic development (or even a direct development) which indicates that it might be a poor disperser. A preliminary study showed that females of *B. seepensis* display an unusual reproductive tract with spermathecae full of spermatozoa, and an ovisac containing large (500 µm) mature oocytes (S. Hourdez & C. Jouin-Toulmond, unpublished data). As a consequence, the absence of speciation in *Branchipolynoe* along the Mid-Atlantic Ridge, although it seems to evolve faster than its host, is somewhat paradoxical and could only be explained by episodic exchanges between the vent sectors. Young (1994) has questioned the validity of indirect inferences, such as egg size, for estimating dispersal potential of deep-sea organisms. Large amounts of yolk reserves could indeed favour the long-term dispersal of lecithotrophic larvae at low temperatures. This could represent a non-negligible advantage for lecithotrophic species dispersing in oligotrophic regions as compared to planktrophic ones.

Levels of divergence and evolutionary rates

Sequence alignments strengthened the interest of using ribosomal transcribed spacers as a fine-tuned marker to separate closely-related hydrothermal-vent species. Levels of divergence obtained between Atlantic and Pacific species of both taxa are high and fall within those obtained between closely-related species of terrestrial invertebrates (Schlötterer et al., 1994). However, this level is 3 times greater for *Branchipolynoe* ($K = 0.110$) than for *Bathymodiolus* ($K = 0.035$). In contrast to the former genus, the level of divergence indicates that mussel isolation may have occurred about 3.5 million years ago, when the Panama seaway closed (Knowlton et al., 1993) assuming that such markers evolve at a high rate in invertebrates (i.e. 1.2% substitutions/Myr: Schlötterer et al., 1994). This result may indicate an earlier geographic isolation in the polynoids that could be due to a difference in the duration of the planktonic phase. However, the observed difference between levels of divergence obtained between Pacific and Atlantic species of both taxa is questionable and could also be due to other biological discrepancies. First, population sizes of both taxa might be unequal, a varying proportion of the mussels being infested. However, this does not imply that the effective population sizes are unequal, since internal fertilization (as compared to the external fertilization in mussels) may compensate for a possible smaller polynoid population size (see: Hedgecock et al., 1994). Second, generation-time effects and/or differing molecular clocks could also explain this difference as mussels and polynoids seem to exhibit different reproductive strategies.

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