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A hybrid zone between hydrothermal vent mussels (Bivalvia: Mytilidae) from the Mid-Atlantic Ridge

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Abstract

This study provides the first example of a hybrid zone between animal taxa distributed along the mid-ocean ridge system. We examined the distribution and genetic structure of deep-sea hydrothermal vent mussels (Bivalvia: Mytilidae) along a 2888-km portion of the Mid-Atlantic Ridge between 37°50′ N and 14°45′ N latitude. Mitochondrial DNA (mtDNA), allozymes and multivariate–morphometric evidence discriminated between individuals of a northern species, *Bathymodiolus azoricus*, and a southern species, *B. puteoserpentis*, that were separated by an intermediate ridge segment almost devoid of mussels. A small sample of mussels from Broken Spur, a vent locality along this intermediate zone, revealed a mixed population with gene frequencies and morphology that were broadly intermediate to those of the northern and southern species. Multilocus clines in mtDNA and allozyme frequencies were centred over the intermediate zone. We consider intrinsic and extrinsic processes that might limit genetic exchange across this hybrid zone.

Keywords: allozymes, *Bathymodiolus azoricus*, *Bathymodiolus puteoserpentis*, hybridization, intergradation, mitochondrial DNA, morphometrics

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Introduction

Hybrid zones between closely related species have been studied extensively in terrestrial and aquatic environments (Harrison 1993; Arnold 1997). Considerable attention has focused on: (i) the origin of these zones, i.e. primary vs. secondary contact; (ii) intrinsic vs. extrinsic forces that act to maintain their stability; and (iii) potentially constructive vs. destructive roles that hybridization may play in speciation (e.g. Anderson & Stebbins 1954; Mayr 1963; Endler 1977; Moore 1977; Barton & Hewitt 1985; Harrison 1990; Rieseberg *et al*. 1995; Dowling & Secor 1997). Until recently, hybrid zones were thought to be less frequent in oceans for several reasons (for a comprehensive review, see Gardner 1997). First, much of the marine environment (except the intertidal) was thought to be relatively continuous, not offering ecotones or steep environmental gradients believed necessary to stabilize hybrid zones. Second, many marine organisms have pelagic larvae with long-distance dispersal abilities that could easily swamp forces acting to maintain hybrid zones (Bert & Harrison 1984). Finally, except for coastal regions and economically important species, much of the world's oceans and its inhabitants are poorly known.

Nonetheless, the view that hybridization is rare in oceans appears to be mistaken, although hybrid zones are not likely to be as narrow or discrete as in terrestrial environments (Gardner 1997). Not surprisingly, discrete hybrid zones are found in fish with relatively limited dispersal abilities (Planes & Doherty 1997; Seeb 1998; Roques *et al*. 2001). Yet, hybrid zones also are found in marine species that possess a pelagic stage capable of long-distance dispersal. For example, closely related species of stone crabs (genus *Menippe*) hybridize at narrow contact zones along the Atlantic and Gulf of Mexico coasts of northern Florida (Bert & Arnold 1995; but see Schneider-Brussard *et al*. 1997). In contrast, northern and southern species of hard-clams (genus *Mercenaria*) hybridize across a broad area ranging from South Carolina to Southern Florida (Bert & Arnold 1995). Species of blue mussels (genus *Mytilus*) hybridize at multiple contact zones, best described as mosaic, in the Atlantic and Pacific Oceans (Skibinski *et al*. 1978; Coustau *et al*. 1991;

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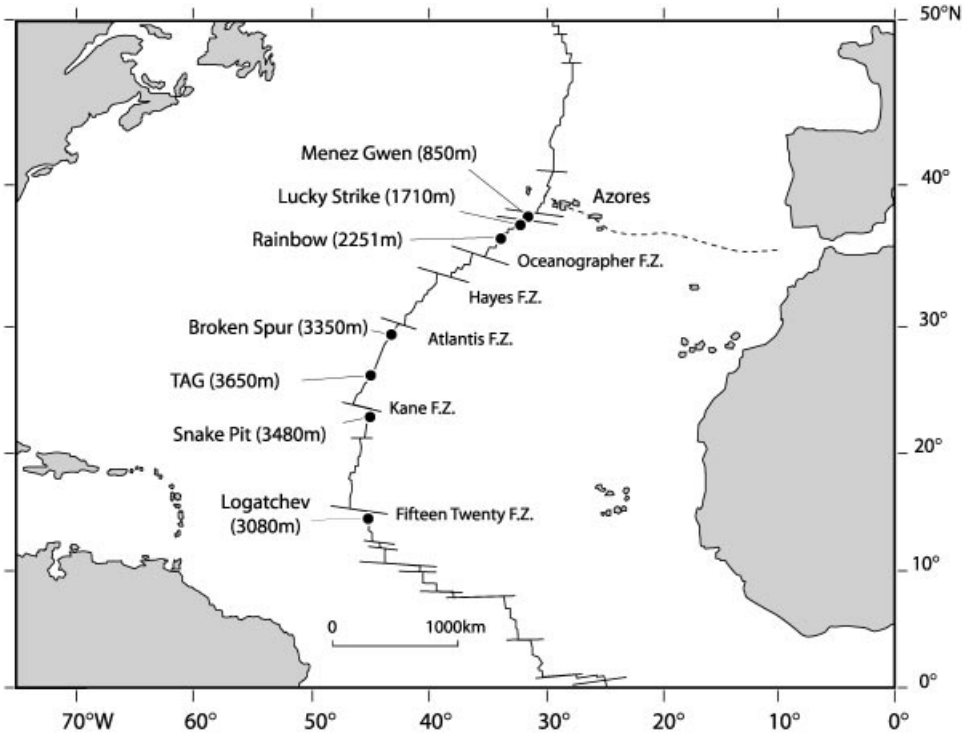

Fig. 1 Mid-Atlantic Ridge hydrothermal vent fields. The base map is from Needham (1996) with additional details from German & Parson (1997).

Väinölä & Hvilsom 1991; Gardner 1994; Inoue *et al*. 1997). Species of soles (genus *Solea*) hybridize broadly in the Mediterranean (She *et al*. 1987). Although the shapes of these zones (i.e. narrow vs. broad, discrete vs. mosaic) may vary greatly, the existence of a pelagic larval stage does not necessarily eliminate opportunities for the formation of marine hybrid zones. Ultimately, dispersal that is realized in the form of gene flow may be considerably less than the dispersal potential inferred from studies of larval life histories (Burton 1986; Hedgecock 1986).

Although a picture of marine hybrid zones is emerging from studies of near-shore and economically important species, little is known about mid-ocean species. In this study, we describe a hybrid zone between two closely related species of *Bathymodiolus* mussels (Bivalvia: Mytilidae) from deep-sea hydrothermal vents along the Mid-Atlantic Ridge (MAR; Fig. 1). Compared with near-shore environments, most of the deep sea is relatively homogeneous in time and space (Gage & Tyler 1991), and thus may not be conducive to genetic divergence and the formation of hybrid zones. Hydrothermal vent habitats provide a notable contrast, however. Individual vent localities are known to be relatively ephemeral and patchily distributed along the global mid-ocean ridge system. Discrete vent fields along the MAR are separated by great distances (> 1000 km), large topographical discontinuities (e.g. fracture zones > 100 km), bathymetric differences (> 2000 m) and shearing oceanic currents that might act as barriers to along-ridge dispersal and favour divergence of populations (Van Dover 1995). Thus, hybrid zones between genetically distinct populations may be more likely along the mid-ocean ridge system than in the rest of the deep sea.

The dense concentration of organisms around hydrothermal vents contrasts sharply with the greater dispersion of organisms in most of the deep sea (Gage & Tyler 1991). In the absence of sunlight and photosynthesis, bacteria that exploit energy-rich compounds concentrated in vent fluid support lush vent communities. Although *Bathymodiolus* mussels are capable of filter feeding, they derive most of their nutrition from endosymbiotic bacteria that oxidize hydrogen sulfide or methane as sources of energy (Fisher *et al.* 1987). These mussels typically occur in large clusters attached to the walls of high-temperature vents or in surrounding areas of diffuse flow, rich in sulfides or methane. Studies of larval shell morphology revealed that the eastern Pacific vent mussel, *Bathymodiolus thermophilus*, has a feeding larval stage with the potential for long distance dispersal (Turner & Lutz 1985). The absence of substantial genetic differentiation in this species was consistent with high rates of gene flow across several thousand kilometres of the northern East Pacific Rise and Galapagos Rift (Craddock *et al*. 1995b).

The MAR differs greatly, however, across a comparable range of several thousand kilometres (Fig. 1). It houses two closely related species of mussels, *B. azoricus* and

B. puteoserpentis, first described from widely separated northern (Lucky Strike; LS) and southern (Snake Pit; SP) type-localities (von Cosel *et al.* 1994, 1999; Craddock *et al.* 1995a). Subsequent molecular studies expanded knowledge of their distributions and discovered that the two species overlap at a geographically intermediate locality, Broken Spur (Jollivet *et al.* 1998; Maas *et al.* 1999), but it was not known that they hybridized. Recent reports (Comtet 1998; Tyler & Young 1999) suggest the two species differ in reproductive mode; *B. azoricus* exhibiting evidence for hermaphroditism and *B. puteoserpentis* being dioecious. The principal goals of this study were: (i) to thoroughly sample known vent fields along the MAR and better define geographical boundaries of the two species; (ii) to examine the potential for hybridization between them; and (iii) to consider potential roles of extrinsic (e.g. ridge topography, bathymetry and geochemistry) and intrinsic factors that might act to maintain their genetic integrity.

Materials and methods

Specimens

During 5–27 July 1997, we used the deep submergence vehicle *Alvin* to search for and collect mussels from seven localities along the MAR (Fig. 1, Table 1). *Alvin*'s robotic manipulators were used to grab specimens and place them in an insulated box containing cold (2 °C) ambient seawater. At the surface, specimens were held in 4 °C filtered seawater for no more than 4 h. We immediately froze (–70 °C) small mussels (max. length = 25 mm). Large mussels (max. length > 25 mm) were dissected, samples of gill, mantle and adductor muscle tissue were frozen separately, and shells were dried, labelled and measured. All frozen samples were shipped on dry ice to Rutgers University and stored at –80 °C prior to genetic analysis.

DNA

Details of DNA extraction, purification, PCR and sequencing are provided in Maas *et al.* (1999). A region of mitochondrial genome that included part of the NADH dehydrogenase subunit 4 (*ND4*) gene was amplified using the following primers: Arg BL (5′-CAAGACCCTTGATTT-CGGCTCA-3′, Bielawski & Gold 1996); and NAP 2H (5′-TGGAGCTTCTACGTGA/GGCTTT-3′, Arevalo *et al.* 1994). GenBank Accession nos AF128533 and AF128533 represent the two major haplotypes that distinguish *Bathymodiolus puteoserpentis* and *B. azoricus* (reported as *B. n.* sp. in Maas *et al.* 1999).

We examined a 646-bp region of DNA from 32 individuals and six locations (Table 1). The fragment included the first third of the *ND4* gene and 129 bp of sequence upstream to its ATG start codon. The upstream sequence appears to contain tandomly arranged transfer RNAs for methionine (tRNA-Met) and valine (tRNA-Val). Sequence divergence within and between populations was estimated as the average of all pairwise distances after correction with the Jukes–Cantor algorithm. To increase the sample size, we examined diagnostic restriction fragment length polymorphism (RFLP) profiles (see Maas *et al.* 1999) in 48 additional individuals from five locations (Table 1).

Allozymes

Details of the allozyme methods are provided in Maas *et al.* (1999). Fourteen putative gene loci were examined in the earlier study. For this study we examined the products of five polymorphic loci that that were represented by 14 alleles (*Lap***A*, **B*, **C*; *Mdh-1***A*, **B*; *Mdh-2***A*, **B*; *Pep-gl***A*, **B*, **C*; and *Pgi***A*, **B*, **C*, **D*). The remaining nine loci were essentially monomorphic within and between species.

Unless otherwise noted, the program GENEPOP (Version 3.3, Raymond & Rousset 1995) was used for population genetic analyses. We used the 'exact tests' of Rousset & Raymond (1995) to assess deviations from random mating expectations and to test for heterozygote deficiencies or excesses. Genic differentiation between populations was assessed with an 'exact test' (Goudet *et al.* 1996). Genotypic disequilibrium between pairs of allozyme loci was assessed by the contingency method and significance was determined by an 'exact test' employing the Markov chain

Table 1 Mussels collected during July 1997 along the Mid-Atlantic Ridge (MAR). The total sample collected, and the sample sizes used for mitochondrial sequencing, RFLP, and allozyme analyses are given

Location (abbr.)	Latitude/longitude	No. dives	Depth (m)	Species	Sample size	No. seq.	No. RFLP	No. alloz.
Menez Gwen (MG)	37°50′ N/31°31′ W	2	850	*Bathymodiolus azoricus*	327	5	11	33
Lucky Strike (LS)	37°17′ N/32°15′ W	5	1710	*B. azoricus*	488	5	11	64
Rainbow (RB)	36°14′ N/33°54′ W	2	2251	*B. azoricus*	67	3	6	33
Broken Spur (BS)	29°10′ N/43°10′ W	3	3350	mixed	11	11	0	11
TAG (TAG)	26°8′ N/44°49′ W	2	3650	no mussels	0	0	0	0
Snake Pit (SP)	23°22′ N/44°56′ W	2	3480	*B. puteoserpentis*	180	3	9	42
Logatchev (LO)	14°45′ N/44°58′ W	3	3080	*B. puteoserpentis*	354	5	11	63

method. We used the CND program (Asmussen & Basten 1994) to assess cytonuclear disequilibrium of mitochondrial and allozyme markers. The statistical package JMP (SAS Institute 2000) was used to perform canonical analysis of multilocus allozyme genotypes. To perform this analysis, we transformed the five-locus allozyme genotype of each individual by the 'allelic coding method' (She 1987; Roques *et al*. 2001). A code of 0, 1 or 2 is given for each allele depending, respectively, on whether it is absent, heterozygous, or homozygous. For example, two five-locus allozyme genotypes were recoded as follows.

Individual 1					Individual 2				
A/B	A/B	B/B	A/C	B/C	B/C	B/B	A/B	B/C	B/D
110	11	02	101	0110	011	02	12	011	0101

Individuals with incomplete multilocus genotypes were excluded from the analysis. We were aware that these data are not multivariate normal and used the scores for descriptive purposes only (see Manly 1986).

Morphology

Shell measurements (*A* = anterior length, *H* = maximum height, *L* = max. length, *W* = max. width, *G* = ligament length, and *U* = umbo length) are described in Maas *et al*. (1999). In the earlier study of three populations [LS, SP, Logachev (LO)], a stepwise discriminant analysis of these shell measurements provided almost complete separation of the two species. We used this discriminant formula to compute canonical scores based on natural log transformed measurements of the five shell measurements in individuals from three new localities [Menez Gwen (MG), Rainbow (RB) and Broken Spur (BS)]. To remain consistent with our earlier studies of deep-sea mytilids, we did not adjust for size by estimating ratios or using regression residuals (see Gustafson *et al*. 1998). Such adjustments 'have little if any useful role in most morphometric analyses ... (size) does not explain somehow irrelevant variance but rather perfectly meaningful covariance' (Bookstein *et al*. 1985; p. 27). Statistical analyses were performed with JMP (SAS Institute 2000).

Results

Distribution and abundance of MAR mussels

We searched for *Bathymodiolus* mussels at seven MAR vent fields that were known to us during July 1997 (Table 1). Dense populations of mussels were observed at northern and southern localities but not in the middle of the sampled range. The two northernmost localities had mounds of mussels that included a broad range of sizes from small recruits to large adults (shell lengths, MG = 14–121 mm; LS = 20–115 mm; see Table 2 for means). Mussels were somewhat less abundant at RB, and the size range was narrower (39–101 mm). In contrast, very few mussels were observed along the intermediate segment between the Atlantis and Kane Fracture Zones. Only 11 individuals were sampled during three dives at BS. No mussels were observed during the first two dives (*Alvin* dive nos 3123–4). The third dive (no. 3125) was devoted entirely to searching for mussels that were observed as solitary individuals attached to an actively venting, sulfide edifice, the 'Spire.' The specimens collected were large (55–112 mm), and new recruits were not evident despite searches of sulfide rubble and fissures in areas of diffuse venting.

Table 2 Variation of shell length, mitochondrial DNA, and five polymorphic allozyme loci in MAR *Bathymodiolus*. Individual locus (f_i) and multiple-locus average (F_{IS}) heterozygote deficiencies are given for the allozyme loci

Populations Character	MG	LS	RB	BS	SP	LO
Length (mm)						
mean	63.9	57.2	67.9	80.3	104.9	81.3
SD	23.3	21.2	16.9	20.4	18.9	20.8
N	141	123	33	10	40	110
mtND4 (No.)						
az	16	16	9	7	—	—
pu	—	—	—	4	12	16
Lap (Freq.)						
**A*	0.788	0.543	0.625	0.364	0.021	0.040
**B*	0.212	0.435	0.354	0.409	0.688	0.540
**C*	—	0.022	0.021	0.227	0.292	0.420
fi	0.289	0.675**	0.245	−0.074	−0.206	−0.416
Pep-gl (Freq.)						
**A*	0.172	0.127	0.121	0.136	0.048	0.025
**B*	0.813	0.849	0.848	0.818	0.929	0.910
**C*	0.016	0.024	0.030	0.045	0.024	0.066
fi	0.209	0.160	−0.131	−0.127	−0.047	−0.070
Pgi (Freq.)						
**A*	0.054	0.017	0.052	0.045	—	—
**B*	0.518	0.542	0.500	0.773	0.881	0.893
**C*	0.375	0.407	0.362	0.136	0.119	0.074
**D*	0.054	0.034	0.086	0.045	0.000	0.033
fi	0.103	−0.282	0.055	−0.149	−0.123	−0.083
Mdh-1 (Freq.)						
**A*	0.550	0.655	0.650	0.889	0.891	0.980
**B*	0.450	0.345	0.350	0.111	0.109	0.020
fi	0.408*	0.253	0.210	−0.067	−0.100	†
Mdh-2 (Freq.)						
**A*	0.786	0.759	0.750	0.944	0.913	0.900
**B*	0.214	0.241	0.250	0.056	0.087	0.100
fi	0.169	−0.300	0.392	†	−0.073	0.351
overall F_{IS}	0.229*	0.115	0.162	−0.09	−0.134	−0.164

*$P < P'_6 = 0.0083$; **$P < P'_{28} = 0.0018$. See text for Bonferroni correction method. †No estimate, because only one heterozygote was observed.

Table 3 Mean sequence divergence ($\hat{d}$) within and between populations (standard errors in parentheses) of MAR *Bathymodiolus*. The six collection sites are labelled with abbreviations (see Table 1). The *azoricus* and *puteoserpentis* haplotypes found at the BS locality were treated separately

	B. azoricus			Hybrid population		*B. puteoserpentis*	
Locality	MG	LS	RB	BS *az*	BS *pu*	SP	LO
MG	0.0039 (0.0024)	0.0030 (0.0021)	0.0027 (0.0019)	0.0043 (0.0025)	0.1024 (0.0133)	0.1024 (0.0133)	0.1013 (0.0132)
LS		0.0021 (0.0018)	0.0019 (0.0016)	0.0037 (0.0023)	0.1038 (0.0134)	0.1038 (0.0135)	0.1026 (0.0134)
RB			0.0016 (0.0016)	0.0034 (0.0021)	0.1038 (0.0134)	0.1038 (0.0134)	0.1026 (0.0133)
BS *az*				0.0052 (0.0028)	0.1030 (0.0134)	0.1030 (0.0134)	0.1018 (0.0133)
BS *pu*					0.0000 (0.0000)	0.0000 (0.0000)	0.0011 (0.0011)
SP						0.0000 (0.0000)	0.0011 (0.0011)
LO							0.0021 (0.0018)

The TAG hydrothermal mound appeared to be devoid of mussels; none were observed or collected during two *Alvin* dives. However, we observed dense clumps of mussels again at localities south of the Kane Fracture Zone. Mussels from SP (58–141 mm) and LO (24–139 mm) were larger, on average, than samples taken from northern localities. Although our sampling efforts were not consistent, the samples taken from these seven areas roughly reflect local densities.

Mitochondrial DNA (mtDNA) analysis and haplotypic distribution

To identify mussels that predominate at each locality, we examined mitochondrial *ND4* sequences that discriminate between the two species (Maas *et al*. 1999). First, we examined 646 bp sequences from three to five individuals from each locality, except BS for which we sequenced the entire sample of 11 (Table 1). To increase sample sizes, we subsequently used species-diagnostic *Cac*8I restriction profiles to type specimens (Fig. 4 in Maas *et al*. 1999). Each of the 15 unique *ND4* sequences could be assigned to one of the two major haplotypes: *az* corresponding to *B. azoricus* and *pu* to *B. puteoserpentis*. The average Jukes–Cantor distance ($\hat{d}$) between *az* and *pu* sequences was 10.3% (Table 3), which included 13 inferred amino acid substitutions. A neighbour-joining tree illustrates high divergence between vs. low divergence within each species (Fig. 2). We found three variants of the *pu* haplotype (mean = 0.21%), two of which (*pu*1 and *pu*2) shared a nonsynonymous substitution. We found 12 variants (*az*1 to *az*12) of the *az* haplotype (mean = 0.32%), all of which were defined by synonymous substitutions.

Based on this marker, the two species segregated along the north/south axis of the MAR (Table 1). The *az* haplotypes occurred at three northern localities (MG, LS and RB), *pu* haplotypes occurred at two southern localities (SP and LO). Both were present at BS, an intermediate locality with seven *az* and four *pu* haplotypes.

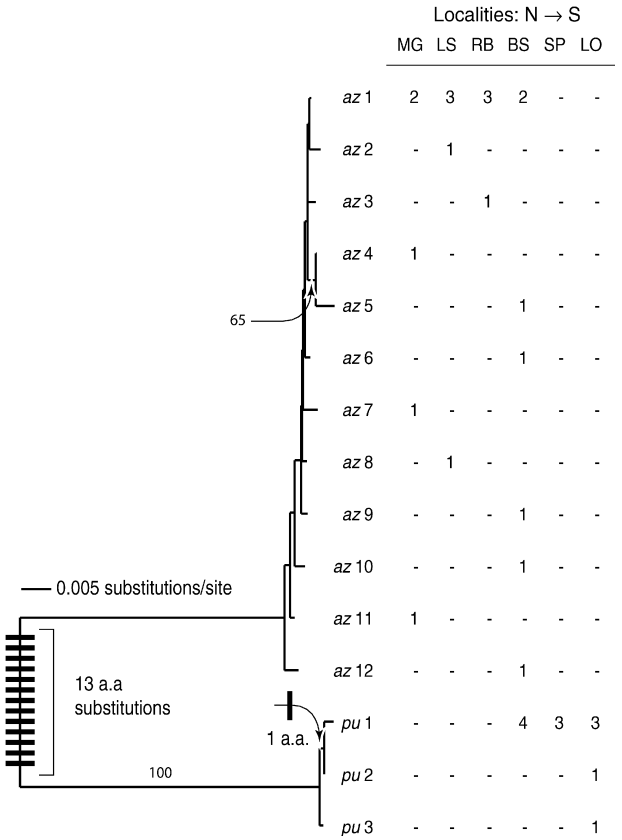

Fig. 2 Neighbour-joining tree of *ND4* haplotypes and their distribution at Mid-Atlantic Ridge vent localities. The tree was based on pairwise sequence divergence (Jukes–Cantor correction) and is rooted along the midpoint of its longest branch. Black hashes indicate amino acid substitutions. Small numbers on the tree represent percentage of 1000 bootstrap replications.

Allozymes and gene frequency clines

To assess whether the BS mussels comprised a mixture of the parental species or an intergrade population, we examined allozymes encoded by five loci (Table 2). Overall, allelic frequencies did not differ significantly

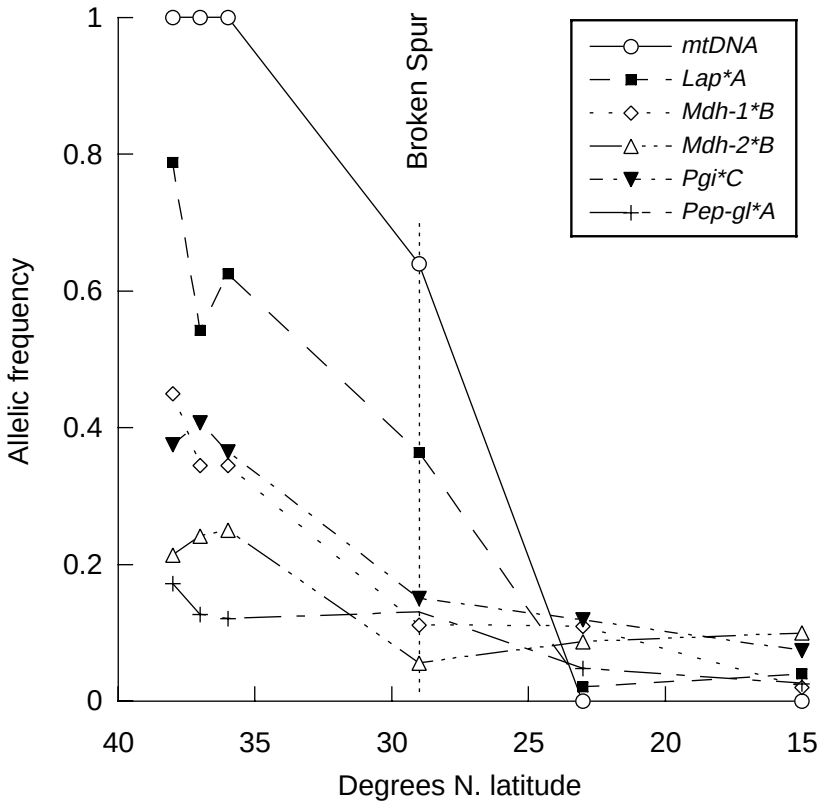

Fig. 3 Representative gene frequency clines exhibited by alleles at five polymorphic loci and mitochondrial *ND4*. See Table 2 for a complete listing of allelic frequencies.

among samples (MG, LS and RB) identified as *B. azoricus* ($P_{all\ loci\ combined}$ = 0.441). However, examination of individual loci revealed that *Lap* frequencies differed among the *B. azoricus* samples (P_{Lap} = 0.031). Overall, allelic frequencies did not differ significantly between the samples (SP and LO) identified as *B. puteoserpentis* ($P_{all\ loci\ combined}$ = 0.224) or at individual loci. In contrast, comparisons involving all the samples revealed significant genic heterogeneity. Significant differences existed at four loci: $P_{Lap} = P_{Pgi} = P_{Mdh\text{-}1}$ = 0; and $P_{Pep\text{-}gl}$ = 0.005), and the fifth was marginally significant (P_{Mdh2} = 0.07). The combined test was highly significant ($P_{all\ loci\ combined} \approx 0$). Although no locus exhibited 'species-diagnostic' alleles, differences in allelic frequencies were manifested as clines that changed with latitude (Fig. 3). Frequency shifts centred over the geographically intermediate BS population.

Discriminant analysis was used to reduce the multilocus allozyme genotype of individuals to a value that could be arrayed along a single canonical axis (Fig. 4a). BS mussels were not used to generate the discriminant function:

$$A = 0.212\ Lap^*A + 0.131\ Lap^*B + 0.044\ Pep\text{-}gl^*A + 0.039\ Pep\text{-}gl^*B + 0.092\ Pgi^*A - 0.055\ Pgi^*B + 0.023\ Pgi^*C - 0.051\ Mdh\text{-}1^*A - 0.023\ Mdh\text{-}2^*A.$$

Only $k - 1$ of k alleles at each polymorphic locus were used to generate this function. When this function was applied to BS individuals, they were broadly intermediate to northern and southern populations, as would be expected for a hybrid population. The mitochondrial haplotypes (a = *az* and p = *pu*) associated with these genotypes are listed above each bar in the histogram. It is noteworthy that the three leftmost individuals, predicted by discriminant analysis to be *B. azoricus*, also had *az* mitochondrial haplotypes. They may represent 'pure' *B. azoricus*. Three individuals also with *az* haplotypes were *Lap**A/C* heterozygotes, a combination that occurred in no other population because the **A* and **C* alleles are nearly diagnostic of the 'pure' species (see *Linkage disequilibrium and hybridization*, below).

Allozymes and breeding systems

Systematic deviations from the genotypic proportions expected under random-mating can provide insights into the breeding system of a population (Weir 1996). We observed slight excesses of heterozygotes at most polymorphic loci in the SP and LO samples of *B. puteoserpentis*, which is reported to be dioecious (Tyler & Young 1999). The fixation index (f_i), a measure of heterozygote deficiency, was negative in eight of nine cases where it could be estimated (Table 2). Situations in which we found only one heterozygote (e.g. *Mdh-1* in LO) were excluded from the analysis. Following application of a table-wide sequential

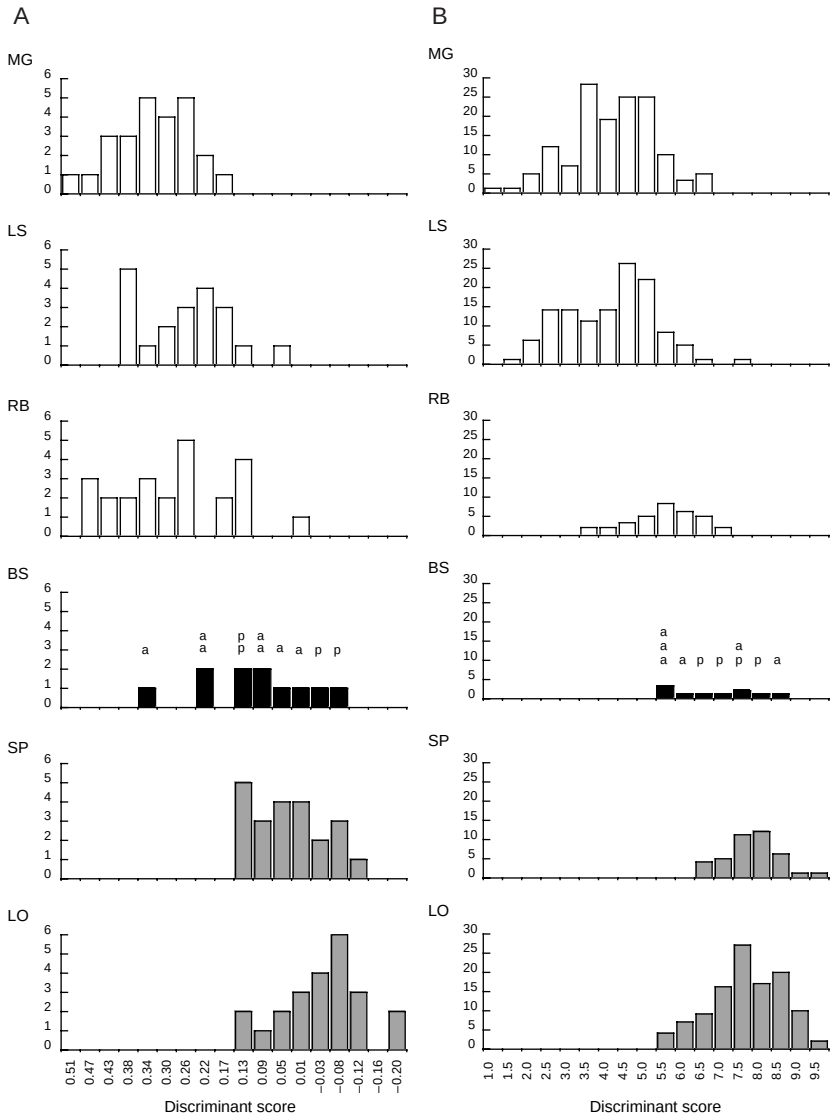

Fig. 4 Discriminant analyses of genetic and morphometric traits in Mid-Atlantic Ridge mussels: (a) allozyme genotypes; and (b) shell measurements. Open bars are *Bathymodiolus azoricus*; grey bars are *B. puteoserpentis*; and black bars are from the hybrid zone. Letters above the black bars indicate the mitochondrial types (a = *az* and p = *pu*) found in each class.

Bonferroni (SB) correction ($P'_{28} = 0.05/28 = 0.0018$), no f_i values were statistically significant in these two samples (for method see Rice 1989). Multilocus fixation indices (F_{IS}) also were negative in these two populations (−0.134 and −0.164), but not statistically significant after application of a separate SB correction for this level of analysis ($P'_6 = 0.05/6 = 0.0083$). Testing against the alternative hypothesis of heterozygote excess did not increase the number of significant tests for f_i or F_{IS} values; however, the combined probability (Fisher's method) across the two populations was significant ($P = 0.0301$). The observation of heterozygote excesses is unusual in population genetic studies of marine bivalves (discussed below). All individuals were examined at least twice for each locus, and we do not believe these excesses resulted from a problem of scoring because heterozygote excesses have not been observed in other species of *Bathymodiolus* that exhibit similar allozyme phenotypes (Craddock *et al*. 1995a,b; below).

A previous investigation of *B. azoricus* revealed that some individuals are hermaphroditic (Comtet 1998), a condition observed in several other *Bathymodiolus* species (Le Pennec & Beninger 1997). However, these morphological examinations could not resolve whether the mussels were simultaneous or sequential hermaphrodites. The results of our allozyme analysis revealed an overall pattern of heterozygote deficiency that is consistent with some degree of selfing. The f_i values were positive in 12 of 15 cases (Table 2), but only one ($f_i = 0.675$ for *Lap* in LS; $P = 0.0003 < P'_{28} = 0.0018$) was significant after application of the table-wide SB correction. Multilocus F_{IS} values also were elevated in the three *B. azoricus* samples, but only the highest value ($F_{IS} = 0.229$ for MG; $P = 0.0066 < P'_6 = 0.0083$) was significant. Testing against the alternative hypothesis

of heterozygote deficiency did not increase the number of significant tests for f_i or F_{IS} values; however, the combined probability across all three *B. azoricus* populations was significant ($P'_1 = 0.0221$).

If some selfing occurs in *B. azoricus*, we could estimate its proportion (S) from the relationship $f_e = S/(2 - S)$, which holds at equilibrium (Hedrick 2000). Substituting F_{IS} values for f_e and solving for S, we estimated that 21–30% of matings in *B. azoricus* populations could be from selfing. However, other factors are believed to cause the heterozygote deficiencies commonly observed in marine bivalves, e.g. null alleles, aneuploidy, cryptic population subdivision (Wahlund effects) in time and space, etc. (see for example, Foltz 1986; Thiriot-Quievreux *et al*. 1988; Gaffney *et al*. 1990; Li & Hedgecock 1998).

If cryptic subdivision exists in *B. azoricus* populations, we might also expect to observe linkage disequilibrium between loci (Ohta 1982). A test of genotypic disequilibrium between all pairs of polymorphic loci in the six population samples revealed only one potentially significant association (i.e. $P < 0.05$) of 50 tests. None were significant after a table-wide sequential Bonferroni correction, but this adjustment for type I errors left us with little power to reject the null hypothesis given our sample sizes. Nevertheless, none of the combined *P*-values (Fisher's method) across these six populations was significant for any locus-pair (range of combined *P*-values: 0.398–0.999). Thus, we found no evidence for cryptic subdivision in any of the six samples.

Linkage disequilibrium and hybridization

The absence of species-diagnostic nuclear markers limited our opportunity to analyse admixture. However, the *Lap* locus was informative: *Lap*A* was almost completely restricted to *B. azoricus*; *Lap*C* was nearly restricted to *B. puteoserpentis*; and *Lap*B* occurred in both species although it was most frequent in *B. puteoserpentis* (Table 2). Excluding the BS sample, the *az* and *pu* mitochondrial types were tightly coupled with *Lap*A* and *Lap*C*, respectively, in 98.2% of the 56 individuals examined for both markers. If the parental types regularly dispersed into the hybrid zone, we expected to see an excess of the parental combinations (*az*/*Lap*A* and *pu*/*Lap*C*) in the BS sample. The sample of 11 mussels was too small for a multiallelic test, so we lumped the *Lap*B* and **C* alleles which together are nearly fixed in the southern populations (Table 4). The likelihood ratio χ^2 (*G*-test) of the gametic matrix was marginally significant ($\chi^2 = 3.405$, d.f. = 1, $P = 0.065$). We also used the CNP programs (Asmussen & Basten 1994; Basten & Asmussen 1997) to test for disequilibrium. The combination of southern *Lap* genotypes with northern *az* mtDNA was marginally deficient (= −0.141; $nr^2 = 4.055$, $P = 0.087$), but a sample size of at least 26 individuals was needed to detect the observed level of disequilibrium with power of $1 - \beta = 0.9$ and type I error of $\alpha = 0.05$. Clearly, a larger sample was needed, but unfortunately collecting mussels at a depth of 3000 m with a manned submersible does not always produce the desired sample size when individuals are scarce.

Table 4 Observed (expected) zygotic and gametic proportions of nuclear *Lap** and mitochondrial *ND4* genotypes observed in the Broken Spur sample. Expected values were generated by the contingency method

	Mitochondrial type		
*Lap**	*az*	*pu*	Total
Zygotic			
A/*A*	1 (0.6)	0 (0.4)	1
A/—	5 (3.8)	1 (2.2)	6
—/—	1 (2.6)	3 (1.4)	4
total	7	4	11
Gametic			
A	7 (5.1)	1 (2.9)	8
—	7 (8.9)	7 (5.1)	14
total	14	8	22

Nevertheless, examination of the BS genotypes provided some insight regarding admixture. When mitochondrial types were superimposed on the canonical allozyme scores, several BS individuals appeared to be parental types. The leftmost three individuals in Fig. 4a carried *az* mtDNA and a corresponding allozyme score and might have been parental types. Others mussels had mixed genotypes; in particular, three individuals with the *az* haplotype were *Lap*A*/*C* heterozygotes. The *Lap*A* and **C* alleles were essentially fixed in *B. azoricus* and *B. puteoserpentis*, respectively.

Morphology

Maas *et al*. (1999) showed that stepwise discriminant analysis effectively separates *B. azoricus* and *B. puteoserpentis* individuals. Using a canonical function based on four shell measurements,

$$C = 1.12 \ln A - 38.16 \ln U + 34.39 \ln L + 4.71 \ln H,$$

individuals with values of $C < 5.5$ could be classified as *B. azoricus* and those with values of $C > 6.5$ could be classified as *B. puteoserpentis*. Discrimination between the two species was due in part to differences in size. *B. puteoserpentis* were larger, on average, than *B. azoricus* (Table 2), and the *C*-score was correlated with shell length ($r = 0.671$). Nevertheless, all size measures were highly correlated (*r*-values among $\ln L$, $\ln H$ and $\ln U$ all were > 0.971). However, the size measures cancel one another in the canonical function (above), leaving character *A* with

the strongest effect on *C* ($r = 0.871$). This character, the length of shell anterior to the umbo, was visibly greater in all size classes of *B. puteoserpentis*.

We applied this discriminant function to shell measurements from all the populations examined in this study. Excluding the BS mussels, 93.2% of the combined sample of 442 individuals were correctly assigned to species. Many individuals could not be measured because their fragile shells were badly damaged during collection. The *B. azoricus* mussels from MG were essentially identical to those from LS (ANOVA of *C*-values, $F_{1,261} = 0.1218$; $P = 0.724$). However, *C*-scores of RB mussels were shifted to the right of MG and LS ($F_{2,293} = 24.2886$; $P < 0.0001$), in part a consequence of their slightly larger size.

The *C*-scores of *B. puteoserpentis* mussels from SP and LO did not differ significantly from one another ($F_{1,151} = 0.8300$; $P < 0.3637$). However, combined, they were highly significantly different from the combined *B. azoricus* samples ($F_{1,442} = 932.0226$; $P < 0.0001$). These dramatic shifts to higher C-scores also were associated in part with larger sizes.

C-Scores of the BS mussels were shifted mostly into the *B. puteoserpentis* range, although they differed significantly from SP and LO ($F_{2,160} = 3.8420$; $P = 0.0235$). The rightmost individual from BS (Fig. 4b) was classified by the discriminant analysis as *B. puteoserpentis* ($C = 8.95$), but it carried *az* mtDNA and a *B. azoricus* allozyme score ($A = 0.24$). Apparently, these morphological phenotypes were decoupled from mitochondrial and allozyme genotypes.

Discussion

Our genetic and morphometric analyses revealed a hybrid zone between two mussel species from hydrothermal vent localities along the MAR (Fig. 1). One species, *Bathymodiolus azoricus* occurred at three neighbouring localities, MG, LS and RB, north of the Oceanographer Fracture Zone. The second species, *B. puteoserpentis*, occurred at two disjunct localities, SP and LO, south of the Kane Fracture Zone. Intermediate ridge segments spanning these regions are nearly devoid of mussels, although two highly active hydrothermal fields (BS and the TAG Hydrothermal Mound) were explored during our 1997 expedition. Previous molecular studies suggested the two species overlap at BS (Jollivet *et al*. 1998; Maas *et al*. 1999), although it was not known that they hybridized. Of the 11 mussels we collected from BS, seven carried a mitochondrial haplotype associated with *B. azoricus* and four carried a haplotype associated with *B. puteoserpentis*. A multivariate analysis of allozyme genotypes (Fig. 4a) revealed the BS mussels as broadly intermediate to the 'pure' species. Furthermore, alleles at several independent loci exhibited coincident clines that centred over the intermediate locality (Fig. 3), a common feature of plant and animal hybrid zones (Endler 1977; Barton & Hewitt 1985).

It is difficult to determine whether a hybrid zone arose as a consequence of primary or secondary contact (Endler 1977, 1982). With our data, we cannot exclude the hypothesis that the BS population represents a transitory stage left over from primary divergence between northern *B. azoricus* and southern *B. puteoserpentis* populations. It is conceivable that latitudinal clines in mtDNA and allozymes are maintained by differential selection that covaries with the different bathymetries of the species. Except for the BS mussels, the known depth range of *B. azoricus* is 850–2250 m, and *B. puteoserpentis* is 3080–3650 m. It should be noted that the steepest allozyme cline occurred at *Lap*, a locus encoding the enzyme leucine aminopetidase. In near-shore mussels (*Mytilus*) *Lap* polymorphisms are known to covary with salinity gradients, and involvement of this enzyme in osmoregulation is believed to form a basis for differential selection across these gradients (Koehn *et al*. 1980; Gardner & Kathiravetpillai 1997). Temperature and salinity can vary greatly across a few centimetres at MAR hydrothermal fields, as ambient seawater (2 °C) mixes with brines and freshwater resulting from phase separation in hydrothermal vents (Desbruyères *et al*. 2000). Mussels tend to cluster in more dilute seawater (Desbruyères *et al*. 2001). If *Lap* variation in these vent mussels is maintained by selection, the cline could have arisen from primary divergence or secondary contact between differentially adapted taxa. Adaptive clines do not provide exclusive evidence for primary contact.

We favour the secondary contact hypothesis for several reasons. *B. azoricus* and *B. puteoserpentis* are separated by an immense distributional gap (≈ 1800 km) containing several hydrothermal fields that appear to be relatively inhospitable for mussels. Compared with other MAR vent fields, Broken Spur may expose vent-endemic organisms to wide fluctuations in habitat quality (Copley *et al*. 1997). During a 1993 expedition to BS, observers identified a small aggregation of mussels and many broken shells near the base of 'Spire', a 12-m sulfide edifice (Murton & Van Dover 1993). No mussels were seen 15 months later, although different areas of this vent field were explored (Copley *et al*. 1997). During our expedition, we carefully explored the BS hydrothermal field and observed only a small number of solitary mussels on the flanks of Spire and in areas of diffuse flow around Mound K. Species that are ubiquitous at other MAR vents, e.g. swarming shrimp (*Rimicaris exoculata*) and bythograeid crabs (*Segonzacia mesatlantica*), also were not abundant at BS. The relatively large mussels sampled there may have been remnants of a waning vent community, as mussels are among the last organisms to disappear at a 'dying' vent (Hessler *et al*. 1988; Fisher 1995). The mussels were covered with rust-coloured sediments enriched in polymetalic sulfides and other

potentially toxic minerals. A high particle flux of these materials is suspected to foul the gills of filtering species such as mussels (Desbruyères *et al*. 2000). Similarly, TAG hydrothermal mound may present an inhospitable environment for mussels. TAG has the highest particle flux measured along the MAR (Desbruyères *et al*. 2000). The immense sulfide edifice (*c*. 35 m high) at the centre of this mound is dominated by dense swarms of shrimp and bythograeid crabs, highly motile organisms that may be less affected by high particle fluxes (Desbruyères *et al*. 2000). Mussel shell debris was observed at the flanks of the large sulfide edifice on TAG, and one mussel was collected by geologists in 1985, but the preserved specimen has not been located (P. Rona, personal communication). Subsequent American (1993) and Russian (1994) expeditions to TAG observed no mussels (Galkin & Moskalev 1990; Van Dover 1995), and we found none there in 1997.

This intermediate ridge segment appears to comprise a 'fitness trough' for *Bathymodiolus*, a hostile region in which both hybrids and nonhybrids are poorly adapted. Nevertheless, some reproduction must occur there, because the Broken Spur sample contained genetically mixed individuals. Alternatively, these mussels might have dispersed from an undiscovered source along this intermediate segment, but this hypothesis seems unlikely because a newly discovered, off-axis, hydrothermal field near Broken Spur ('Lost City' 30° N) lacked mussels and other vent macrofauna (Kelley *et al*. 2001). Nevertheless, periodic volcanic and tectonic activity along this ridge segment may generate suitable hydrothermal habitats that are colonized by *B. azoricus* and *B. puteoserpentis* larvae from northern and southern refugia. Mussels of this family appear to have exceptional long-distance dispersal abilities across comparable ranges in the eastern Pacific (Craddock *et al*. 1995b). Local reproduction at these 'ephemeral' sites could generate mixed populations that persist for a few generations and then die out. In contrast, the dense mussel populations observed north and south of this inhospitable region exhibited a broad range of size classes and evidence for local reproduction and recruitment (Comtet & Desbruyères 1998; Trask & van Dover 1999).

Numerous well-studied hybrid zones appear to be stable in time and space, which raises many questions about stabilizing mechanisms (Harrison 1990). If a hybrid zone is maintained by regular dispersal of parental types and selection against hybrids, a 'tension zone', we expect to observe linkage disequilibrium due to correlations of alleles at different loci (Barton & Hewitt 1985). Disequilibrium should be particularly evident for nuclear genes and correlated mitochondrial haplotypes, especially if hybridization is asymmetrical with regard to the sexes (Asmussen & Basten 1994; Arnold 1997). Unfortunately, with our small sample from Broken Spur, we lacked the statistical power needed to reject equilibrium hypotheses for nuclear or cytoplasmic genes. Nevertheless, examination of individual allozyme and mitochondrial genotypes suggested that some BS mussels were genetically mixed and colleagues may be nonhybrids, a result that is consistent with the tension zone hypothesis.

Evidence that populations along this intermediate ridge segment contribute to introgressive hybridization is mixed. Alternative mitochondrial markers appeared to be fixed north and south of the intermediate zone, suggesting no introgression, but mitochondrial sample sizes are small and we cannot rule out some introgression. Examination of the multivariate distributions of the allozyme and morphometric data (Fig. 4) suggest that limited introgression may have occurred. The multivariate means tended to shift centrally the closer a population was to Broken Spur. The degree of introgression across a hybrid zone depends primarily on: (i) the intensity of selection acting on each gene locus; and (ii) the rate of recombination between loci (Barton 1983). Thus, multilocus clines will tend to converge on a sharp step at their centres even when selection is weak relative to recombination. This convergence can act as a barrier to further gene flow, and the strength of the barrier increases with selection acting at more loci. Such intrinsic barriers can act in the presence of high dispersal rates (Hewitt 1988; Barton & Hewitt 1989; Gardner 1997).

Reproductive differences might also limit wholesale introgression between *B. azoricus* and *B. puteoserpentis*. For example, differential timing of reproduction can determine whether a hybrid zone will, or will not, form between closely related species of near-shore bivalves (Gardner 1997). Reproductive timing is difficult to determine in deep-sea organisms however, because sampling opportunities are limited and sample sizes typically are small (Tyler & Young 1999). Nevertheless, a 'sexual pause' has been reported for *B. azoricus* and recruitment appears to be synchronized across localities (Comtet 1998; Comtet & Desbruyères 1998). Different breeding systems might also limit introgression, as *B. puteoserpentis* is dioecious (Le Pennec & Beninger 1997), whereas *B. azoricus* includes hermaphrodites (Comtet 1998). The significant heterozygote deficiencies observed in *B. azoricus* populations may have resulted from some degree of selfing, although other potential causes of heterozygote deficiencies frequently occur in marine bivalves (Gaffney *et al*. 1990). It is curious in this regard, that slight (nonsignificant) heterozygote excesses occurred in *B. puteoserpentis*. Further research into the breeding systems of these mussels is warranted, preferably with noncoding nuclear DNA markers that are unlikely to be foci of selection (see for example, Karl & Avise 1992; Karl *et al*. 1996).

The MAR hybrid zone matches some aspects of the tension zone model of Key (1968). Tension zones rely upon the interplay between dispersal of parental forms and selection against intermediate genotypes (Barton & Hewitt 1985). This interplay produces stable concordant clines and

a sink for hybrid individuals, although in this case the intermediate zone appears to be equally inhospitable to parental types. Tension zones tend to become trapped by local geographical barriers and reside in areas of low population density, i.e. 'density troughs' (Barton & Hewitt 1985). Based on observations made during numerous French, Japanese, British and American explorations of known MAR vent habitats, it appears certain that this intermediate ridge segment constitutes a density trough for *Bathymodiolus* mussels. Although it is probable that the peculiar chemistry of vent fluids and the high flux of mineral particles in this region may lead to inhospitable habitats for mussels (Desbruyères *et al*. 2000), additional studies are needed to assess potential stress factors.

The MAR provides a sharp contrast with the rest of the deep sea (Gage & Tyler 1991). Rift valleys may serve as corridors for dispersal of vent organisms (Marsh *et al*. 2001). Along the MAR, these valleys can be wide (10s of km) with high walls (1–2 km) and ridge flanks composed of rugged parallel mountain ranges (Kennett 1982). Yet vent communities occupying different ridge segments can be separated bathymetrically by 1000s of metres, and large fracture zones may create discontinuities that limit dispersal and trap hybrid zones. In the north, three major fracture zones (Oceanographer, Hayes and Atlantis) separate dense populations of *B. azoricus* (depth 850–2250 m) from an intermediate segment (depth 3350–3650 m) that is nearly devoid of mussels. In the south, the Kane Fracture Zone (145 km wide) separates this intermediate segment from dense *B. puteoserpentis* populations (depth 3080–3480 m). Shearing ocean currents may interact with these fracture zones to increase opportunities for isolation, but more studies of mid-ocean circulation patterns are needed to assess this possibility (Reid 1994; Thurnherr *et al*. 2001).

We saw no evidence for evolutionary divergence or intergradation of molluscan populations along similar lengths of the northern East Pacific Rise (Vrijenhoek 1997). Fracture zones in this region have low relief, and bathymetry is relatively homogeneous (2500 ± 200 m depth), thus it is not surprising that these topographical features are not associated with divergence of endemic mussels, clams or limpets (Craddock *et al*. 1995b, 1996; Karl *et al*. 1996). EPR rift valleys are narrow with low or absent walls and ridge flanks that grade smoothly to the abyssal plain. Finally, vent habitats along the fast-spreading EPR system tend to be ephemeral, persisting for a few decades (Lalou & Brichet 1982). In contrast, some MAR vent fields may be thousands of years old (Lalou *et al*. 1993). Increased longevity and greater topographical and geochemical complexity of vent fields along slow-spreading systems such as the MAR are believed to create more opportunities for adaptive divergence and speciation (Craddock *et al*. 1995a,b; Van Dover 1995; Desbruyères *et al*. 2000). Consequently, primary and secondary intergradation may be more likely along the MAR than other ridge systems. Phylogeographic studies of other vent-endemic taxa with parallel distributions (e.g. bresiliid shrimp, bythograeid crabs, *Phymorhynchus* gastropods and polychaetes) may reveal whether certain topographical and geochemical features of the MAR present common dispersal barriers or unique barriers that depend on a species' distinct dispersal mode (see for example, Vrijenhoek 1997).

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References

Anderson E, Stebbins G (1954) Hybridization as an evolutionary stimulus. *Evolution*, **8**, 378–388.

Arevalo E, Davis SK, Sites JW Jr (1994) Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloperus grammicus* complex (Phrynosomatidae) in Central Mexico. *Systematic Biology*, **43**, 387–418.

Arnold ML (1997) *Oxford Series in Ecology and Evolution: Natural Hybridization and Evolution*. Oxford University Press, Oxford.

Asmussen MA, Basten CJ (1994) Sampling theory for cytonuclear disequilibria. *Genetics*, **138**, 1351–1363.

Barton NH (1983) Multilocus clines. *Evolution*, **37**, 454–471.

Barton NH, Hewitt GM (1985) Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, **16**, 113–148.

Barton NH, Hewitt GM (1989) Adaptation, speciation and hybrid zones. *Nature*, **341**, 497–503.

Basten CJ, Asmussen MA (1997) The exact test for cytonuclear disequilibria. *Genetics*, **146**, 1165–1171.

Bert TM, Arnold WS (1995) An empirical test of predictions of two competing models for the maintenance and fate of hybrid zones: both models are supported in a hard clam hybrid zone. *Evolution*, **49**, 276–289.

Bert TM, Harrison RG (1984) Hybridization in western Atlantic stone crabs (genus *Menippe*): evolutionary history and ecological context influence species interactions. *Evolution*, **42**, 528–544.

Bielawski JP, Gold JR (1996) Unequal synonymous substitution rates within and between two protein-coding mitochondrial genes. *Molecular Biology and Evolution*, **13**, 880–992.

Bookstein F, Chernoff D, Elder R *et al*. (1985) *Special Publication: Morphometrics in Evolutionary Biology*. The Academy of Natural Sciences of Philadelphia, Philadelphia, PA.

Burton RS (1986) Evolutionary consequences of restricted gene flow among populations of the copepod, *Tigriopus californicus*. *Bulletin of Marine Science*, **39**, 526–535.

Comtet T (1998) Structure des populations, reproduction, croissance et phylogeographie des Mytilidae des champs hydrothermaux Lucky Strike et Menez Gwen (37°17′ N et 37°50′ N sur al dorsale medio-atlantique). These de doctorat, de l'Universite de Bretagne Occidentale, Brest, France.

Comtet T, Desbruyères D (1998) Population structure and recruitment in mytilid bivalves from the Lucky Strike and Menez Gwen hydrothermal fields (37°17′ N and 37°50′ N on the Mid-Atlantic Ridge). *Marine Ecology Progress Series*, **163**, 165–177.

Copley JTP, Tyler PA, Murton BJ, Van Dover CL (1997) Spatial and interannual variation in the faunal distribution at Broken Spur vent field (29° N, Mid-Atlantic Ridge). *Marine Biology*, **129**, 723–733.

von Cosel R, Comtet T, Krylova E (1999) *Bathymodiolus* (Bivalvia: Mytilidae) from hydrothermal vents on the Azores Triple Junction and the Logatchev Hydrothermal Field, Mid-Atlantic Ridge. *Veliger*, **42**, 218–248.

von Cosel R, Métivier B, Hashimoto J (1994) Three new species of *Bathymodiolus* (Bivalvia: Mytilidae) from hydrothermal vents in the Lau Basin and the Fiji Basin, Western Pacific, and the Snake Pit Area, Mid-Atlantic Ridge. *The Veliger*, **37**, 374–392.

Coustau C, Renaud F, Delay B (1991) Genetic characterization of the hybridization between *Mytilus edulis* and *M.galloprovincialis*. *Marine Biology*, **111**, 87–93.

Craddock C, Hoeh WR, Gustafson RG *et al.* (1995a) Evolutionary relationships among deep-sea mytilids (Bivalvia: Mytilidae) from hydrothermal vents and cold-water methane/sulfide seeps. *Marine Biology*, **121**, 477–485.

Craddock C, Hoeh WR, Lutz RA, Vrijenhoek RC (1995b) Extensive gene flow in the deep-sea hydrothermal vent mytilid *Bathymodiolus thermophilus*. *Marine Biology*, **124**, 137–146.

Craddock C, Lutz RA, Vrijenhoek RC (1996) Patterns of dispersal and larval development of archaeogastropod limpets at hydrothermal vents in the eastern Pacific. *Journal of Experimental Marine Biology and Ecology*, **210**, 37–51.

Desbruyères D, Almeida A, Comtet T *et al.* (2000) A review of the distribution of hydrothermal vent communities along the northern Mid-Atlantic Ridge: dispersal vs. environmental controls. *Hydrobiologia*, **440**, 201–216.

Desbruyères D, Biscoiti M, Caprais J-C *et al.* (2001) Variations in deep-sea hydrothermal vent communities on the Mid-Atlantic ridge near the Azores plateau. *Deep Sea Research Part I*, **48**, 1325–1346.

Dowling TE, Secor CL (1997) The role of hybridization and introgression in the diversification of animals. *Annual Review of Ecology and Systematics*, **28**, 593–619.

Endler J (1977) *Geographic Variation, Speciation and Clines*. Princeton University Press, Princeton, NJ.

Endler JA (1982) Problems in distinguishing historical from ecological factors in biogeography. *American Zoologist*, **22**, 441–452.

Fisher CR (1995) Towards an appreciation of hydrothermal vent animals: their environment, physiological ecology and tissue stable isotope values. In: *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions* (eds Humphris SE, Zierenberg RA, Mullineaux LS, Thomson RE), pp. 222–247. American Geophysical Union, Washington DC.

Fisher CR, Childress JJ, Oremland RS, Bidigare RR (1987) The importance of methane and thiosulphate in the metabolism of the bacterial symbionts of two deep-sea mussels. *Marine Biology*, **96**, 59–71.

Foltz DW (1986) Null alleles as a possible cause of heterozygote deficiencies in the oyster *Crassostrea virginica* and other bivalves. *Evolution*, **40**, 869–870.

Gaffney PM, Scott TM, Koehn RK, Diehl WJ (1990) Interrelationships of heterozygosity, growth rate and heterozygote deficiencies in the coot clam, *Mulinia lateralis*. *Genetics*, **124**, 687–699.

Gage JD, Tyler PA (1991) *Deep Sea Biology: A Natural History of Organisms at the Deep-Sea Floor*. Cambridge University Press, Cambridge.

Galkin SV, Moskalev LI (1990) Hydrothermal fauna of the Mid-Atlantic Ridge. *Okeanologia*, **30**, 842–847.

Gardner JPA (1994) The structure and dynamics of naturally occurring hybrid *Mytilus edulis* Linnaeus, 1758 and *Mytilus galloprovincialis* Lamarck, 1819 (Bivalvia, Mollusca) populations: review and interpretation. *Archives of Hydrobiology Supplement*, **99**, 37–71.

Gardner JPA (1997) Hybridization in the sea. *Advances in Marine Biology*, **31**, 1–78.

Gardner JPA, Kathiravetpillai G (1997) Biochemical genetic variation at a leucine aminopeptidase (LAP) locus in blue (*Mytilus galloprovincialis*) and Greenshell (*Perna canaliculus*) mussel populations along a salinity gradient. *Marine Biology*, **128**, 619–625.

German CR, Parson LM (1997) Distribution of hydrothermal activity along the Mid-Atlantic Ridge: interplay of magmatic and tectonic controls. *Earth and Planetary Science Letters*, **160**, 327–341.

Goudet J, Raymond M, DeMeeüs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.

Gustafson RG, Turner RD, Lutz RA, Vrijenhoek RC (1998) A new genus and five species of mussels (Bivalvia, Mytilidae) from deep-sea sulfide/hydrocarbon seeps in the Gulf of Mexico. *Malacologia*, **40**, 63–113.

Harrison R (1990) Hybrid zones: windows on evolutionary process. *Oxford Surveys in Evolutionary Biology*, **7**, 69–128.

Harrison RG (1993) *Hybrid Zones and the Evolutionary Process*. Oxford University Press, Oxford.

Hedgecock D (1986) Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? *Bulletin of Marine Science*, **39**, 550–564.

Hedrick PW (2000) *Genetics of Populations*, 2nd edn. Jones and Bartlett, Sudbury, MA.

Hessler RR, Smithey WM, Boudrias MA *et al.* (1988) Temporal change in megafauna at the Rose Garden hydrothermal vent (Galapagos Rift; eastern tropical Pacific). *Deep Sea Research Part II*, **35**, 1681–1709.

Hewitt GM (1988) Hybrid zones — natural laboratories for evolutionary studies. *Trends in Ecology and Evolution*, **3**, 158–167.

Inoue K, Odo S, Noda T *et al.* (1997) A possible hybrid zone in the *Mytilus edulis* complex in Japan revealed by PCR markers. *Marine Biology*, **128**, 91–95.

Jollivet D, Comtet T, Chevaldonné P *et al.* (1998) Unexpected relationships between dispersal strategies and speciation within the association *Bathymodiolus* (Bivalvia)–*Branchipolynoe* (Polychaeta) inferred from the rDNA neutral ITS2 marker. *Cahiers de Biologie Marine*, **39**, 359–362.

Karl SA, Avise JC (1992) Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. *Science*, **256**, 100–102.

Karl SA, Schutz SJ, Desbruyères D, Lutz RA, Vrijenhoek RC (1996) Molecular analysis of gene flow in the hydrothermal-vent clam *Calyptogena magnifica*. *Molecular Marine Biology and Biotechnology*, **5**, 193–202.

Kelley DS, Karson JA, Blackman DK *et al*. (2001) An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. *Nature*, **412**, 145–149.

Kennett J (1982) *Marine Geology*. Prentice Hall, Englewood Cliffs, NJ.

Key KHL (1968) The concept of stasipatric speciation. *Systematic Zoology*, **17**, 14–22.

Koehn RK, Newell RIE, Immerman F (1980) Maintenance of an aminopeptidase allele frequency cline by natural selection. *Proceedings of the National Academy of Sciences of the USA*, **77**, 5385–5389.

Lalou C, Brichet E (1982) Ages and implications of East Pacific Rise sulfide deposits at 21° N. *Nature*, **300**, 169–171.

Lalou C, Reyss J-L, Brichet E *et al*. (1993) New age data for Mid-Atlantic Ridge hydrothermal sites: TAG and Snakepit chronology revisited. *Journal of Geophysical Research*, **98**, 9705–9713.

Le Pennec M, Beninger PG (1997) Ultrastructural characteristics of spermatogenesis in three species of deep-sea hydrothermal mytilids. *Canadian Journal of Zoology*, **75**, 308–316.

Li G, Hedgecock D (1998) Genetic heterogeneity, detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. *Canadian Journal of Fisheries and Aquatic Science*, **55**, 1025–1033.

Maas PAY, O'Mullan GD, Lutz RA, Vrijenhoek RC (1999) Genetic and morphometric characterization of mussels (Bivalvia: Mytilidae) from Mid-Atlantic hydrothermal vents. *Biological Bulletin*, **196**, 265–272.

Manly BFJ (1986) *Multivariate Statistical Methods, A Primer*. Chapman & Hall, London.

Marsh AG, Mullineaux LS, Young CM, Manahan DT (2001) Larval dispersal potential of the tubeworm *Riftia pachyptila* at deep-sea hydrothermal vents. *Nature*, **411**, 77–80.

Mayr E (1963) *Animal Species and Evolution*. Belknap Press, Cambridge, MA.

Moore WS (1977) An evaluation of narrow hybrid zones in vertebrates. *Quarterly Review of Biology*, **52**, 263–277.

Murton BJ, Van Dover CL (1993) *ALVIN* dives on the Broken Spur hydrothermal vent field at 29°10′ N on the Mid-Atlantic Ridge. *BRIDGE Newsletter*, **5**, 11–14.

Needham HD (1996) Some features of the North America–Africa plate boundary. *Journal of Continents Abstracts*, **1**, 834–835.

Ohta T (1982) Linkage disequilibrium due to random genetic drift in finite subdivided populations. *Proceedings of the National Academy of Sciences of the USA*, **79**, 1940–1944.

Planes S, Doherty P (1997) Genetic and color interactions at a contact zone of *Acanthochromis polyachanthus*: a marine fish lacking pelagic larvae. *Evolution*, **51**, 1232–1243.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Reid JL (1994) On the total geostrophic circulation of the North-Atlantic Ocean — flow patterns, tracers, and transports. *Progress in Oceanography*, **33**, 1–92.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rieseberg L, Van Fossen C, Desrocers A (1995) Hybrid speciation accompanied by genomic reorganization in wild sunflowers. *Nature*, **375**, 313–316.

Roques S, Sévigny J-M, Bernatchez L (2001) Evidence for broad-scale introgressive hybridization between two redfish (genus *Sebastes*) in the North-west Atlantic: a rare marine example. *Molecular Ecology*, **10**, 149–165.

Rousset F, Raymond M (1995) Testing heterozygote excess and deficiency. *Genetics*, **140**, 1413–1419.

SAS Institute (2000) *JMP® User's Guide*, 4th edn. SAS Institute Inc, Cary, NC.

Schneider-Brussard R, Felder DL, Chlan CA, Neigel JE (1997) Tests of phylogeographic models with nuclear and mitochondrial DNA sequence variation in the stone crabs, *Menippe adina* and *Menippe mercenaria*. *Evolution*, **52**, 1671–1678.

Seeb L (1998) Gene flow and introgression within and among three species of rockfishes, *Sebastes auriculatus*, *S. caurinus*, and *S. maliger*. *Journal of Heredity*, **89**, 393–404.

She X (1987) *Analyses moléculaires et numériques de la structure et de la differentiation génétiques des populations naturelles (exemples des genres Solea et Mus)*. Université de Montpellier II, Montpellier.

She X, Autem M, Kotulas G, Pasteur N, Bonhomme F (1987) Multivariate analysis of genetic exchanges between *Solea aegyptiaca* and *Solea senegalensis* (Teleosts, Soleidae). *Biological Journal of the Linnaean Society*, **32**, 357–371.

Skibinski DOF, Beardmore JA, Ahmad M (1978) Genetic evidence for naturally occurring hybrids between *Mytilus edulis* and *Mytilus galloprovincialis*. *Evolution*, **32**, 354–364.

Thiriot-Quievreux C, Noel T, Bougrier S, Dallot S (1988) Relationships between aneuploidy and growth rate in pair matings of the oyster *Crassostrea virginica*. *Aquaculture*, **75**, 89–96.

Thurnherr AM, Richards KJ, German CR, Lane-Serff GF, Speer KG (2001) Flow and mixing in the rift valley of the Mid-Atlantic Ridge. *Oceanography*, in press.

Trask J, Van Dover CL (1999) Site-specific and ontogenetic variations in nutrition of mussels from the Lucky Strike hydrothermal vent field, Mid-Atlantic Ridge. *Limnology and Oceanography*, **44**, 334–343.

Turner RD, Lutz RA (1985) Growth and distribution of mollusks at deep-sea vents and seeps. *Oceanus*, **27**, 55–62.

Tyler PA, Young CM (1999) Reproduction and dispersal at vents and cold seeps. *Journal of the Marine Biological Association of the UK*, **79**, 193–208.

Väinölä R, Hvilsom MM (1991) Genetic divergence and a hybrid zone between Baltic and North Sea *Mytilus* populations (Mytilidae; Mollusca). *Biological Journal of the Linnaean Society*, **43**, 127–148.

Van Dover CL (1995) Ecology of Mid-Atlantic Ridge hydrothermal vents. In: *Hydrothermal Vents and Processes* (eds Parson LM, Walker CL, Dixon DR), pp. 257–294. Geological Society Special Publication no. 87, London.

Vrijenhoek RC (1997) Gene flow and genetic diversity in naturally fragmented metapopulations of deep-sea hydrothermal vent animals. *Journal of Heredity*, **88**, 285–293.

Weir BS (1996) *Genetic Data Analysis*, 2nd edn. Sinauer Associates, Sunderland, MA.

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