ORIGINAL ARTICLE

Recent population expansion and connectivity in the hydrothermal shrimp *Rimicaris exoculata* along the Mid-Atlantic Ridge

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ABSTRACT

Aim Deep-sea hydrothermal vents are unstable habitats that are both spatially and temporally fragmented. In vent species, a 'short-term insurance' hypothesis would lead us to expect mostly self-recruitment, limiting the loss of larvae in the deep ocean or water column and increasing genetic differentiation over the time elapsed since colonization. Alternatively, a 'long-term insurance' hypothesis would support the prediction of selection for large-scale dispersal, to ensure long-term persistence in these ephemeral habitats. The main goal of this study was to infer the spatial and temporal distribution of genetic diversity of the shrimp *Rimicaris exoculata*, which forms high-density local populations on hydrothermal vents along the Mid-Atlantic ridge.

Location Deep-sea hydrothermal vents along the Mid-Atlantic Ridge.

Methods We used sequences of mitochondrial cytochrome *c* oxidase subunit I (COI, 710 bp) to assess the spatio-temporal distribution of genetic diversity across five hydrothermal fields from 36° N to 4° S.

Results In contrast to previous results from pioneer studies, very high haplotype diversity was observed in vents across the entire region (i.e. 0.69–0.82), indicating current large effective population size and low drift. The star-like shape of the network of haplotypes, the lack of spatial genetic structure and the significance of tests reflecting demographic effects, together with the fitting of a population expansion model, all support a recent population expansion.

Main conclusions Our results suggest a very recent common history of *R. exoculata* populations/demes along the Mid-Atlantic Ridge, derived after a common bottleneck or founder event and followed by a concomitant demographic expansion. This study therefore suggests a large effective population size and/or high dispersal capacity, as well as a possible recent (re)colonization of Mid-Atlantic hydrothermal vents by *R. exoculata*.

Keywords
Bottleneck, deep-sea biogeography, dispersal, genetic diversity, hydrothermal vents, marine biogeography, Mid-Atlantic Ridge, *Rimicaris exoculata,* spatio-temporal distribution.

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INTRODUCTION

Dispersal plays a central role in both the dynamics and evolution of species. Effective dispersal (i.e. gene flow) attenuates genetic differences *among* populations and increases the genetic variability *within* populations (Billiard &

Lenormand, 2005). Dispersal is a risky strategy, as immigrants may never reach a suitable habitat, or may face higher mortality and lower reproductive success in the receiver population (Ims & Andreassen, 2000; Garant *et al.*, 2007). Dispersal can, however, offer clear advantages, particularly in fragmented ephemeral habitats, as it allows areas to be explored for more

favourable habitats, and extinction to be avoided by permitting escape from locally deteriorating physical or biotic conditions, such as competition (Hamilton & May, 1977; Olivieri *et al.*, 1995). By enabling populations to randomly spread their progeny more evenly among different sites, dispersal may indeed represent a favourable bet-hedging strategy in temporally variable or ephemeral ecosystems (Ronce & Olivieri, 2004).

Deep-sea hydrothermal vents are an extreme example of a spatially and temporally unstable environment. Environments of this type are scattered world-wide along the global mid-ocean ridge system, and are sometimes thousands of kilometres apart (Tunnicliffe & Fowler, 1996; Van Dover, 2000). They are extreme and unique habitats, with communities composed of specialized animals, dependent on chemoautotrophic microorganisms that exploit the reduced compounds (mainly H_2S and CH_4) abundant in vent fluids (Desbruyères *et al.*, 2000). Each hydrothermal vent has a characteristic profile that may differ in depth, temperature and/or chemical composition (Van Dover, 2000), adding abiotic heterogeneity to the spatial component of habitat fragmentation that potentially influences the patchy community distribution and limited effective dispersal. In addition to becoming geographically and genetically isolated because of the distance separating successive hydrothermal vents, communities may also become genetically isolated across transform faults, which act as potential barriers between populations and limit effective dispersal among sites (Plouviez *et al.*, 2009). This hypothesis is supported by the high levels of endemism found across biogeographic provinces (Van Dover *et al.*, 2002; Fabri *et al.*, 2006; Bachraty *et al.*, 2009; Moalic *et al.*, submitted), as well as by the rather high genetic differentiation found across some neighbouring sites (e.g. Jollivet *et al.*, 1999; Won *et al.*, 2003; Hurtado *et al.*, 2004; Matabos *et al.*, 2008; Young *et al.*, 2008; Plouviez *et al.*, 2009), which indeed suggest a limited effective dispersal over large distances. On the other hand, depending on the sea floor geology and magmatic and tectonic events, individual hydrothermal vents have a relatively short life span of 1–100 years for fast-spreading ridges such as the East Pacific Rise (Hessler *et al.*, 1988; Lalou *et al.*, 1993), and of up to 300 years for more stable, slow-spreading ridges such as the Mid-Atlantic Ridge (Brazelton *et al.*, 2010). These common temporal fluctuations in vent activity and changes in the geochemical profile of their habitat prevent long-term local population persistence (Tunnicliffe, 1991; Jollivet, 1996). Those species that escape local extinction owing to habitat disappearance and that are capable of (re)colonizing newly active sites may also have the capacity for long-distance effective migration. Species associated with hydrothermal vents should therefore also have good colonization abilities, with high rates of dispersal, growth and fecundity (Vrijenhoek, 1997; Tyler & Young, 1999). Indeed, in 1991 the formation of new hydrothermal vents in the East Pacific Rise was studied and, astonishingly, microbe-derived material was found in active areas of the vent immediately following their formation. Within the first year, mobile vent and non-vent fauna such as amphipods, copepods, crabs and non-vent shrimps proliferated (Shank *et al.*, 1998a). These vastly differing sources of influence lead to contradictory expectations concerning strategy, which have yet to be tested. On the one hand, low effective dispersal and high self-recruitment may be favoured across limited distances owing to the patchy nature of vent distribution and abiotic characteristics. On the other hand, larger-scale dispersal potential may be selected for in the long term, allowing species persistence despite the instability of vent activity. Finally, temporal instability (Desbruyères *et al.*, 1998), although apparently less pronounced in the Mid-Atlantic Ridge (MAR) than in the East Pacific Rise (EPR) (Copley *et al.*, 2007 on one site), may also result in short-term changes in genetic composition.

Along the MAR, vent communities are dominated by shrimps, mussels, anemones and crabs (Tunnicliffe, 1991; Van Dover, 1995). The first described and the most abundant species is the shrimp *Rimicaris exoculata* Williams & Rona, 1986. This species is often present in high-density swarms (>1500 individuals m^{-2}) around high-temperature sulphide chimneys at deeper hydrothermal vent sites (Segonzac *et al.*, 1993; Copley *et al.*, 1997). The species has highly modified eyes that form a single dorsal organ, which has been suggested to be able to detect the black-body radiation associated with high-temperature vents (Van Dover *et al.*, 1989; Van Dover, 1995). This shrimp does not seem to be a scavenger or a predator because its gut is filled with minerals, mainly iron oxides and sulphurs, and its nutrition is hypothesized to be derived from several epibiotic bacterial sources, mainly those located in the gill chambers and gut (see Durand *et al.*, 2010, and references therein). Although hydrothermal vents at MAR have been known since 1985 (Rona *et al.*, 1986), little is known about the population genetic structure of its most abundant shrimp species. Genetic studies conducted to date have focused on clarifying the systematic status of the juvenile morphotype of *R. exoculata* (Creasey *et al.*, 1996; Shank *et al.*, 1998b). The allozyme analyses conducted in these studies showed very low genetic diversity within sites and essentially no genetic differentiation between populations, suggesting that there is high gene flow.

The main goal of this study was to infer the spatial and temporal distribution of genetic diversity of *R. exoculata* from different hydrothermal vents along the MAR, in order to assess the extent of effective population sizes, rates of dispersal and recent demographic histories at these sites. We used mitochondrial cytochrome *c* oxidase subunit I (COI) as a molecular marker to estimate the level of genetic variability in natural populations, to test for genetic subdivision and to obtain insights into population history, dynamics and demography at the ridge level. In order to assess the effect of the potential temporal instability of hydrothermal activity and the population dynamics of associated species, two of the five populations studied were analysed at time intervals of one or ten years.

MATERIALS AND METHODS

Sampling and DNA extraction

Samples were collected from five locations corresponding to different hydrothermal vent fields (Rainbow, Logatchev, TAG, Ashadzé and South MAR) along the MAR (Fig. 1, Table 1). For two locations (Rainbow and Logatchev), samples from different years were used to assess temporal variability; a time interval of 10 years (Rainbow 1997 and 2007) and three consecutive 1-year intervals (Logatchev 2005, 2006, 2007) were used. Samples were collected using a slurp-gun from the ROV (remotely operated vehicle) or the Nautile. Prior to each dive, the bowls used for collecting the shrimps were aseptically washed with ethanol (96%) before being filled with sterile seawater. Once on board, live specimens were either entirely frozen or immediately dissected into body components under sterile conditions and frozen or stored in 70% alcohol. DNA extraction was performed using the CTAB (cetyl trimethyl ammonium bromide) method (Doyle & Doyle, 1990) on muscle tissue. Sampling size could not be predefined to a constant value across all sampling sites and times owing to the overall difficulty in obtaining samples from deep hydrothermal vents; at some sites, sampling size was thus limited to the shrimp samples available from Ifremer research cruises for each particular location and time, and (for the 4° S location) to some samples kindly provided by the Max Plank Institute (N. Dubilier).
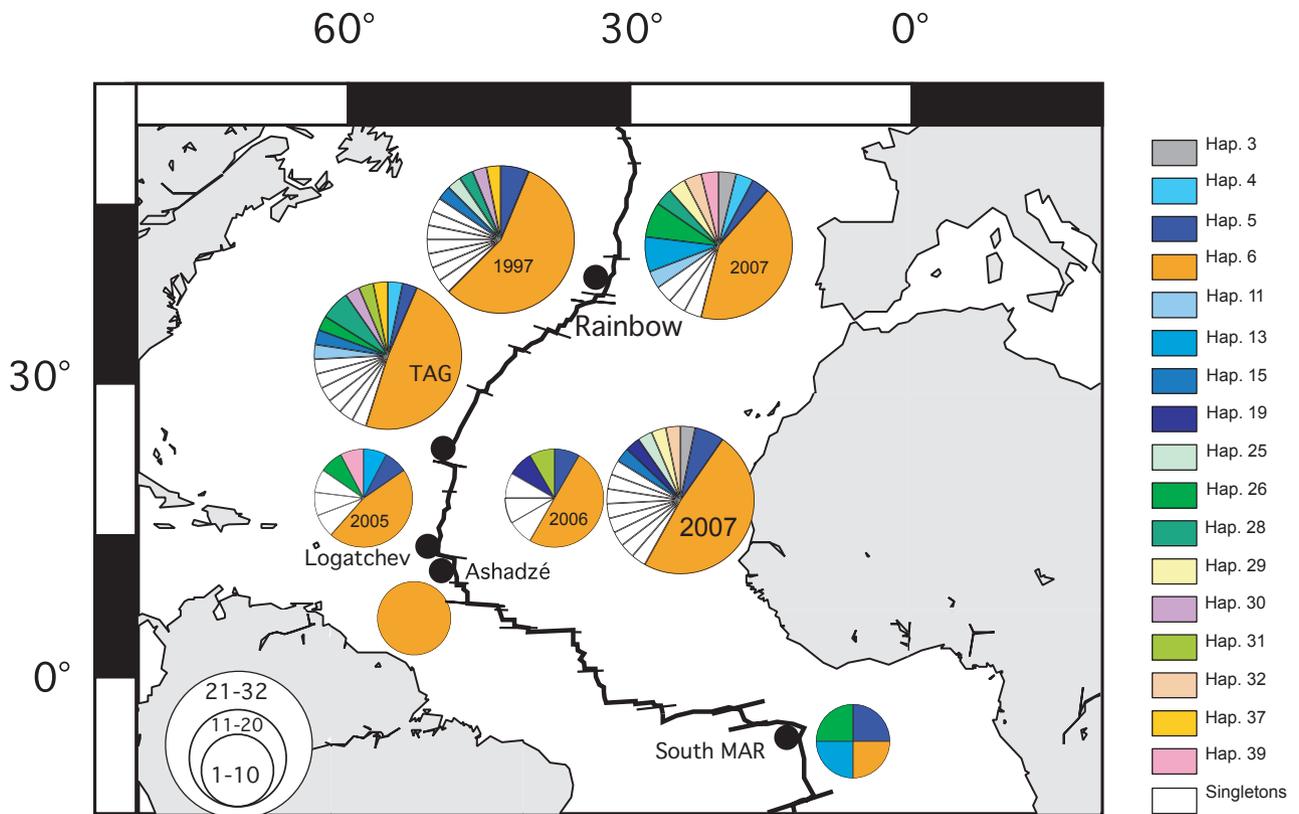
Figure 1 Location of the *Rimicaris exoculata* populations sampled along the Mid-Atlantic Ridge, and the respective mtDNA haplotype distribution (pie charts) per population. The size of the pie charts is proportional to a class of individuals; these classes were defined as small – 1 to 10 individuals, medium – 11 to 20 individuals and large – 21 to 32 individuals according to the number of individuals sampled from each population. The colours in the pie charts represent different haplotypes; the singletons (haplotypes that appear only once in one population) are coded as white, the same haplotype found in different populations is represented by the same colour in the corresponding pie charts. The colour key for the haplotypes found is on the right.

Table 1 Geographic coordinates, depth and cruise details for the populations of *Rimicaris exoculata* sampled along the Mid-Atlantic Ridge.

Population	Year	GPS coordinates	Depth	Cruise
Rainbow	2007	36°08′44″ N; 34°00′02″ W	2200 m	MoMAR; ROV Victor6000; RV *Pourquoi pas?*
TAG	2005	26°02′34″ N; 44°54′00″ W	3650 m	EXOMAR; ROV Victor6000; RV *L'Atalante*
Logatchev (Irina2)	2007	14°44′62″ N; 46°35′59″ W	2860 m	SERPENTINE; ROV Victor6000; RV *Pourquoi pas?*
Ashadzé (SE1)	2007	12°50′71″ N; 44°54′47″ W	3200 m	SERPENTINE; ROV Victor6000; RV *Pourquoi pas?*
South MAR	2006	4°47′ S; 12°22′ W	3048 m	M64/1; ROV Meteor/Quest

Polymerase chain reaction and sequencing

Mitochondrial COI was amplified using the universal primers LCO1490 and HCO2198 described by Folmer *et al.* (1994). All polymerase chain reaction (PCR) amplifications were carried out in 50-µL volumes containing 50 ng of DNA, 1× reaction buffer (GoTaq, Promega, Madison, WI, USA), 0.2 mM of each dNTP, 2.5 mM MgCl$_2$, 0.4 U Taq DNA polymerase (GoTaq, Promega) and 0.6 µM of each primer. The PCR amplification was conducted in a Perkin-Elmer Gene Amp System 7200 (Waltham, MA, USA) with the following program: 2 min at 95 °C; 35 cycles of 1 min denaturation at 95 °C, 1 min at 52 °C and 1.5 min elongation at 72 °C; followed by a final elongation step of 7 min at 72 °C. PCR products were sent to be purified and sequenced commercially at Macrogen, Inc. (Seoul, Korea). Sequences were proofread and aligned using GENEIOUS software (Drummond *et al.*, 2009). The 18S rDNA (*c.* 1.7 kb) was amplified for four individuals of all sampled locations and years (except for the Ashadzé population, for which only the three individuals available were amplified), using 18S-82F/18S-1498R primers (López-García *et al.*, 2003), and sequenced in an ABI 3130 XL automatic sequencer (Applied Biosystems, Foster City, CA, USA), to provide insights into nuclear variation. The nuclear regions internal transcribed spacer (ITS) 1 and 2 (*c.* 931 bp) were also amplified for four individuals of three populations (Rainbow, Logatchev and South MAR) using the primer pair ITS 1 and 4 (Kumar & Shukla, 2005). The obtained sequences were cloned (PGem-T easy vector, Promega) following the supplier's protocol; four clones per individual were sequenced using an ABI 3130 XL automatic sequencer (Applied Biosystems).

No diversity was detected across the four individuals from the five sites sequenced for 1200 bp of 18S rDNA. The amplified regions ITS 1 and 2 proved to be too variable to be used: all the sequences obtained were different, including sequences from different clones of the same individual.

Data analysis

ARLEQUIN 3 (Schneider *et al.*, 2000) was used to estimate gene diversity and conduct statistical tests. For each population, the following statistics were computed: haplotype (*h*) (Nei, 1987) and nucleotide (π_2) (Nei, 1987) diversities, and mean number of pairwise differences (π_1) (Tajima, 1983). For populations with significantly different sample sizes, a re-sampling method was applied using R to generate files for 100 random re-samplings of the population haplotype frequencies to a minimum common size. The 100 generated files were then analysed with the batch file function of ARLEQUIN software to re-estimate gene diversity in these subsamples. To assess population differentiation, pairwise F_{ST} values were calculated following the method of Hudson *et al.* (1992), and exact tests of differentiation were conducted following the method of Raymond & Rousset (1995).

In order to analyse and illustrate the phylogenetic relationships among haplotypes: (1) a median-joining network was constructed using NETWORK 4.1.0.9 (Bandelt *et al.*, 1999) to infer the most parsimonious branch connections between the sampled haplotypes, and (2) a neighbour-joining phylogram was constructed using the mutation model HKY (GENEIOUS software; Drummond *et al.*, 2009) and the bresiliid sister species *Alvinocaris* sp. as an outgroup.

We tested whether the sampled populations fitted mutation drift equilibrium expectations, and assessed which population demographic model was most appropriate; that is, the constant population size model or the expansion model. We determined Fu's F_S (Fu, 1996) and Tajima's *D* (Tajima, 1989), which are sensitive to departures both from selective neutrality and from population size equilibrium caused by expansions or bottlenecks (Tajima, 1996; Fu, 1997). Both statistics result in negative values after a population expansion (Ray *et al.*, 2003) or a selective sweep, whereas positive values are expected under balancing selection of recent bottlenecks. ARLEQUIN was used to perform tests and estimate the associated *P*-values. Mismatch curves were drawn for each population, and observed values were compared with the expected curves fitted for the constant population size model and for the population growth/decline model, using DNASP 4.0 (Rozas *et al.*, 2003).

RESULTS

Genetic diversity and population differentiation

A total of 47 haplotypes were recovered from the 152 individuals analysed, 30 (63.8%) of which were 'private'; that is, unique to a single locality (Table 2; Fig. 1; accession numbers HM125910 to HM125956). The most common haplotype was present in all population samples and was the most abundant haplotype across all populations, identified in 49% of all the sampled individuals across the entire study region. This main haplotype was also central to all other haplotypes, most of which were represented by a single individual and divergent by a single point mutation, leading to this haplotype being the core of a star-like cluster topography (Fig. 2). The remaining haplotypes (36.2%), although not unique, were mainly (27.7%) represented by only one individual in each population. Accordingly, the neighbour-joining phylogram revealed extremely unresolved clusters supported by poor bootstrap values (see Appendix S1 in Supporting Information).

At a temporal scale of a 10-year sampling interval (Rainbow 1997 and 2007), the unique haplotypes (present in one year but not in the other) represented 56% of the total haplotypes found, whereas on a temporal scale of a one-year sampling interval (Logatchev 2005, 2006, 2007) the proportion of unique haplotypes was higher (88.5%).

Haplotype diversity (*h*) was high, ranging from 0 to 1 in populations with small sample sizes (Ashadzé: three individuals and one haplotype; South MAR: four individuals and four haplotypes) and from 0.69 to 0.82 in populations with larger sample sizes (>11 individuals). Considering these rather high values of haplotype diversity, nucleotide diversity (π_2) was low, ranging from 0 to 0.0033 (Table 2). The highest diversities

Table 2 Genetic diversity indices, calculated for each sampled population and year, of the deep-sea shrimp *Rimicaris exoculata* sampled along the Mid-Atlantic Ridge. n, sample size; Nh, number of haplotypes; Nph, number of private haplotypes (i.e. the number of haplotypes that only appeared in one population); k, number of polymorphic sites; h, haplotype diversity; π_1, mean number of pairwise differences; π_2, nucleotide diversity. Neutrality and population expansion tests: D, Tajima's D-test; F_S, Fu's F_S test. All values obtained for the neutrality tests were significant at the 5% level.

Population	Year	n	k	Nh	h	π_1	π_2	D	F_S	Nph
Rainbow	2007	26	18	14	0.82 ± 0.07	1.67	0.0033	−2.292 (0.002)	−11.01 (0)	3
	1997	32	14	14	0.69 ± 0.09	1.05	0.0021	−2.298 (0.001)	−13.87 (0)	7
Logatchev	2007	31	20	16	0.77 ± 0.08	1.41	0.0028	−2.487 (0)	−15.38 (0)	8
	2006	12	9	7	0.77 ± 0.13	1.50	0.0030	−2.016 (0.004)	−3.52 (0.003)	3
	2005	13	8	8	0.81 ± 0.11	1.36	0.0027	−1.831 (0.012)	−5.25 (0)	3
TAG	2005	31	16	16	0.77 ± 0.08	1.09	0.0022	−2.454 (0)	−18.32 (0)	6
Ashadzé	2007	3	0	1	0	0	0	0	0	0
South MAR	2006	4	3	4	1.00 ± 0.18	1.50	0.0030	−0.754 (0.23)	−2.37 (0.008)	0
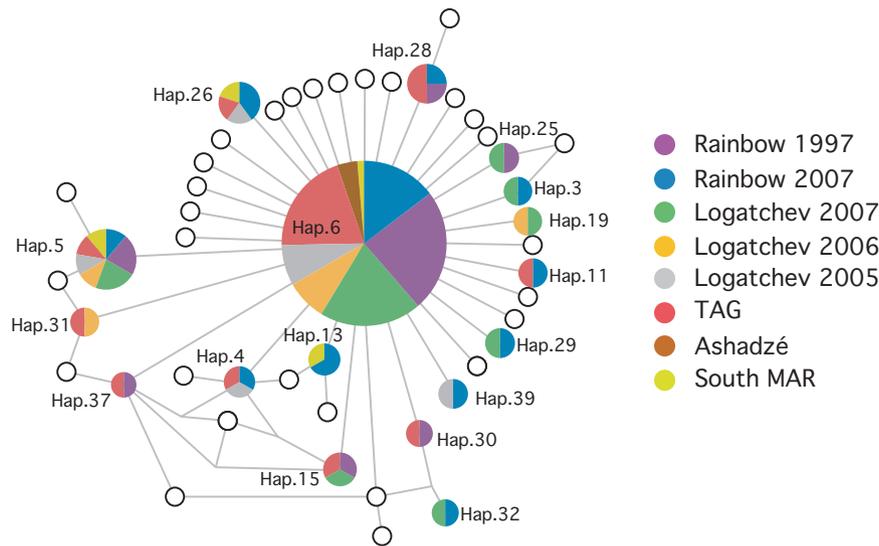
Figure 2 Haplotype network of the obtained mtDNA haplotypes of *Rimicaris exoculata* sampled along the Mid-Atlantic Ridge. Each circle represents a different haplotype, the size of each circle is proportional to the number of individuals displaying that particular haplotype. The white circles represent singletons (haplotypes that occur only once, represented by a single individual), the haplotypes found in more than one individual are identified and represented by pie charts, the colours used represent the locations where that haplotype was found. The segment size is proportional to the relative frequency of a haplotype in each population where it is present. The colour key to the locations where each haplotype was present is on the right.

were recorded in the South MAR population, although the sample size was very low (four individuals), followed by the Rainbow 2007 population. Populations sampled in more than one year presented different diversity indices across years. The Rainbow population sampled in 2007 showed more polymorphic sites, and higher haplotype and nucleotide diversity than when it was sampled in 1997, even though the number of samples was lower in 2007 (26 individuals) than in 1997 (32 individuals). A random re-sampling method was therefore not needed for Rainbow, as the sample with the fewest individuals already presented the higher diversity indices. Random re-sampling was, however, performed for Logatchev, where

Table 3 Genetic diversity indices for the temporal samples of the population of *Rimicaris exoculata* from Logatchev. Diversity indices for the sampling years of 2005 and 2007 are given as averages (±SD) owing to the random re-sampling (100 times) to a minimum common size of 12 individuals per population. Nh, number of haplotypes; k, number of polymorphic sites; h, haplotype diversity; π_2, nucleotide diversity.

Year	Nh	k	h	π_2
2005	5.26 ± 1.1	5.1 ± 1.46	0.74 ± 0.12	0.0024
2006	7	9	0.77 ± 0.13	0.0030
2007	5.9 ± 1.51	6.78 ± 2.25	0.73 ± 0.15	0.0026

Table 4 Pairwise F_{ST} values based on haplotype frequencies obtained for *Rimicaris exoculata* sampled along the Mid-Atlantic Ridge. None of the F_{ST} values was significant at the 5% level.

Population	Rainbow07	Rainbow97	Logatchev07	Logatchev06	Logatchev05	TAG	Ashadzé	South MAR
Rainbow07								
Rainbow97	0.00245							
Logatchev07	−0.0115	−0.01243						
Logatchev06	−0.0184	−0.0221	−0.0298					
Logatchev05	−0.0335	−0.0144	−0.0227	−0.0360				
TAG	−0.0147	−0.0138	−0.0122	−0.0262	−0.0260			
Ashadzé	0.0480	−0.0230	0.0140	0.0145	0.0331	0.0140		
South MAR	−0.0589	0.0452	0.0031	−0.0147	−0.0495	0.0031	0.250	
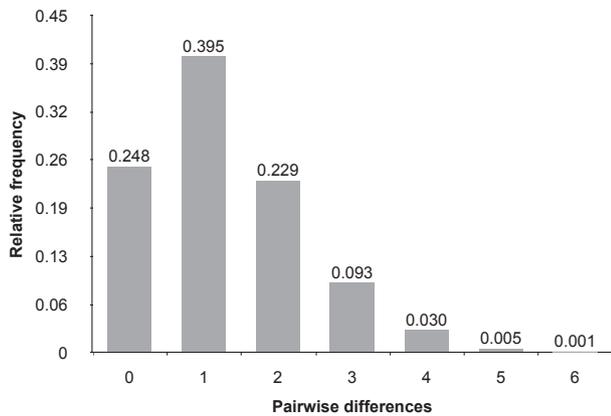
Figure 3 Mismatch distribution of nucleotide pairwise differences for all the mitochondrial cytochrome *c* oxidase subunit I sequences analysed (152) for *Rimicaris exoculata* sampled along the Mid-Atlantic Ridge.

samples had more contrasting sizes (12–31 individuals) with no obvious diversity trend through time. In order to dissociate a putative biological effect from a sampling size effect, random re-sampling (100 times) was therefore performed to a minimum common size of 12 individuals per sampling time, and no significant differences were found in any of the diversity indices over time (Table 3).

No significant population differentiation was detected between any of the sampling sites or years using pairwise F_{ST} estimates (Table 4).

Historical demography

Demographic analyses suggested the occurrence of population expansions, as highly significant negative values for Tajima's *D* and Fu's F_S were obtained for all populations (Table 2). Mismatch distribution curves were consistent with models of population expansion, displaying a unimodal distribution (Fig. 3). When comparing the expected and observed mismatch curves under constant population size versus the growth/decline model (Fig. 4), there is an excess of rare variants in the observed curves in relation to the number expected under constant population size. Furthermore, the observed curves seem to follow the expected curve better for the growth/decline model.
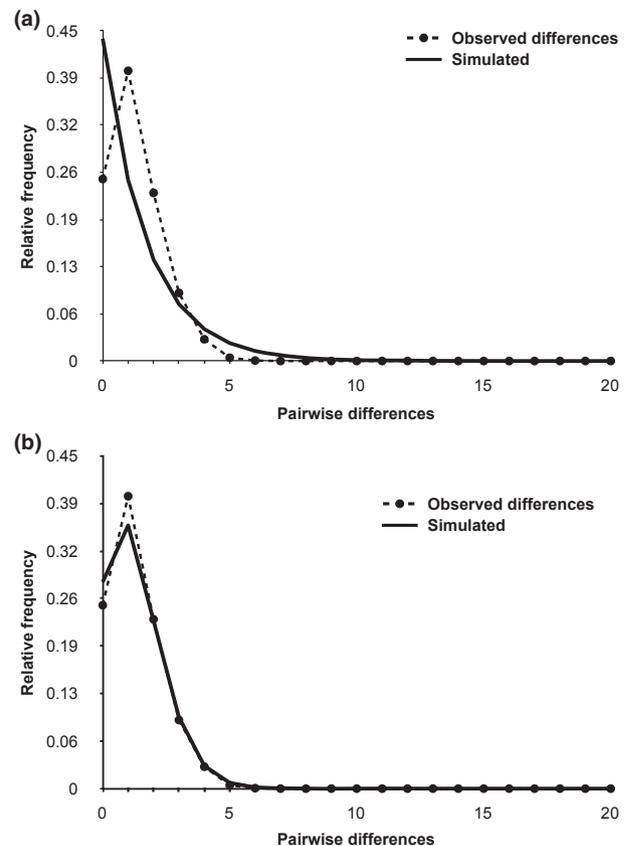
Figure 4 Observed and simulated mismatch curves of nucleotide pairwise differences of mitochondrial cytochrome *c* oxidase subunit I sequences of *Rimicaris exoculata* sampled along the Mid-Atlantic Ridge. The graphs represent a comparison of the observed curve of nucleotide pairwise differences to (a) a simulated curve assuming constant population size, and (b) a simulated curve assuming a population growth/decline model.

DISCUSSION

The analysis of mtDNA haplotypes from a total of 152 individuals from five discrete vent localities along the MAR showed an overall high genetic variability within the studied populations. Both the number of haplotypes per population (1–16) and the gene diversity (0.69–0.82) are within the range

of values found for other vent organisms studied with the same marker, including tubeworms (e.g. *Riftia pachyptila*, *Alvinella pompejana*), limpets (e.g. *Lepetodrillus elevatus*) and bivalves (e.g. *Bathymodiolus azoricus*, *Calyptogena magnifica*) (e.g. Hurtado *et al.*, 2004; Johnson *et al.*, 2006; Plouviez *et al.*, 2009). However, these results contradict those obtained for *Rimicaris exoculata* with allozymes, where genetic variability was amongst the lowest reported for vent organisms (Creasey *et al.*, 1996). This may be a result of the different properties of the distinct markers, as allozymes may exhibit lower polymorphism than mtDNA, possibly because of protein constraints. Mitochondrial DNA polymorphism is classically considered to be less influenced by possible selective processes (Ballard & Whitlock, 2004), although it was recently demonstrated that this may not be a systematic rule (Galtier *et al.*, 2009). In any case, DNA sequences retain more polymorphisms than proteins, as polymorphic DNA sequences include synonymous mutations; higher variability can, therefore, be detected by DNA sequences than by allozymes. On the other hand, a differential imprint of demographic events on mitochondrial versus nuclear DNA may be evoked, although lower diversity is usually expected for mtDNA in relation to nuclear DNA, owing to the difference in effective population size. Such discrepancies may also be affected by the more restricted sampling that was available for the allozyme study, which included only two sampling locations despite the higher overall number of samples analysed (448 individuals).

The rather unresolved phylogram, with low bootstrap values, shows a generally low divergence without a geographical imprint. Together with the non-significant F_{ST} values obtained in this study, the results indicate a lack of geographic partitioning of the numerous but slightly divergent haplotypes among the sampled populations. This lack of genetic structure may be the result of several non-mutually exclusive factors: high levels of effective dispersal (gene flow) between all populations of the MAR (i.e. panmixia at the ridge level); large effective population sizes (N_e); and/or a recent common population history. The life cycle of *Rimicaris* is poorly understood. Some authors have suggested that *R. exoculata* might feed in the upper water column during larval dispersal (Dixon & Dixon, 1996), and others that the juvenile stages of these shrimp differ physiologically from the adult stage (Pond *et al.*, 1997; Allen *et al.*, 1998) in traits such as wax-ester reserves and possibly photosynthetically derived lipids. These findings led the authors to hypothesize that the species had a long planktotrophic phase with long-distance migration achieved via the surface, so that some of the barriers usually considered (depth, seafloor topology and transform faults) would not constrain this species. Such a hypothesis, however, implies either a huge risk factor in the case of passive dispersal, or an extremely sophisticated mechanism of active and deterministic migration (which has not been identified so far) allowing a return to hydrothermal habitat at the end of larval development. In any case, whether dispersal occurs via the surface or not, the little information available to date on *R. exoculata* suggests a significant dispersal potential either at the larval or at the adult stage (although no observations of adults away from hydrothermal sites have been reported so far).

The low degree of genetic differentiation among populations may also be attributed to a relatively recent common history and large effective population sizes, which limit genetic drift (Crow, 1986). The scenarios (1) of high levels of gene flow and (2) of low gene flow since a recent divergence and large N_e may both leave similar signatures of low genetic structure of populations. The hypothesis of large effective population sizes limiting drift is concordant with the population biology of this species, as *R. exoculata* is reported to have remarkably high population densities of over 1500 individuals m^{-2} (Copley *et al.*, 1997). Such large population sizes may, in the absence of temporal fluctuation or significant variance in reproductive success, result in large effective population sizes, limiting the differentiating effects of drift. However, the haplotype phylogram and network, together with the analyses of population history, indicate a recently very low effective population size as they show: (1) a star-like haplotype network in which many new haplotypes are derived from one dominant haplotype via single mutation steps, indicating that they are the product of recent mutation events; (2) negative and highly significant values of Tajima's *D* and Fu's F_S tests, as expected under population bottleneck/ expansion scenarios; and (3) the expected and observed mismatch curves that follow a growth/decline model. This profile supports a scenario of relatively young populations sharing a recent common history of bottleneck or founder events, and expansion. A recent study of epibionts of the *R. exoculata* gill chamber (Petersen *et al.*, 2009) showed spatial clustering of bacterial sequences across the different sites (Rainbow, Logatchev and South MAR). Such geographical segregation of the symbionts compared to host homogeneity may be attributable either to the influence of environment on symbionts or to the very recent common history of *R. exoculata* populations, with recent divergence detectable only on short-generation symbionts and still undetectable on the genetics of their hosts.

The analysis of temporal variability revealed a lack of genetic differentiation among samples separated by time steps of one (Logatchev) and ten (Rainbow) years, although within-population diversity increased within a decade in Rainbow. In contrast to Logatchev, where no significant differences were observed across three consecutive years, Rainbow exhibited a significantly higher genetic diversity in 2007 than it did 10 years earlier, despite the lower sample size and equal number of (but more evenly distributed) haplotypes in 2007. Genetic diversity, quantified in terms of both allelic richness and heterozygosity, increased significantly over the 10-year interval in Rainbow, in agreement with a possible ongoing effect of population expansion (Table 2). Such trends were not detected in the diversity indices for the one-year-interval samples, possibly indicating that temporal variations in diversity are not sufficiently large

to be detected over the shorter time scale of one-year intervals. The pattern of variability across temporal samples therefore suggests a contemporary expansion, over one decade, at least in Rainbow. However, population growth tests are known not to perform well when population expansion is very recent (Ramos-Onsins & Rozas, 2002), and any expansion episodes detected using the growth/decline model are, therefore, likely to be older than a decade. Altogether, with the star-like cluster of haplotypes revealing the accumulation of mutations over a recent evolutionary scale, these results imply either successive episodes of expansion or one long-term expansion episode affecting the pattern of haplotype diversity at both ecological and evolutionary time scales.

Considering the demographic history of these apparently young genetic lineages, which have probably undergone recent recolonization and/or expansion events, the lack of genetic differentiation among sites means that we cannot make inferences about the ongoing occurrence of extensive or restricted gene flow among the hydrothermal sites distributed along the MAR. However, in the absence of any selective hypothesis explaining the star-like pattern of COI polymorphism, the hypothesis of such a recent and global (re)colonization also implies a high dispersal potential *per se* in the recent past. Recent bottlenecks and/or expansion have also been observed across populations of several hydrothermal invertebrates along other oceanic ridges (Young *et al.*, 2008; Faure *et al.*, 2009; Plouviez *et al.*, 2009), and such profiles of young lineages may be the rule rather than the exception for hydrothermal vent-associated species, owing to the dynamic nature of vents subject to recurrent extinction events. In other ridges, such as the EPR, most species studied to date display a clear north/south genetic differentiation, and in some cases even highly significant F_{ST} values within the north and south populations (Hurtado *et al.*, 2004; Matabos *et al.*, 2008; Plouviez *et al.*, 2009). This genetic differentiation reported among populations of various vent species may be the result of a lower dispersal potential and/or effective population size of these organisms, or of a higher occurrence or strength of physical and abiotic barriers to gene flow, such as transform faults, in the EPR than in the MAR (but see O'Mullan *et al.*, 2001). Other species, however, do not exhibit such a cleavage (Won *et al.*, 2003; Hurtado *et al.*, 2004; Plouviez *et al.*, 2009), which may indicate a species-specific characteristic (such as dispersal potential or population size), or might simply reflect different sampling schemes. Large gaps in the sampling were indeed observed for most species where a north/south cleavage was shown, and such a sampling scheme was demonstrated by Audzijonyte & Vrijenhoek (2010) to have the potential to result in artificially inflated F_{ST} values. However, in the study of the sister mussel species *Bathymodiolus azoricus* and *B. puteoserpentis* and their hybrids (O'Mullan *et al.*, 2001), the clear distinction in species distribution between north (Rainbow, Lucky strike and Menez Gwen vents) and south (Snake Pit and Logatchev

vents) along the MAR suggests the existence of an ancient vicariance event that apparently did not affect *R. exoculata*, possibly owing to a more recent history, higher effective population sizes, or higher dispersal potential in this shrimp species.

As few species have been studied so far in the MAR compared with the more extensively studied EPR, a generalized comparison is not yet possible, but a species-specific hypothesis may be put forward to explain the lack of structure observed for *R. exoculata*. High dispersal potential, including long-distance migration as a bet-hedging strategy enabling escape from extinction, is thought to be a factor of risk in a fragmented environment owing to the possible loss of progeny in unsuitable habitats. In *R. exoculata*, this risk may be compensated by a high fecundity, as suggested for other species (Llodra *et al.*, 2000), and/or by an environmentally driven delay in larval development, suggested by O'Connor *et al.* (2007) to be a possible adaptation associated with large-scale dispersal. Moreover, it has been suggested that *R. exoculata* may be able to detect the black-body radiation associated with high-temperature vents (Van Dover *et al.*, 1989; Herring *et al.*, 1999). This may be a specific adaptation of this species, enabling it to locate its exceptional habitat during long-distance dispersal and thus allowing the benefit of large-scale dispersal to be retained while minimizing the associated risk of loss at the time of settlement. However, empirical data have shown that the detection of temperature in seawater may be possible only within a small area around the chimneys where the seawater is actually warmer (Segonzac *et al.*, 1993). The putative mechanism that may allow *R. exoculata* larvae to settle in their habitat after this hypothetical long-distance dispersal is therefore not elucidated: it may be temperature-associated or based on chemical signals linked to the presence of adults, as has been demonstrated for other marine invertebrates such as the oyster *Ostrea puelchana*, for which preferential settlement of spat occurs close to the adults owing to a chemical stimulus (Pascual & Zampatti, 1995).

This study makes a novel contribution to our understanding of the biogeography of the MAR by providing new insight into the population biology and biogeographic structure of a key species of shrimp from the unique, extreme and remote marine habitats of hydrothermal vents. Our results indicate that, across this very remote biogeographic region, all vent populations of this shrimp have a recent common history derived from a bottleneck or founder event, followed by a demographic expansion towards apparently large effective population sizes. Associated with high dispersal potential, such a demographic history may explain the absence of significant drift effects. Interestingly, these results may indicate a possible common source or centre of origin of a (re)colonization event at the origin of the lineages sampled from the MAR in our study. The identification of such a potential source population is, unfortunately, rendered particularly difficult in these ecosystems because the sampling of hydrothermal vents is necessarily as fragmented in space and time as the ecosystems themselves are. Finally, more species should be studied on the

MAR in order to compare results with the more extensively studied EPR, so as to test for the possible influence of the shape of ridges and transform faults on the pattern of evolution and genetic differentiation of species associated with hydrothermal vents.

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SUPPORTING INFORMATION

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

Appendix S1 Neighbour-joining phylogram constructed under the HKY mutation model with 152 COI mitochondrial sequences of *Rimicaris exoculata*. The outgroup used was the bresiliid shrimp *Alvinocaris* sp.

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BIOSKETCHES

The researchers involved in this study have a long-standing interest in the biogeography, ecology and evolution of marine organisms and especially in deep-sea hydrothermal vent communities (D.D.) and their symbiotic relationships (M.-A.C.B).

Author contributions: S.A.-H. and D.D. conceived the ideas; M.-A.C.-B. collected most of the samples; S.T. collected the data; S.T., S.A.-H. and E.S. analysed the data; and all authors contributed to the writing.

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