

# Distinct patterns of genetic differentiation among annelids of eastern Pacific hydrothermal vents

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## Abstract

Population genetic and phylogenetic analyses of mitochondrial COI from five deep-sea hydrothermal vent annelids provided insights into their dispersal modes and barriers to gene flow. These polychaetes inhabit vent fields located along the East Pacific Rise (EPR) and Galapagos Rift (GAR), where hundreds to thousands of kilometers can separate island-like populations. Long-distance dispersal occurs via larval stages, but larval life histories differ among these taxa. Mitochondrial gene flow between populations of *Riftia pachyptila*, a siboglinid worm with neutrally buoyant lecithotrophic larvae, is diminished across the Easter Microplate region, which lies at the boundary of Indo-Pacific and Antarctic deep-sea provinces. Populations of the siboglinid *Tevnia jerichonana* are similarly subdivided. *Oasisia alvinae* is not found on the southern EPR, but northern EPR populations of this siboglinid are subdivided across the Rivera Fracture Zone. Mitochondrial gene flow of *Alvinella pompejana*, an alvinellid with large negatively buoyant lecithotrophic eggs and arrested embryonic development, is unimpeded across the Easter Microplate region. Gene flow in the polynoid *Branchiopolynoe symmytilida* also is unimpeded across the Easter Microplate region. However, *A. pompejana* populations are subdivided across the equator, whereas *B. symmytilida* populations are subdivided between the EPR and GAR axes. The present findings are compared with similar evidence from codistributed species of annelids, molluscs and crustaceans to identify potential dispersal filters in these eastern Pacific ridge systems.

**Keywords:** *Alvinella pompejana*, *Branchiopolynoe symmytilida*, COI, hydrothermal vents, mitochondrial gene flow, phylogeography, *Oasisia*, *Riftia*, *Tevnia*

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## Introduction

Dispersal of deep-sea hydrothermal vent animals in the vast ocean basins has been an intriguing and elusive issue for biologists (Tyler & Young 1999; Van Dover *et al.* 2002). For most vent animals little is known about the longevities or transport modes of dispersing larvae or juveniles. Similarly, the roles that deep oceanic currents play in facilitating or constraining larval transport among vents also remain poorly understood, although progress has been made (Kim *et al.* 1994; Kim & Mullineaux 1998; Marsh *et al.* 2001;

Thomson *et al.* 2003). Nevertheless, the constrained distribution of deep-sea hydrothermal vent communities provides remarkable opportunities to study the effects of benthic topography, hydrography and geographical distance on the dispersal of marine animals (Vrijenhoek 1997; Van Dover *et al.* 2002). Phylogeographical analyses of vent species should shed light on the effective dispersal of individual species and on the areas where gene flow is restricted which may, in turn, help to identify dispersal barriers encountered by these organisms in the deep sea.

Vent communities occur worldwide at marine hot springs distributed along the global mid-ocean ridge system, in back arc spreading centres and on volcanically active seamounts (Tunnicliffe & Fowler 1996; Van Dover 2000). Because most vent-endemic animals depend on free-living or symbiotic chemolithoautotrophic microbes for nutrition, they must

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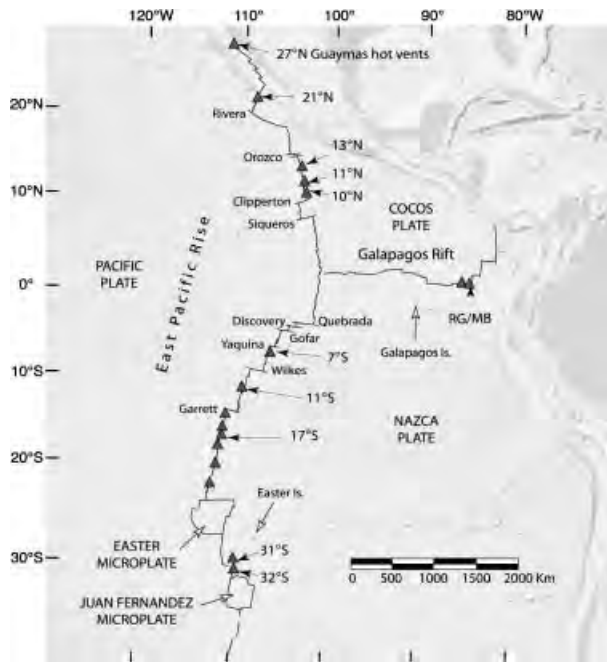


Fig. 1 East Pacific Rise and Galapagos Rift. Triangles represent known vent fields; names of transform faults are indicated.

live in or near hydrothermal effluents. This restriction creates island-like populations distributed intermittently along tectonically or volcanically active spreading segments of the global mid-ocean ridge system as illustrated by the distribution of vent fields along the East Pacific Rise (EPR) and Galapagos Rift (GAR; Fig. 1). Discrete vent fields are separated typically by tens to hundreds of kilometres along an actively spreading ridge segment and by hundreds of kilometres between disjunct ridge segments that are often displaced by large transform faults. Finally, populations can be located on separate ridge systems that are separated by large habitat gaps (e.g. EPR vs. GAR). Furthermore, frequent volcanic eruptions and tectonic events along the EPR and GAR can destroy local vent communities and create nascent vents that are rapidly colonized and persist for few decades (Haymon *et al.* 1993; Lutz *et al.* 1994; Shank *et al.* 1998). Therefore, the animals found in these ephemeral environments are expected to have exceptional colonization abilities including high rates of dispersal, rapid individual growth rates and high fecundity (Lutz *et al.* 1994; Vrijenhoek 1997; Tyler & Young 1999).

The adults of many vent animals are sessile or relatively immobile, and long-distance dispersal takes place mainly through the larvae. Negatively buoyant larvae are expected to move primarily with along-axis bottom currents constrained to the axial valley of the ridge system (Kim & Mullineaux 1998; Pradillon *et al.* 2001; Thomson *et al.* 2003). In contrast, positively buoyant vent larvae can disperse in hydrothermal plumes that rise several 100 m above the sea-

floor (Kim *et al.* 1994). Megaplumes generated by volcanic eruptions rise as much as 1000 m above the axial walls and can potentially transport buoyant larvae across vast distances (Mullineaux *et al.* 1995). Oceanic strata that transport larvae expose them to a variety of current vectors that vary with height above the seafloor (Tyler & Young 1999; Van Dover *et al.* 2002).

Population genetic studies of vent animals from the northern EPR and GAR have revealed different patterns of population structure, some of which appear to be related to ridge topography and geographical distance (France *et al.* 1992; Black *et al.* 1994; Craddock *et al.* 1995; Jollivet *et al.* 1995; Karl *et al.* 1996; Craddock *et al.* 1997; Vrijenhoek 1997; Hurtado *et al.* 2003; Won *et al.* 2003). Although differences were not surprising, given the disparate life histories of these annelids, molluscs and crustaceans, most species disperse effectively throughout the northern region. Genetic studies of southern EPR vent animals have examined fewer species, but present evidence suggests that two important biogeographical filters might exist there. Populations of *Calymene magnifica* clams and *Bathymodiolus thermophilus* mussels exhibit concordant divergence across a region of the southern EPR encompassing 15°S latitude (Hurtado *et al.* 2003; Won *et al.* 2003). Divergence of these clam and mussel populations was hypothesized to result from strong cross-axis currents that could disrupt along-axis gene flow in this region (Lupton & Craig 1981; Hautala & Riser 1993; Lupton 1998). Further south, species-level divergence between sister-lineages separated by the Easter Microplate region is observed for *Oasisia* tubeworms (Hurtado *et al.* 2002), bythograeid crabs (Guinot *et al.* 2002; Guinot & Hurtado 2003) and *Bathymodiolus* mussels (Won *et al.* 2003). Phylogeographical studies of other vent-endemic taxa that cross these potential biogeographical filters are needed to assess the generality of these findings.

The present report examines patterns of mitochondrial differentiation and gene flow in five vent-endemic annelids from the EPR (27°N to 32°S latitude) and GAR. We examined three siboglinid tubeworms (*Riftia pachyptila*, *Tevnia jerichonana* and *Oasisia alvinae*), one alvinellid worm (*Alvinella pompejana*) and a commensal polynoid (*Branchiopolynoe symmytilida*) that lives in the mantle cavity of mussels. For each of these species, we amplified a portion of the mitochondrial cytochrome c oxidase subunit I gene (*mtCOI*) and examined patterns of genetic differentiation to assess geographical subdivision and gene flow.

## Materials and methods

### Biological specimens

Specimens were collected during *Alvin/Atlantis* expeditions to 12 hydrothermal vent fields along GAR and EPR axes (Table 1). The northern EPR and GAR were sampled

**Table 1** East Pacific Rise and Galapagos Rift samples

Region, vent field	Latitude	Longitude	Depth (m)	Species*				
				<i>Rp</i>	<i>Tj</i>	<i>Oa</i>	<i>Ap</i>	<i>Bs</i>
NEPR								
27°N	27°00'N	111°24'W	2008	10	—	—	—	—
21°N	20°50'N	109°06'W	2636	10	—	22	7	—
13°N	12°49'N	103°57'W	2636	†	12	11	5	†
11°N	11°25'N	103°47'W	2515	†	9	†	††	†
9°N	9°48'N	104°15'W	2525	19	12	10	21	10
GAR								
GAR	0°48'N	86°09'W	2486	9	—	—	—	22
SEPR								
7°S	7°25'S	107°49'W	2747	26	28	—	—	8
11°S	11°18'S	110°32'W	2669	24	†	—	10	†
14°S	13°59'S	112°29'W	2626	—	—	—	3	†
17°S	17°25'S	113°12'W	2578	25	28	—	21	4
21°S	21°34'S	114°18'W	2834	††	††	—	†	—
32°S	31°51'S	112°03'W	2331	12	26	—	26	17

\*Sample sizes: (*Rp*) *R. pachyptila*; (*Tj*) *T. jerichonana*; (*Oa*) *O. alvinae*; (*Ap*) *A. pompejana*; and (*Bs*) *B. symmytilida*.

—Not recorded for this locality.

†Observed but not sampled for this study.

††Rare.

between 1990 and 1994, 2000, and 2002, and the southern EPR was sampled in 1999.

The siboglinid tubeworms are completely sessile as adults. *R. pachyptila* are large worms that commonly reach 1.5 m in length and live in smooth flexible tubes attached to sulphide chimneys and basalts. This species produces neutrally buoyant, small (~100 µm) lecithotrophic eggs with sufficient energy reserves to persist in the water column for about 38 days (Marsh *et al.* 2001). *T. jerichonana* are thinner worms, reach up to a metre in length and live in narrower rigid tubes attached to solid substrates. *O. alvinae* are relatively small worms (typically < 0.3 m) that live in narrow rigid tubes with wide flutes. They are often attached to basaltic rocks, clumps of *Bathymodiolus* mussels or *Riftia* tubes. No information on the larval biology of *Tevnia* or *Oasisia* is reported.

*A. pompejana* worms live in soft thin-walled tubes attached to sulphide chimneys or to *Riftia* tubes in areas of strong hydrothermal flow. Adults can exit their tubes and swim vigorously, although long-distance dispersal of adults seems improbable. This worm releases large (~200 µm) negatively buoyant lecithotrophic eggs that are expected to drift with bottom currents, and arrested embryonic development appears to increase long-distance dispersal abilities of these benthic larvae (Pradillon *et al.* 2001).

*B. symmytilida* scale worms live in the mantle cavity of *Bathymodiolus* mussels. Larval biology of this species is unknown, but the mid-Atlantic ridge species, *Branchiopolynoe seepensis*, has very large eggs (~500 µm) and lecithotrophic larvae (Tyler & Young 1999).

### Molecular methods

We examined mitochondrial cytochrome c oxidase subunit I (*mtCOI*) sequences in this study because: (i) universal invertebrate primers were available for a portion of this gene (Folmer *et al.* 1994); (ii) intermolecular genetic recombination appears to be absent; and (iii) this gene region has provided useful variation for previous genetic studies of vent taxa (e.g. Craddock *et al.* 1995; Hurtado *et al.* 2002, 2003; Won *et al.* 2003). Although doubly uniparental inheritance (DUI) of mitochondria is reported in some marine bivalves (Zouros *et al.* 1994 and Passamonti & Scali 2001), it has not been observed in hydrothermal vent bivalves (Goffredi *et al.* 2003; Hurtado *et al.* 2003; Won *et al.* 2003). Strictly maternal transmission of mitochondria in the annelids examined in this study was assumed because we found no evidence for heteroplasmy in any of the observed sequences. Thus, our conclusions about gene flow apply only to females, although we have no reason to suspect that differential dispersal occurs between the sexes. Previous studies of *mtCOI* and nonmitochondrial gene markers in vent species have revealed concordant patterns of population structure (Craddock *et al.* 1995; O'Mullan *et al.* 2001; Hurtado *et al.* 2003; Won *et al.* 2003).

Total DNA from tissue samples was extracted using the DNEasy kit (Qiagen, Inc., Chatsworth, CA, USA). A 710-base pairs (bp) region of mitochondrial COI (*mtCOI*) was amplified using primers and conditions reported by Folmer *et al.* (1994). Polymerase chain reaction (PCR) products

were cleaned with the Qiaquick PCR Kit™ (Qiagen, Inc., Valencia, CA, USA) and sequenced in both directions with ABI 377 or Licor 4000 L sequencers. Sequences were proof-read and aligned with SEQUENCHER version 4.1 (Gene Codes Corp., Ann Arbor, MI, USA). All *mtCOI* DNA sequences were translated to amino acid sequences and no terminal codons or indels were found among these sequences; thus, we are confident to have amplified and sequenced *mtCOI* and not a pseudogene.

For *Riftia*, most individuals were scored by restriction digests of PCR products (see explanation in Results section) using the *BsiE* I enzyme according to manufacturer's protocols (New England Biolabs, Beverly, MA, USA), and fragment patterns were visualized on 1% agarose gels.

### Population genetic and phylogenetic analyses

ARLEQUIN 2000 (Schneider *et al.* 2000) was used to estimate gene diversity and conduct statistical tests. For each species the following statistics were calculated: haplotype diversity,  $h$  (equ. 8.6, Nei 1987); mean number of pairwise differences,  $\pi_1$  (Tajima 1983); and nucleotide diversity,  $\pi_2$  (equ. 10.6 Nei 1987).  $F_{ST}$  and  $Nm$  were estimated following the method of Hudson *et al.* (1992) and exact tests of differentiation were conducted following the method of Raymond & Rousset (1995). Kimura-2-parameter (K2P) genetic distances are reported (Kimura 1980) to correct for mutational saturation, although at the within-species level this is unlikely to present a problem. These K2P distances were not used in any analyses, and are shown for comparative purposes only. Phylogenetic analyses were conducted with the rcs program (Clement *et al.* 2000), which was used to construct statistical parsimony networks of mitochondrial haplotypes. This method appears to provide better representations of gene genealogies at the population level than other phylogenetic inference methods (Templeton *et al.* 1992).

### Demographic analyses

Tests were conducted to assess whether the populations examined fit expectations for mutation-drift equilibrium, and whether they fit a population size stationary model vs. a population expansion model. Tajima's  $D$  (Tajima 1989) and Fu's  $F_S$  (Fu 1996) statistics were estimated to assess evidence of population expansions. Although these statistics were developed as tests of selective neutrality, they are very powerful for detecting departures from population size equilibrium caused by population expansions or bottlenecks (Aris-Brosou & Excoffier 1996; Tajima 1996; Fu 1997; Ray *et al.* 2003). ARLEQUIN was used to conduct these tests and calculate the corresponding  $P$ -values. Mismatch distributions of DNA sequences (Harpending 1994; Schneider & Excoffier 1999) were also examined. Ragged and erratic distributions are expected for stationary popu-

lations, whereas smooth distributions are expected for populations that experienced range expansions (Harpending 1994). ARLEQUIN estimates parameters related to a population growth expansion, such as expansion time  $\tau$  ( $= 2ut$ , where  $u$  is the mutation rate and  $t$  is the number of generations since the expansion),  $\theta_0 = 2uN_0$ , and  $\theta_1 = 2uN_1$  (where  $N_0$  and  $N_1$  are the population sizes before and after the expansion). The values reported for  $\theta_0$  and  $\theta_1$  are mean values based on 100 replicates and an alpha value of 0.05. A model of population expansion is assumed if  $\tau > 0$  and  $\theta_1 > \theta_0$ , whereas a model of population stationarity is assumed if  $\tau = 0$  or  $\theta_1 = \theta_0$ . Validity of the estimated demographic model is tested by obtaining the distribution of a test statistic SSD (the sum of squared differences) between the observed and an estimated mismatch distribution obtained by a bootstrap approach (Schneider & Excoffier 1999). The  $P$ -value of the SSD statistic is computed as the proportion of simulated cases that show a SSD value larger than the original. A significant SSD value ( $P$ -values  $< 0.05$ ) is taken as evidence for departure from the estimated model of population expansion (when  $\tau > 0$  and  $\theta_1 > \theta_0$ ), or from a model of population stationarity (when  $\tau = 0$  or  $\theta_1 = \theta_0$ ). Demographic tests were conducted first for each locality and secondly for pooled localities that were determined to be genetically homogeneous by the exact tests of population differentiation. The same groups of pooled localities were used to conduct Analysis of Molecular Variance (AMOVA, Excoffier *et al.* 1992), also using ARLEQUIN, to assess the proportion of genetic variation attributable to differentiation within groups.

## Results

### Geographic distributions

*R. pachyptila* has the broadest known distribution of these five polychaetes, ranging from 27°N (Guaymas Basin) to 32°S on the EPR and on the GAR (Table 1). *T. jerichonana* ranges from 13°N to 32°S on the EPR and is not known from the GAR. *Oasisia* is a polytypic genus with highly divergent evolutionary lineages that probably comprise undescribed species (Hurtado *et al.* 2002). *O. alvinae sensu stricto* has been found between 21°N and 9°N on the northern EPR. Hurtado *et al.* (2002) report the presence of two other very divergent lineages of *Oasisia* and suggest they may constitute different species. One restricted to vents south of the Easter Microplate (31–32°S) and the other found at 9°N vents, with *mtCOI* divergences of ~9% and ~6% from *O. alvinae s. s.*, respectively. The present analysis was restricted to what we regard as *O. alvinae s. s.* samples, as they formed a distinct, well-supported monophyletic group (maximum *mtCOI* K2P divergence = 3.0%) that excluded the other two divergent lineages. An earlier allozyme study revealed no evidence for cryptic subdivision

**Table 2** Mitochondrial COI diversity

Species	<i>N</i>	<i>L</i>	<i>K</i>	<i>h</i>	<i>k</i>	$\pi_1$	$\pi_2$
<i>R. pachyptila</i>	41	640	3	0.3305 (0.0817)	2	0.3390 (0.3434)	0.0005 (0.0005)
<i>T. jerichonana</i>	115	630	9	0.5545 (0.0333)	9	1.1210 (0.7361)	0.0018 (0.0013)
<i>O. alvinae</i>	43	649	20	0.8893 (0.0345)	33	9.2270 (4.3256)	0.0142 (0.0074)
<i>A. pompejana</i>	93	648	40	0.9054 (0.0254)	48	5.2382 (2.5567)	0.0081 (0.0044)
<i>B. symmytilida</i>	61	641	38	0.9601 (0.0161)	40	3.8727 (1.9716)	0.0060 (0.0034)

*N* = number of sequences; *L* = sequence length; *K* = number of haplotypes; *h* = haplotype diversity; *k* = number of polymorphic sites;  $\pi_1$  = mean number of pairwise differences;  $\pi_2$  = nucleotide diversity. Standard errors in parentheses.

in this subset of *Oasisia* samples (Black *et al.* 1998). No *Oasisia* tubeworms have been observed or collected between 9°N and the Easter Microplate region of the EPR (~3000 km). *Oasisia*-like tubeworms were observed at GAR vents in 1988, but specimens were not obtained then for genetic analyses (C. Fisher, personal communication). The Pompeii worm, *A. pompejana*, ranges from 21°N to 32°S on the EPR and is not known from GAR. The range of the commensal scale worm *B. symmytilida* coincides with that of its host, the mussel *B. thermophilus*, from 13°N to 32°S along the EPR and on the GAR.

#### Mitochondrial diversity and phylogeography

Altogether, 353 *mtCOI* sequences were obtained from the five worm species (Table 2). All different haplotypes were deposited in GenBank (Accession nos AY645949–AY646057). Mitochondrial diversity varied greatly across the five species. *R. pachyptila* and *T. jerichonana* exhibited very low haplotypic diversity relative to *O. alvinae*, *A. pompejana* and *B. symmytilida*, which have more segregating sites across this 630–649 bp portion of *mtCOI*.

*R. pachyptila*. A preliminary sequence analysis of EPR individuals from 27°N (*n* = 10), 21°N (*n* = 10) and 31–32°S (*n* = 12) identified only one polymorphic site. One of the haplotypes was observed in 25 individuals (*Rp-A*) and the other (*Rp-B*) in seven. Because so little sequence variation was observed, 94 additional specimens were screened from EPR localities using the restriction enzyme *BsiE-I* that cuts the polymorphic site in the *Rp-B* haplotype. In total, 112 individuals had the *Rp-A* haplotype in EPR localities north of the Easter Microplate (27°N to 17°S), and only two individuals from 7°S had the *Rp-B* haplotype. However, the frequencies of the two haplotypes shifted dramatically

**Table 3** *Riftia pachyptila*. Pairwise  $F_{ST}$  (above) and *Nm* values (below diagonal). Bold-type  $F_{ST}$  values are statistically significant ( $P < 0.05$ ) based on exact tests

Locality	27°N	21°N	9°N	GAR	7°S	11°S	17°S	32°S
27°N		0	0	0	0.01	0	0	<b>0.52</b>
21°N	*		0	0	0.01	0	0	<b>0.52</b>
9°N	*	*		0	0.02	0	0	<b>0.50</b>
GAR	*	*	*		0.02	0	0	<b>0.62</b>
7°S	*	*	21.4	*		0.04	0.04	<b>0.48</b>
11°S	*	*	*	*	13.6		0	<b>0.66</b>
17°S	*	*	*	*	12.7	*		<b>0.66</b>
32°S	0.5	0.5	0.3	0.5	0.6	0.3	0.3	

\**Nm* is undefined and approaches panmixia.

to the south of the Easter Microplate, at 31–32°S, where five individuals were observed with the *Rp-A* haplotype and seven with the *Rp-B* haplotype. Nine individuals from GAR were also sequenced and eight of them had the *Rp-A* haplotype and the remaining individual had a third haplotype (*Rp-C*) that differs from the common *Rp-A* by only one position (Fig. 2A).

Limited genetic variability may limit our conclusions about gene flow in this species. However, the severe shift observed in the frequency of the two EPR haplotypes between the population south of the Easter Microplate (31–32°S) and the other populations to the north suggests very restricted gene flow across the Easter Microplate (Table 3; Fig. 2F). Rates of gene flow between localities north of the Easter Microplate appear to be high, since the *Rp-A* haplotype was essentially fixed at these localities.

*T. jerichonana*. Sequences from 115 individuals yielded nine polymorphic sites and nine haplotypes (Table 2). A cluster of haplotypes (shown blue in Fig. 2B) differed by single nucleotide substitutions from the most common haplotype (*Tj-D*). A second group of haplotypes (shown red in Fig. 2B) connects to the blue group through a 'missing' haplotype. Frequencies of the two groups of haplotypes were distributed as a north–south cline (Fig. 2G). As in *R. pachyptila*, populations of *T. jerichonana* from south of the Easter Microplate (31–32°S) also differed from all populations to the north (Table 4).  $F_{ST}$  values between these groups ranged from 0.38 to 0.74, and estimated rates of gene flow were low ( $Nm < 0.8$ ). The *T. jerichonana* group to the north of the Easter Microplate is subdivided into two subgroups separated by the Equator (i.e. 9–13°N vs. 7–17°S).  $F_{ST}$  values between these two groups ranged from 0.09 to 0.22 and estimates of gene flow between groups were at intermediate levels ( $Nm$  range: 1.8–5.2).

*O. alvinae*. Sequences from 43 *O. alvinae* individuals yielded 33 polymorphic sites and 20 haplotypes. The statistical

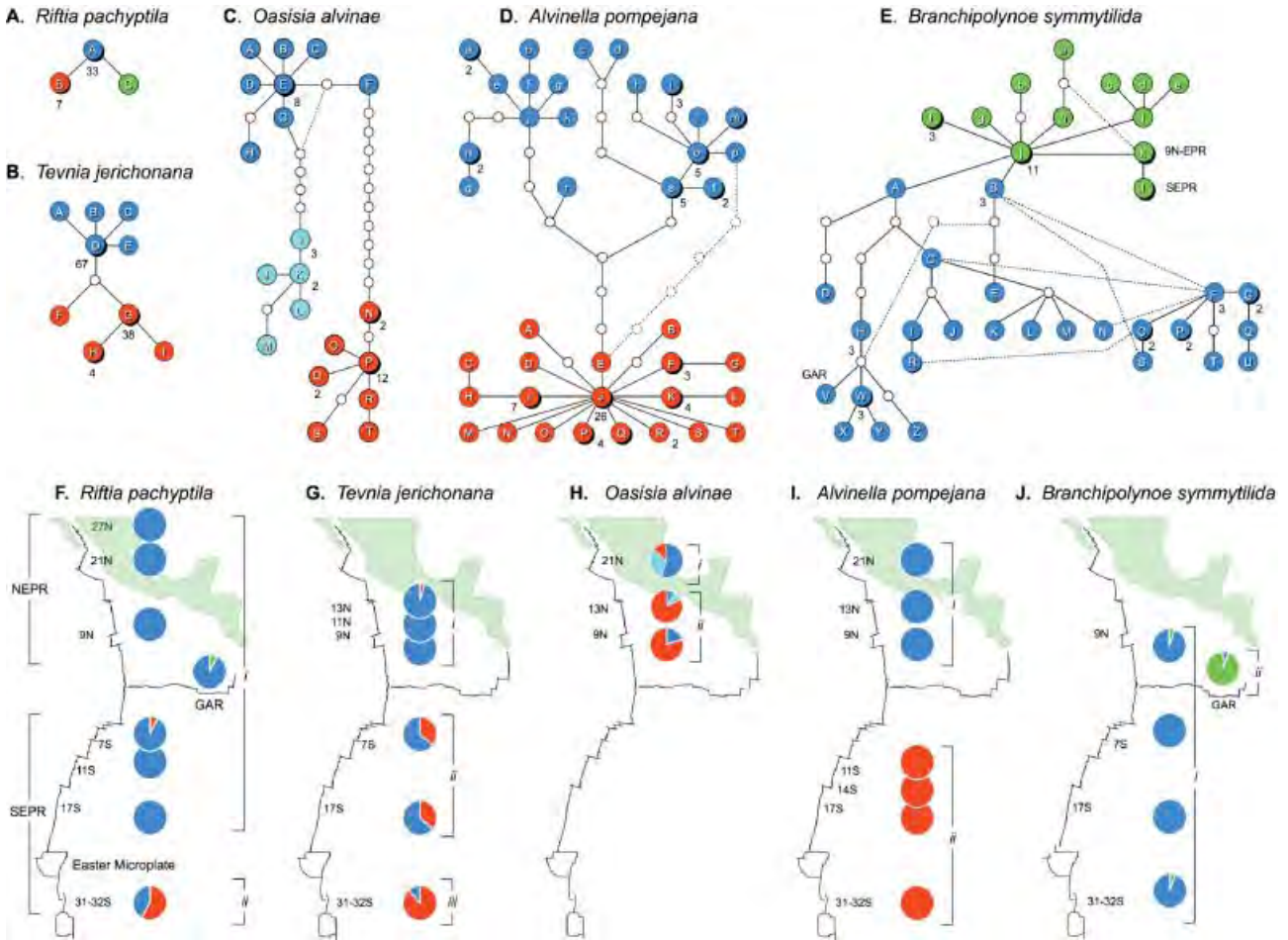


Fig. 2 Haplotype networks and frequencies for five eastern Pacific hydrothermal vent annelids. (A–E) Haplotype networks. (F–J) Distribution of haplotype frequencies. Colour codes used for haplotypes in the networks correspond with those used in the pie-diagrams.

**Table 4** *Tevnia jerichonana*. Pairwise  $F_{ST}$  (above) and  $Nm$  values (below diagonal). Bold-type  $F_{ST}$  values are statistically significant ( $P < 0.05$ ) based on exact tests

Locality	13°N	11°N	9°N	7°S	17°S	32°S
13°N		0.00	0.00	0.09	<b>0.11</b>	<b>0.68</b>
11°N	117.0		0.01	<b>0.17</b>	<b>0.20</b>	<b>0.71</b>
9°N	*	34.9		<b>0.19</b>	<b>0.22</b>	<b>0.74</b>
7°S	5.2	2.4	2.1		0.00	<b>0.39</b>
17°S	4.0	2.1	1.8	*		<b>0.38</b>
32°S	0.2	0.2	0.2	0.8	0.8	

\* $Nm$  is undefined and approaches panmixia.

parsimony network was partitioned into three clusters (shown light blue, dark blue and red in Fig. 2C). The red and dark blue clusters are connected by 10 ‘missing’ haplotypes, and the light and dark blue clusters are linked by four haplotypes. Mean K2P divergence between the red and blue clusters is 2.5%. Significant genetic differentiation

**Table 5** *Oasisia alvinae*. Pairwise  $F_{ST}$  (above) and  $Nm$  values (below diagonal). Bold-type  $F_{ST}$  values are statistically significant ( $P < 0.05$ ) based on exact tests

Locality	21°N	13°N	9°N
21°N		<b>0.48</b>	<b>0.49</b>
13°N	0.55		0.00
9°N	0.52	*	

\* $Nm$  is undefined and approaches panmixia.

was observed between populations separated by the Rivera Fracture Zone (21°N vs. 9–13°N; Table 5; Fig. 2H).

*A. pompejana*. Sequences from 93 individuals yielded 48 polymorphic sites and 40 haplotypes. The statistical parsimony network revealed a clear separation between northern and southern EPR localities (red vs. blue, Fig. 2D). Two equally parsimonious paths separate the red and blue

**Table 6** *Alvinella pompejana*. Pairwise  $F_{ST}$  (above) and  $Nm$  values (below diagonal). Bold-type  $F_{ST}$  values are statistically significant ( $P < 0.05$ ) based on exact tests

Locality	21°N	13°N	9°N	11°S	14°S	17°S	32°S
21°N		0.01	0.00	<b>0.65</b>	<b>0.46</b>	<b>0.70</b>	<b>0.73</b>
13°N	32.34		0.00	<b>0.75</b>	<b>0.64</b>	<b>0.76</b>	<b>0.78</b>
9°N	*	*		<b>0.53</b>	<b>0.44</b>	<b>0.58</b>	<b>0.61</b>
11°S	0.27	0.17	0.45		0.00	0.00	0.01
14°S	0.60	0.29	0.65	*		0.00	0.00
17°S	0.22	0.16	0.37	*	*		0.07
32°S	0.19	0.14	0.32	38.26	*	6.63	

\* $Nm$  is undefined and approaches panmixia.

clusters. The red cluster exhibited a star-like phylogeny with short branches from the most frequent haplotype (*Ap-J*), whereas the blue cluster exhibited a more complex branching topology. As expected, the northern and southern EPR samples differed significantly (Table 6; Fig. 2I).  $F_{ST}$  values between these groups ranged from 0.44 to 0.78, and estimated rates of gene flow were low ( $Nm$  range: 0.14–0.65). This dramatic partitioning of mitochondrial lineages across the Equator contrasted with homogeneity within subdivisions. For both the northern and southern regions, pairwise  $F_{ST}$  values revealed no appreciable genetic differentiation and extensive gene flow within each subdivision. Although larger samples from some localities would have improved the  $F_{ST}$  estimates, sample sizes of these worms generally were beyond our control. Nonetheless, this species exhibited a large number of polymorphic sites (mean number of pairwise differences =  $5.2 \pm 2.6$ ) that may have compensated for low sample size in some of the pairwise estimates of  $F_{ST}$ .

*B. symmytilida*. Sequences from 61 individuals yielded 40 polymorphic sites and 38 haplotypes. The statistical parsimony network was complex (Fig. 2E), exhibiting multiple equally parsimonious links. One discrete cluster of haplotypes (shown green in Fig. 2E) was restricted almost entirely to the GAR; however, green haplotypes were nearly absent from the EPR, which houses a poorly resolved complex network of haplotypes (blue). As expected, *B. symmytilida* from the GAR differed significantly from the EPR worms.  $F_{ST}$  values between these groups ranged from 0.19 to 0.48 and estimated gene flow was fairly low ( $Nm$  range: 0.55–2.19). However, unlike the other worms, *B. symmytilida* exhibited no genetic differentiation along its entire range on the EPR (Table 7; Fig. 2J). Pairwise  $F_{ST}$  values indicated extensive gene flow across the EPR. Like *A. pompejana*, this species also exhibited a large number of polymorphic sites (mean number of pairwise differences =  $3.9 \pm 2.0$ ) that may have compensated for low sample size in some of the pairwise estimates of  $F_{ST}$ .

**Table 7** *Branchipolynoe symmytilida*. Pairwise  $F_{ST}$  (above diagonal) and  $Nm$  values (below diagonal). Bold-type  $F_{ST}$  values are statistically significant ( $P < 0.05$ ) based on exact tests

Locality	9°N	7°S	17°S	32°S	GAR
9°N		0	0	0	<b>0.19</b>
7°S	*		0	0	<b>0.32</b>
17°S	*	*		0	<b>0.48</b>
32°S	*	*	*		<b>0.32</b>
GAR	2.19	1.06	0.55	1.06	

\* $Nm$  is undefined and approaches panmixia.

### Demographic analyses

Demographic tests were conducted for each locality separately and on groups of localities (Roman numerals in Fig. 2 and Table 8) that were genetically homogeneous as determined by previous exact tests of population differentiation. Both methods provided similar results, but because some localities had small sample sizes, we report only the results for pooled localities. AMOVAS for each species (not shown) revealed that the proportion of genetic variation due to heterogeneity among samples within these groups of localities did not differ from zero. Demographic analyses suggested that population expansions might have occurred in *T. jerichonana*, *A. pompejana* and *B. symmytilida* (Table 8). Significant Tajima's  $D$  and Fu's  $F_S$  statistics were obtained for the northern EPR population of *T. jerichonana*, the southern EPR population of *A. pompejana*, and the GAR population of *B. symmytilida*, whereas only significant  $F_S$  values were obtained for the northern EPR population of *A. pompejana* and the EPR population of *B. symmytilida*. Significant test statistics in *T. jerichonana* from the northern EPR and in *B. symmytilida* from the GAR and EPR resulted from the presence of a few individuals (assumed to be migrants) from very divergent clades. As expected (see Ray *et al.* 2003), the  $D$  statistic was less sensitive than  $F_S$  to departures from population equilibrium.

Mismatch statistics of populations with significant  $D$  and  $F_S$  statistics were consistent with models of population expansion. Mismatch distributions for these populations were unimodal (not shown). Evidence of population expansion (i.e.  $\tau > 0$ ;  $\theta_1 > \theta_0$ ) was found in nearly all tests except for the SEPR (7–17°S) grouping of *T. jerichonana*, where the SSD statistic was significant, therefore rejecting the population expansion hypothesis. The mismatch graph was somewhat ragged (not shown), and the  $D$  and  $F_S$  statistics were not significant. The results for *O. alvinae* were more complicated.  $D$  and  $F_S$  were not significant and the mismatch graphs may be interpreted as ragged (not shown), suggesting population stationarity. However, mismatch parameters were consistent with a model of population expansion ( $\tau > 0$ ;  $\theta_1 > \theta_0$ ). Demographic analyses were not

**Table 8** Population expansion tests:  $D$  = Tajima's  $D$ -test;  $F_S$  = Fu's  $F_S$  tests.  $P$ -values follow each test (significant are in bold type). Population growth expansion parameters:  $\tau$ ,  $\theta_0$  and  $\theta_1$ . SSD = sum of square deviation and corresponding  $P$ -value

Species/group	$D$	$P$	$F_S$	$P$	$\tau$	$\theta_0$	$\theta_1$	SSD	$P$
<i>R. pachyptila</i>									
i (EPR, 27°N–17°S)	-1.15	0.15	-1.18	0.06					
ii (SEPR, 31–32°S)	1.38	0.95	1.15	0.62					
<i>T. jerichonana</i>									
i (NEPR, 9–13°N)	-2.00	<b>0.00</b>	-3.89	<b>0.00</b>	3.01	0.04	76.77	0.00	0.37
ii (SEPR, 7–17°S)	-0.12	0.47	0.07	0.53	2.57	0.10	641.03	0.14	0.04
iii (SEPR, 31–32°S)	-0.85	0.23	-0.52	0.29	2.02	0.11	546.76	0.00	0.69
<i>O. alvinae</i>									
i (NEPR, 21°N)	-0.41	0.40	-2.69	0.13	4.39	2.58	78.90	0.03	0.49
ii (NEPR, 9–13°N)	-0.47	0.35	0.62	0.62	6.19	0.54	31.20	0.06	0.36
<i>A. pompejana</i>									
i (NEPR, 9–21°N)	-0.89	0.21	-7.21	<b>0.01</b>	9.57	0.12	17.78	0.00	0.87
ii (SEPR, 7–32°S)	-2.22	<b>0.00</b>	-19.38	<b>0.00</b>	1.41	0.27	3005.66	0.01	0.07
<i>B. symmitilida</i>									
i (EPR, 13°N–32°S)	-1.23	0.08	-3.60	<b>0.00</b>	3.76	0.42	2087.79	0.00	0.37
ii (GAR)	-2.05	<b>0.00</b>	-5.84	<b>0.00</b>	1.94	0.06	2034.22	0.00	0.79

conducted for *R. pachyptila*, because most of the specimens were scored using a restriction enzyme. Nevertheless, extremely reduced mitochondrial variability observed among the individuals of this species suggests that a very strong bottleneck or mitochondrial selective sweep has occurred recently in this species. Results of an earlier allozyme study suggest that bottlenecks might have impacted northern populations of *R. pachyptila* (Black *et al.* 1994). Polymorphic loci carry only two alleles and the least frequent allele generally exceeded 0.10 in frequency. Rare alleles are lost rapidly during bottlenecks and founder events, but heterozygosity due to remaining common alleles need not diminish greatly if the population has a high intrinsic rate of increase (Nei *et al.* 1975), or if balancing selection was acting on the polymorphic allozyme loci (Karl & Avise 1992).

## Discussion

Population genetic and phylogenetic analyses of five polychaete annelids revealed patterns of genetic differentiation among vent fields spread across ~7000 km of the East Pacific Rise (EPR) and Galapagos Rift (GAR). Different patterns of mitochondrial population structure exist among these species, suggesting substantial differences in their evolutionary histories, individual life histories and dispersal strategies. The results revealed four barriers to dispersal that affected one or more of these annelid species: (i) a dispersal filter around the Easter Microplate region; (ii) a dispersal filter separating EPR and GAR populations; (iii) a dispersal filter around the Equator separating northern and southern EPR populations; and (iv) a dispersal filter

around the Rivera Fracture Zone. However, no evidence was found among these annelids for restricted gene flow across the 15°S latitude region of the EPR, where strong cross-axis currents are hypothesized to disrupt along-axis dispersal of the vent bivalves *C. magnifica* and *B. thermophilus* (Hurtado 2002; Hurtado *et al.* 2003; Won *et al.* 2003). The four dispersal filters affecting these vent annelids are discussed below, and our findings are compared with genetic and biogeographical evidence from other vent-endemic macroinvertebrates. The location of these filters is concordant with genetic shifts or distributional limits of other vent species.

### The Easter Microplate region

Mitochondrial evidence suggests that the siboglinid tube-worms *R. pachyptila* and *T. jerichonana* are subdivided across the Easter Microplate region, but the polynoid *B. symmitilida* and alvinellid *A. pompejana* are not. Mitochondrial and allozyme evidence indicate that populations of *Bathymodiolus* mussels also are isolated across the Easter Microplate region and may comprise distinct species (Won *et al.* 2003). Other vent animals exhibit evidence for an older historical barrier across the Easter Microplate. *Oasisia* tubeworms from south of the Easter microplate appear to comprise a distinct species from those found on the northern EPR (Hurtado *et al.* 2002). The bythograeid crabs *Allograea tomentosa* and *Bythograea vrijenhoeki* have only been found south of the microplate, whereas their respective sister-species, *Cyanograea praedator* and *Bythograea laubieri*, are distributed widely to the north (Guinot *et al.* 2002;

Guinot & Hurtado 2003). The Easter Microplate region also coincides with the distributional boundaries of several vent animals. As mentioned previously, two bythograeid crabs, a mussel and an *Oasisia* tubeworm species appear to be endemic to vents south of the microplate. Conversely, several northern species appear to reach their southern limits just north of the Easter Microplate (e.g. the previously mentioned vent crabs *Bythograea thermydron* and *C. praedator*). We did not find the clam *C. magnifica* south of the Easter Microplate (Hurtado *et al.* 2003). However, the clam appears to be a late colonizer at nascent vents (Shank *et al.* 1998), and perhaps the 31–32°S vents had not reached a sufficiently late stage of succession at the time of our visit in 1999.

The Easter Microplate may constitute a historical dispersal barrier for some taxa. It was formed between 5.25 and 2.47 million years ago (Mya) (Naar & Hey 1991; Rusby & Searle 1995). Transform faults on the north and south flanks of the microplate, and chains of young seamounts extending to the east and west (Searle *et al.* 1989), create topographical features that appear to entrain strong cross-axis currents (Fujio & Imasato 1991). However, high levels of molecular divergence in *Oasisia* tubeworms (Hurtado *et al.* 2002) and bythograeid crabs (Guinot *et al.* 2002; Guinot & Hurtado 2003) suggest that their divergence began prior to formation of the microplate. This region lies at a well-known zoogeographical boundary separating Indo-Pacific and Antarctic deep-sea faunas, and defined by the Antarctic Circumpolar Current (ACC) (Mironov *et al.* 1998; Vinogradova 1979). Transitions between biogeographical provinces are often associated with phylogeographical breaks in taxa that cross provincial boundaries (reviewed by Avise 2000). The ACC provides a link between the Pacific, Atlantic and Indian Oceans that originated with the opening of the Tasmanian Gateway between Australia and Antarctica 34 Mya and the Drake Passage between South America and Antarctica 20 Mya (Barker & Burrell 1977; Lawver *et al.* 1992). A molecular clock used to estimate the time of divergence of the *Oasisia* lineage from 31–32°S (Hurtado 2002), which is up to 10% divergent from northern lineages, indicates that divergence of this lineage occurred at least 18–21.6 Mya (assuming that the substitution rate for *mtCOI* in siboglinids is 0.43–0.48%/MY; Chevaldonné *et al.* 2002). This estimate overlaps with the time the ACC began crossing the EPR.

#### *A filter around the Equator*

Significant mitochondrial divergence exists between populations of the alvinellid *A. pompejana* separated by a habitat gap that spans the Equator. Northern and southern EPR populations of this worm are reciprocally monophyletic, which suggests long-standing separation. Although less dramatic, mitochondrial frequencies shift significantly between *T. jerichonana* populations from north and south

of the Equator. In contrast, the annelids *R. pachyptila* and *B. symmytilida*, the clam *C. magnifica* (Hurtado *et al.* 2003) and the mussel *B. thermophilus* (Won *et al.* 2003) show no evidence for restricted gene flow across the Equator. The distributional limits of two bythograeid crabs suggest the presence of a historical dispersal filter around the Equator. *Bythograea microps* appears to be restricted to northern EPR (21–9°N) vents, whereas *B. laubieri* to southern EPR vents (11–32°S) (Guinot *et al.* 2002; Guinot & Hurtado 2003).

A strong, eastward, deep-sea, equatorial current creates abrupt northern and southern gyres where it crosses the EPR (Reid 1997). Such currents might present a barrier for dispersal of pelagic larvae, which crabs are likely to possess, but these currents should not impede dispersal of negatively buoyant larvae that travel near the bottom, such as the larvae of *A. pompejana*, a species with restricted dispersal across this region. Topographical features associated with the triple junction at the EPR and Galapagos Rift convergence (e.g. the Hess Deep, a 6000 m depression), where the Galapagos Microplate is formed (Lonsdale 1988), may present a barrier to dispersal for species such as *A. pompejana*.

#### *The Galapagos barrier*

Hydrographic and topographical features of the equatorial region may also create a dispersal filter between the GAR and EPR. For example, *B. symmytilida* from the GAR was nearly fixed for a unique mitochondrial lineage. Other taxa also reveal evidence for restricted dispersal between the EPR and GAR. *C. magnifica* clams from the GAR are fixed for a unique mitochondrial variant (Hurtado *et al.* 2003), and extremely reduced levels of gene flow occur between GAR and EPR populations of the amphipod *Ventiella sulfuris* (France *et al.* 1992). Restricted gene flow between the EPR and GAR populations was also suggested for the alvinellid *Paralvinella grassleii* (Jollivet *et al.* 1995). On the other hand, gene flow is not restricted between *B. thermophilus* mussels from GAR and northern EPR populations (Craddock *et al.* 1995; Won *et al.* 2003). Distributions of several vent species also suggest isolation of the GAR. The vent annelids *T. jerichonana* and *A. pompejana*, and bythograeid crabs *C. praedator* and *B. laubieri* (Guinot & Hurtado 2003) are not found on the GAR. Conversely, *Bythograea galapagensis* (a possible synonymy of *Bythograea intermedia*) is endemic to the GAR, and this species is 9.6% divergent from its closest relative, *B. thermydron*, which is distributed widely at EPR and GAR vents (Guinot & Hurtado 2003).

#### *The Rivera Fracture Zone*

The Rivera Fracture Zone (FZ) (Fig. 1) is the longest transform fault (240 km) along the EPR axis. It lacks hydrothermal habitats and coincides with a strong westward current at 2500 m depth (Reid 1997). Mitochondrial

frequencies in *O. alvinae* shift significantly across this region. The amphipod *V. sulfuris* also exhibits shifts in allozyme frequencies across this region (France *et al.* 1992). The Rivera FZ corresponds with the northern limit for a number of EPR taxa, including the annelids *T. jerichonana* and *B. symmytilida* and the mussel *B. thermophilus*, even though the chemical milieu at 21°N appears to be adequate for the missing species (Van Dover 2000). Strong currents in this region may divert the larvae of some EPR species away from the ridge axis.

#### Larval histories and genetic patterns

Unfortunately, larval life histories of most vent invertebrates are poorly understood (Tyler & Young 1999; Van Dover 2000), which severely limits our ability to interpret the present genetic patterns. In addition, it is not possible to infer modes of larval dispersal based on similarities of genetic differentiation patterns, because they are very different in the species analysed. The worm *A. pompejana* releases large negatively buoyant eggs (~200 µm) that are expected to drift with bottom currents (Pradillon *et al.* 2001), and larvae are believed to be benthic (Jollivet *et al.* 1998). Delayed metamorphosis in cold water and movement through along-ridge axis currents appear to increase long distance dispersal abilities of these benthic larvae (Pradillon *et al.* 2001). For a species with such larval characteristics, an important dispersal filter is found between 9°N and 11°S in the EPR (i.e. the filter around the Equator), but the Easter Microplate filter does not seem to affect dispersal of this species. In contrast, the Easter Microplate filter appears to restrict gene flow in *R. pachyptila*, a species that produces neutrally buoyant small eggs (~100 µm) with sufficient energy reserves to persist in the water column for about 38 days (Marsh *et al.* 2001). At 9°N the nonfeeding trochophore larvae of *R. pachyptila* are expected to disperse a mean distance of about 100 km, although the dispersal distance does not appear limited by the physiological performance of the larvae but rather by temporal oscillations in the along-axis currents and by larval loss in cross-axis flows. Therefore, dispersal distances of larvae of this species at other vent sites will depend on local current regimes.

Larval biology of *B. symmytilida* is unknown. A related species from the Mid-Atlantic Ridge, *B. seepensis*, has very large eggs (~500 µm) and lecithotrophic larvae (Tyler & Young 1999). *B. symmytilida* shows no genetic differentiation across the Easter Microplate. Based on this similarity with *A. pompejana*, one might suggest similar larval biologies, i.e. negatively buoyant eggs and arrested development. However, population structure of the two species differs north of the Easter Microplate, where *A. pompejana* is subdivided across the Equator, while *B. symmytilida* is not. Similarly, one might speculate that the larvae of *T. jerichonana* are similar to those of its closest relative, *R. pachyptila*,

because both species showed significant mitochondrial subdivision across the Easter Microplate. Another siboglinid, *O. alvinae*, does not appear to disperse long distances, because significant genetic subdivision was observed at comparatively small geographical scales (21°N vs. 9–13°N EPR).

#### Ecology and genetic patterns

A positive correlation between allozyme diversity and levels of occupancy is reported for vent invertebrates from the northern EPR and GAR (Vrijenhoek 1997). Species with established populations at most of the known vent fields are reported to have higher levels of allozyme diversity than species that occupy fewer vent fields. However, site occupancy is confounded with the order in which these species establish colonies at nascent hydrothermal vents (Vrijenhoek *et al.* 1998). Early colonizers (appearing within 2 years of vent formation — *R. pachyptila*, *T. jerichonana*, *P. grasslei*, *Alvinella caudata*, *A. pompejana*, *Lepetodrilus elevatus* and *V. sulfuris*) had high occupancy and nearly twice the allozyme diversity of late colonizers (appearing after 2 years of vent formation — *O. alvinae*, *C. magnifica*, *B. thermophilus* and *Eulepetopsis vitrea*). However, these predictions are not always consistent with the present mitochondrial data obtained from approximately twice the geographical range. For example, lower mitochondrial diversity is observed in *R. pachyptila* and *T. jerichonana*, early colonizers with high site occupancy, than in *O. alvinae*, a late colonizer with low occupancy. Conversely, *B. symmytilida* and its mussel host *B. thermophilus* (Won *et al.* 2003), which are late colonizers with relatively low occupancy, both have high levels of mitochondrial diversity. On the other hand, allozyme and mitochondrial diversity are both high in *A. pompejana*, an early colonizer (Jollivet *et al.* 1995); and the clam *C. magnifica*, a late colonizer with very low occupancy, has remarkably low diversity in allozymes and nuclear DNAs (Karl *et al.* 1996), as well as low mitochondrial diversity (Hurtado *et al.* 2003). Contrasting patterns of allozyme and mitochondrial diversity indicate that overall genetic diversity of vent macroinvertebrates cannot be predicted simply from knowledge of site occupancy and successional order. More complex evolutionary and demographic processes appear to have shaped genetic diversity in these species. Demographic tests suggest that the ephemeral nature of vent habitats in the EPR and GAR has had strong effects on mitochondrial diversity of these vent annelids. For the most part, these populations do not appear to be in mutation-drift equilibrium, due probably to continuous population reductions and expansions.

#### Conclusion

We examined patterns of population genetic differentiation among five vent annelid species in the eastern Pacific and

identified four regions where dispersal filters appear to restrict gene flow. Comparisons with other population genetic and phylogenetic studies indicate these filters may affect also other vent taxa (i.e. crustaceans, molluscs and other annelids). However, these filters work on different time scales and to different degrees among various taxa. For example, the Easter Microplate region has a significant historical component, as it lies at the boundary between two zoogeographical divisions of deep-sea fauna (Vinogradova 1979; Mironov *et al.* 1998). Currents that coincide with the Rivera FZ may create present-day filters for some species with buoyant larvae. The equatorial region exhibits a combination of deep-oceanic currents and topographical features that may limit faunal exchange between the EPR and GAR, and across the Equator along the EPR. Finally, a strong cross-current at 15°S in the EPR, that is hypothesized to severely disrupt along-axis dispersal of *C. magnifica* clams and *B. thermophilus* mussels, does not appear to impede dispersal of the annelids studied here. The present evidence for dispersal barriers based on patterns of mitochondrial differentiation is strengthened by comparative genetic and biogeographical evidence from other vent taxa. Nevertheless, studies using nuclear markers should be conducted to assess whether our results reflect genome-wide patterns of phylogeographical structure.

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