Intergradation between discrete lineages of *Tevnia jerichonana*, a deep-sea hydrothermal vent tubeworm

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ABSTRACT

We describe a broad zone of intergradation between genetically differentiated, northern and southern lineages of the hydrothermal vent tubeworm, *Tevnia jerichonana*. DNA sequences from four genes, nuclear *HSP* and *ATP α* and mitochondrial *COI* and *Cytb* were examined in samples from eastern Pacific vent localities between 13° N and 38° S latitude. Allelic frequencies at these loci exhibited concordant latitudinal clines, and genetic differentiation (pairwise Φ_{ST} 's) increased with geographical distances between sample localities. Though this pattern of differentiation suggested isolation-by-distance (IBD), it appeared to result from hierarchical population structure. Genotypic assignment

tests identified two population clusters comprised of samples from the northern East Pacific Rise (NEPR: 9–13° N) and an extension of the Pacific-Antarctic Ridge (PAR: 31–32° S) with a zone of intergradation along the southern East Pacific Rise (SEPR: 7–17° S). The overall degrees of DNA sequence divergence between the NEPR and PAR populations were slight and not indicative of lengthy isolation. Bayesian assignment methods suggested that the SEPR populations constitute intergrades that connect the NEPR and PAR populations. Though it typically is difficult to distinguish between primary and secondary intergradation, our results were consistent with parallel studies of vent-restricted species that suggest a high degree of demographic instability along the superfast-spreading SEPR axis. Frequent local extinctions and immigration from NEPR and PAR refugia probably shaped the observed pattern of intergradation.

Keywords: Intergrade zone, *Tevnia jerichonana*, Annelida, Polychaeta, Siboglinidae, Hydrothermal vent.

We dedicate this paper to the memory of Professor Peter Rona, a valued colleague and participant in the SEPR-1999 expedition that sampled many of the specimens examined in this and related studies of hydrothermal vent organisms.

1. Introduction

Clines, or geographical gradients in the characteristics of species (Huxley, 1938), can result from adaptive differentiation (primary intergradation) or from the reconnection of genetically differentiated populations (secondary intergradation). The causes are difficult to distinguish, however, in the absence of historical biogeographic information (Endler, 1977, 1982). Examinations of multi-gene data can help to distinguish the causes because primary intergradation is unlikely to generate coincident clines among multiple independent genes, whereas secondary intergradation commonly produces parallel clines (Barton, 1983). Recent secondary intergradation also tends to generate linkage disequilibria among independent genes (Barton and Hewitt, 1985). These patterns have been used to investigate dynamic processes affecting hybrid zones and intergradation in a wide variety of fresh-water and terrestrial plants and animals (reviews in Harrison, 1993; Arnold, 1997). Marine examples are less numerous, however, and mostly limited to commercially important, near-shore taxa (Gardner, 1997).

Only two hybrid zones are known from mid-ocean environments, and both involve deep-sea hydrothermal vent mussels. *Bathymodiolus azoricus* and *B. puteoserpentis* contact one-another and hybridize at an intermediate locality on the Mid-Atlantic Ridge (MAR) (O'Mullan et al., 2001; Won et al., 2003b). *Bathymodiolus thermophilus* and *B. antarcticus* contact one another along the western flank of the Easter Microplate, a

tectonic feature at the southern terminus of the East Pacific Rise (EPR) (Johnson et al., 2013). Both of the hybrid zones exhibit parallel step-clines for multiple genes and significant linkage disequilibria among mitochondrial haplotypes and nuclear genes. Thus, they meet expectations for "tension zones" that are maintained by recent immigration of the parental lineages and possibly "hybrid unfitness" (Barton and Hewitt, 1985). The two hybrid zones occur in geographical regions of low overall fitness for mussels. Demographic sinks, such as these, can focus and stabilize hybrid zones (Barton, 1980)

Here we report a third example, involving multilocus step-clines and intergradation between metapopulation segments of the Jericho tubeworm, *Tevnia jerichonana* (Annelida: Polychaeta: Siboglinidae, Fig. 1). These small to mid-sized worms are pioneers that rapidly colonize nascent and re-activated, low-temperature vents (2–100° C) along the East Pacific Rise (Jollivet, 1993), where they are quickly replaced by the giant siboglinid, *Riftia pachyptila* (Shank et al., 1998; Mullineaux et al., 2000; 2012). As opposed to *R. pachyptila* (Fig. 1 in Coykendall et al., 2011), the short persistence times of *T. jerichonana* results in a spotty distribution of extant colonies along the EPR (Fig. 2A). Details about its dispersal potential are not known, but we can refer to pioneering study of *R. pachyptila* as a model (Marsh et al., 2001). The giant siboglinid produces trochophore larvae that develop for about 28 days before metamorphosis and settlement. With prevailing currents, *R. pachyptila*'s larval duration is expected to result in mean dispersal distances of about 100 km. However, *T. jerichonana* appears to have greater realized dispersal, because estimates of gene flow tend to be higher ($Nm > 25$) than those of *R. pachyptila* ($5 \leq Nm < 25$) (Vrijenhoek, 2010). A study of mitochondrial (mt) DNA

variation in *T. jerichonana* revealed an isolation-by-distance (IBD) signal — i.e. genetic differentiation (F_{ST}) that increases with geographical distances between vents (Hurtado et al., 2004; Audzijonyte and Vrijenhoek, 2010). Nonetheless, the IBD-like pattern does not necessarily indicate an underlying process of "isolation-by-distance" (sensu Wright, 1943). Other processes including adaptive divergence, range expansions, hierarchical subdivision, introgressive hybridization, and sparse sampling can produce similar patterns (Meirmans, 2012; Orsini et al., 2013). To determine if the IBD-like signal is unique to mtDNA or more pervasive, we compared genetic structures revealed by fragments of two independent nuclear genes, ATP synthetase subunit α (*ATP α*) and heat-shock protein (*HSP*), with those of two mitochondrial genes, cytochrome-*c*-oxidase subunit I (*COI*), and cytochrome B (*Cytb*).

2. Methods

2.1. Sample collection and identification

Samples were collected between 1998 and 2005 during expeditions conducted along NEPR, SEPR, PAR, and GAR segments of the southeastern Pacific ridge systems (Fig. 2A). We explored 20 disjunct hydrothermal vent fields with the human occupied vehicle (HOV) *Alvin* (Woods Hole Oceanographic Institution). The present specimens were sampled from seven locations (Table 1). The worms typically occur in dense clusters that could be grasped with HOV *Alvin*'s manipulator arms and deposited in an insulated box containing ambient seawater. Upon recovery at the surface, whole worms were stored in 2° C filtered seawater prior to tissue sampling. A small sample of vestimentum, a tissue normally devoid of endosymbiotic bacteria, was frozen at -70°C or preserved in 95% EtOH.

Tevnia jerichonana commonly co-occurs with two other siboglinid tubeworms, *Riftia pachyptila* and the *Oasisia* species complex. Adults are easily distinguished macroscopically. *Riftia pachyptila* are large (typically >1 m long) robust worms with a long, brilliant red, anterior plume (obturatorium) and a smooth-walled flexible tube. *Tevnia jerichonana* and *Oasisia* species are smaller, thin-bodied worms with a short obturatorium. *Tevnia* tubes are rigid and relatively straight with closely spaced external flanges. *Oasisia* tubes are thinner and more flexible with widely spaced, large, external flanges. Very young, post-settlement juveniles of *Oasisia*, *R. pachyptila*, and *T. jerichonana* were difficult to distinguish macroscopically. All *T. jerichonana* specimens used in this study were distinguished by their unique mitochondrial *COI* sequences (Black et al., 1997; Hurtado et al., 2004).

2.2. Gene sampling, DNA extraction and sequencing

Genomic DNA was extracted using the microplate extraction method of Elphinstone et al. (2003), excepting that we used 100 µL of lysed tissue and 100 µL of elution buffer. Mitochondrial *COI* and *Cytb* were amplified with DNA primers previously known to work with siboglinids (Table 2). Amplifications were conducted with Amplitaq Gold® Fast PCR Master Mix, UP (Life Technologies Corp., Carlsbad, CA). Initial denaturation occurred at 95° C for 10 min, followed by 35 cycles of 94° C for 1 min, 55° C for 1 min, and 72° C for 2 min, with a final extension at 72° C for 7 min.

To identify nuclear gene markers, we screened DNA primers previously reported to amplify the genes from invertebrate animals (Jarman et al., 2002; Audzijonyte and Vrijenhoek, 2010; Coykendall et al., 2011). Inconsistent amplifications and inclusions of one or more introns that produced unscorable length polymorphisms in the heterozygotes

led to the exclusion of many candidate genes. Fragments of two nuclear genes, *ATP α* and *HSP*, provided consistently scorable polymorphisms (Table 2). Due to weak initial amplifications, we designed nested primers to conduct secondary amplifications for both genes. Primary amplifications of *ATP α* were conducted with published primers (Jarman et al., 2002), and secondary amplifications were conducted with newly designed internal primers specific to *T. jerichonana*. The *HSP* primers were newly designed from GenBank sequences for *HSP-70* for *T. jerichonana* (FN86147-8). The primer HSP242F1 was anchored in *HSP-70* exon-1; sequences spanned intron 1, and the primer HSP922R1 was anchored in *HSP-70* exo-2. Translated protein sequences were compared with GenBank protein sequences to ensure that the correct product was analyzed. Primary and secondary amplification mixtures contained 1–2 μ L of DNA template, 12.5 μ L AmpliTaq Gold® Fast PCR Master Mix (Life Technologies, Grand Island, NY), and 1 μ L of each primer at 10 μ mol/ μ L, for a total of 25 μ L. PCR conditions were 95 °C for 10 min, followed by 35 cycles of 3s denaturing at 96 °C, 3s annealing at 56 °C, and 15s extension at 68°C, with a final extension at 72 °C for 7 min.

PCR products were purified using a Multiscreen HTS PCR 96 vacuum manifold system (Millipore Corp. Billerica, MA), then resuspended in 50 μ L of sterile water. DNA sequencing employed the same PCR primers. Purified PCR products were sequenced bidirectionally on an ABI 3100 capillary sequencer using BigDye terminator chemistry (Applied Biosystems Inc., Foster, CA). All sequences were edited and manually aligned in GENEIOUS 8.0.4 (<http://www.geneious.com>, Kearse et al., 2012").

2.3. Statistical analyses

Haplotypes for the mitochondrial fragments were inferred directly from sequence

traces, whereas those for the nuclear gene fragments were reconstructed using the program PHASE v. 2.1 (Stephens et al., 2001). Haplotype networks were constructed with the median-joining (MJ) method implemented in NETWORK v. 4.612 (Bandelt et al., 1999). GENEPOP v3.4 (Raymond and Rousset, 1995) was used to conduct exact tests of Hardy Weinberg equilibrium (HWE) and linkage disequilibrium (LD) for the nuclear loci. We used CNDW (Asmussen and Basten, 1994; Basten and Asmussen, 1997) to test for cytonuclear disequilibrium between the mtDNA and the nDNA genotypes. The branch and bound method (with the default parameters) as implemented in BEAGLE (Lyngsø et al., 2005) was used to reconstruct a genealogical history of recombination for each locus. We used LOSITAN to test each locus for selection. Simulations included 10,000–40,000 replications to estimate the neutral mean F_{ST} 95% confidence intervals under an infinite allele mutation model. Confidence intervals of simulated values were then plotted with empirical values to determine if they were outside of the expected ranges, indicating selection (Beaumont and Nichols, 1996; Antao et al., 2008). We used ARLEQUIN 3.5 (Excoffier and Lischer, 2009) to estimate H (haplotype), π (nucleotide) diversity indices, and Φ_{ST} values, and to conduct hierarchical AMOVAs. Estimates of allelic richness (a') were obtained by the rarefaction methods implemented in HP-RARE v. 10 (Kalinowsky, 2005).

To infer the patterns of population structure without prior knowledge of geomorphological partitioning, we conducted assignment tests with STRUCTURE v. 2.3 (Pritchard et al., 2000). Haplotypes for each of the four genes were treated distinct alleles ($a^1, a^2, \dots a^n$), and a multilocus genotype (MLG) was constructed for each individual. Analyses were first conducted separately for the mitochondrial and nuclear genotypes.

Then, the two haploid mitochondrial genes were concatenated and combined with the two diploid nuclear genotypes. We reported results of the concatenated data because both analyses yielded identical results. The STRUCTURE analyses were conducted for values of K (number of population clusters) ranging from 1 to 7, using an admixture model and sampling locations as prior conditions (LOCPRIOR=1). Analyses were iterated ten times for each value of K with correlated allele frequencies. The first 10^4 generations (burn-in) of each iteration were discarded, and 10^6 MCMC generations were used to estimate the optimum value for K by the delta K method of Evanno et al. (2005).

We conducted Bayesian assignment methods with BAYESASS v.3 (Wilson and Rannala, 2003) and NEWHYBRIDS (Anderson and Thompson, 2002) to estimate ancestries and immigration rates. The BAYESASS method does not assume Hardy-Weinberg equilibrium, which is appropriate in cases that exhibit heterozygote deficiencies and linkage disequilibrium. Analyses were conducted with two populations (EPR and PAR) for 10^{7-8} iterations and sampled every 2000 generations following a burn-in of 10^6 . We seeded the iterations with random numbers, and used default mixing parameters of 0.3 for allele frequencies and 0.5 for the inbreeding coefficient. The Bayesian deviances for each run were plotted and calculated to determine the best results to analyze (Meirmans, 2014). STRUCTURE and BAYESASS results were visualized with GGPLOT2 in R. (Wickham, 2009).

To test for isolation-by-distance (IBD), we examined correlations (r) between genetic (**G**) and geographical distance (**D**) matrices. The **G**-matrix was based on pairwise Φ_{ST} values, estimated with ARLEQUIN. The program GENODIVE v. 2.0b23 (Meirmans and van Teinereen, 2004) was used to generate pairwise geographical distances from the geographical coordinates of samples (Table 1) and to estimate Mantel's r between the **G**

and **D**-matrices. Because the previous AMOVAs supported hierarchical population structure, we assigned the individuals samples to NEPR, SEPR, and PAR subdivisions and applied stratified Mantel tests. This procedure randomly permutes samples within the strata to test the significance of correlations between the **G**- and **D**-matrices.

3. Results

3.1. Genetic diversity

DNA sequences from four gene fragments were used for the present analyses. Mitochondrial *COI* (775 bp) and *Cytb* (320 bp) fragments both exhibited 12 polymorphic sites that distinguished 15 and 12 haplotypes, respectively. A relatively short fragment (173 bp) of nuclear *HSP* spanned a 98-bp intron and a 75-bp exon region. Six of the ten polymorphic sites were located in the exon region, three of which resulted in amino acid substitutions. The first, involving a 2nd-position change (bp 11: C→T: Proline→Leucine), occurred in three individuals. The second, involving a 1st-position change (bp 58: G→A: Glutamic acid→Lysine), occurred in five individuals. The third, involving a 2nd-position change (bp 62: C→T: Alanine→Valine), occurred in two individuals. Together these substitutions allowed us to distinguish 20 haplotypes. The intron-spanning fragment of nuclear *ATP5a* (281 bp) was the least polymorphic of the four gene fragments, with seven polymorphic sites that distinguished eight haplotypes. None of the loci showed any evidence of selection (data not shown). All DNA sequences and alignments were deposited with GenBank (acc. nos. KM270879-KM271776).

Haplotype networks were generated for each gene fragment (Fig. 3). The *COI*, *Cytb*

and *ATP5a* networks exhibited similar star-like phylogenies dominated by two sequences (allelic haplotypes *1 and *2). In each case, only one (*ATP5a*), two (*COI*), or three (*Cytb*) mutational steps separated the leading haplotypes. *COI* exhibited ten singletons and three rare variants (i.e. $q_i \leq 0.05$). *Cytb* exhibited three singletons, nine rare variants, and one minor variant ($q_i = 0.08$). *ATP5a* exhibited only one singleton and five rare variants. Genealogical tests for recombination with branch-and-bound method detected no evidence for recombination within these genes. We also tested for recombination between *COI* and *Cytb*, and as expected for mitochondrial genes, found no evidence for recombination.

The *HSP* network was more complex (Fig. 3C). The *HSP*1* allele dominated the sample ($q_i = 0.41$), four additional alleles (*HSP*2*, *3, *4 and *5) occurred at moderate frequencies ($q_i > 0.05$), eleven were rare ($q_i \leq 0.05$), and four were singletons. The network exhibited considerable homoplasy (multiple mutation paths connecting the haplotypes). Because *HSP* genes are highly duplicated in some marine invertebrates (Zhang et al. 2012), we cannot discount that the homoplasy resulted from paralogous genes. Alternatively, if the observed *HSP* variation resulted from homologous alleles, homoplasy might result from intra-genic recombination. A test using the branch-and-bound method detected possible recombination events at five nucleotide sites, four of which were located in the intron region of the amplicon. The putative recombinants are distributed throughout the depth of the genealogy (Supplemental Fig. 1), and thus do not appear to be products of recent secondary contact and hybridization.

Significant heterozygote deficiencies existed for the nuclear genes (F_{IS} in Table 3), but the deficiencies do not necessarily signify deviations from random mating. True

heterozygotes commonly exhibit allelic dropouts due to mutations in the primer sites, low copy number DNA targets, differential methylation of alleles, and consequences of long-term tissue storage (e.g., Piyamongkol et al., 2003). Though we cannot exclude other technical problems, we found that the random mating hypothesis was supported by tests for linkage disequilibrium (LD) between *HSP* and *ATPsa*. Only one of the seven samples (N9) exhibited a significant LD-test ($P = 0.029$). Tests for cytonuclear disequilibrium (CND) between *HSP* and mitochondrial *COI* or *Cytb* were not significant in any of the population samples. However, three CND-tests for genotypic disequilibrium involving *ATPsa* were marginally significant in SEPR samples: with *COI* in S7 ($P = 0.041$), and with *Cytb* in S7 ($P = 0.045$) and in S17 ($P = 0.042$) samples. As subsequently discussed, cytonuclear disequilibrium is commonly observed in admixed populations.

Allelic frequencies exhibited latitudinal clines. For each locus, frequencies of the leading 'northern' alleles (colored blue in Fig. 2B) decreased in a southward progression, whereas the corresponding 'southern' alleles (white) increased. Slopes for the latitudinal clines were statistically significant and broadly congruent (Fig. 4A). Regionally limited alleles also complemented the clines. For example, some alleles (light blue and green, Fig. 2B) were mostly limited to the NEPR region, whereas other alleles (magenta, pink and orange, Fig. 2B) were most frequent in the PAR region. Private alleles (black) occurred in all samples except the northern most (N9 and N13), and singletons (yellow) occurred throughout the sampled range.

Diversity indices tended to be lowest at the northern and southern margins of the species' range (Table 3). When summed across loci, the S7, S17 and S31 samples exhibited the most haplotypes. The S7 and S17 samples exhibited relatively high

haplotypic (h) and nucleotide (π) diversities for *COI* and *Cytb*, whereas N11 had the highest h and π indices for *HSP*. Diversity indices for *ATPsa* were greatest in the southernmost samples: h was greatest in S31, and π was greatest in S32. Rarefaction estimates, adjusted to the smallest sample size ($N = 8$), identified the greatest allelic richness (a') and private allelic richness (P_a) in the S7 sample.

3.2. Population structure

The STRUCTURE analysis (Fig. 5A) identified two clusters ($K = 2$) that distinguished the NEPR (N13, N11 and N9) and PAR (S31 and S32) populations. Individuals from the SEPR region (S7-S17) were assigned NEPR, PAR, or as mixed genotypes. Because we sampled only two nuclear genes, and experienced missing data due presumably to allelic dropouts, the NEWHYBRIDS analyses were inconclusive in assigning genotypes to F1, F2, Bx, etc. generations (data not reported). Instead, we conducted a BAYESASS analyses with three genes, *COI*, *HSP*, and *ATPsa* (Fig. 5B). Mitochondrial *Cytb* was completely correlated with *COI* and not included. Bayesian deviances calculated with trace files of iterative runs differed less than 0.04% despite differences in the starting points (random seeds) or number of generations; the analysis with the lowest deviance was portrayed. Although we had limited statistical power to discriminate among genotypic assignments, the vast majority of NEPR and PAR individuals appeared to be "pure" (non-migrants) genotypes. In contrast, the SEPR populations were a combination of EPR non-migrants and PAR 1st- and 2nd-generation immigrants. These results clearly illustrated the SEPR as a region of intergradation between NEPR and PAR populations.

The BAYESASS analysis also clarified a pattern in the distribution of heterozygote deficiencies for the nuclear loci. Fixation indices ($F_{IS} \pm \text{s.e.}$) were greatest in the EPR

(0.2013 ± 0.0329) samples and less in the PAR (0.1558 ± 0.0365) samples. As previously noted, allelic dropouts might be responsible for part of these deficiencies, but they are not likely to explain the differences between the NEPR, SEPR and PAR clusters. Allelic dropouts should be relatively homogeneous, unless the samples were affected by differential storage. All the samples were frozen at -80°C for different time-periods, yet the newest (PAR) and the oldest (EPR) samples had the lowest fixation indices. Though we cannot exclude the possibility that some unknown technical problems caused these deficiencies, the elevated F_{IS} indices might have resulted from Wahlund effects in the SEPR samples. More independent nuclear loci and fresh samples would help to clarify some of these questions.

3.3. Test for Isolation-by-Distance

Highly significant correlations (Fig. 4B) existed between pairwise Φ_{ST} -values and geographical distances for *COI* (Mantel's $r = 0.981$, $P = 0.001$), *Cytb* ($r = 0.928$, $P = 0.002$), and *HSP* ($r = 0.881$, $P = 0.003$), but not *ATP5a* ($r = 0.298$, $P = 0.0971$).

However, when samples were assigned to their respective ridge axes (NEPR, SEPR, and PAR), stratified Mantel test revealed no significant correlations with geographical distances. These apparent IBD patterns were attributable to hierarchical population structure.

4. Discussion

Multilocus analyses revealed that *T. jerichonana* genotypes could be assigned to northern and southern lineages that occupied the NEPR and PAR regions of the southeastern Pacific ridge system. Connecting segments of the SEPR axis represented a

region of step-clines and intergradation between the two lineages (Fig. 5). The significant cytonuclear disequilibrium (CND) observed between *ATP5a* and mtDNA genes at SEPR also indicated admixture in this region, although no significance was found at *HSP*.

Latitudinal clines that produced an IBD-like signal were observed, as previously reported for *COI* (Audzijonyte and Vrijenhoek, 2010). *Cytb* exhibited an identical signal because the mitochondrial genes are coupled in the non-recombinant mitochondrial genome. In addition, two independent nuclear genes, *HSP* and *ATP5a*, also exhibited IBD-like signals, though the slopes of Φ_{ST} on latitude were shallower (Fig. 4). Stepping-stone dispersal (SSD) among discrete vent fields could create the IBD-like signals (Slatkin, 1993). Strong selection gradients also could result in a convergence of clines among independent loci (Barton, 1983), although there was no evidence of selection on any locus examined. The geochemical composition of vent effluents can vary greatly on very small spatial scales (Luther et al., 2001), but the extremes of these local conditions are very broad. In contrast, the temperatures and salinities of ambient (non-vent) seawater are relatively homogeneous at depths occupied by *T. jerichonana* (2235–2747 m).

4.1. The Refuge/re-invasion hypothesis

A regional extinction event (or events) followed by secondary invasion of the SEPR axis with *T. jerichonana* colonists from NEPR and PAR refugia could have generated the observed patterns. According to the refuge/re-invasion hypothesis, the NEPR and PAR lineages were temporarily split when the conjoining SEPR populations were extinguished. We can only guess about the geotectonic events that might have led to extinction along this superfast-spreading axis. Evidence for regional instability derives

from population genetic studies of other vent-restricted animals. For example, SEPR populations of *Bathymodiolus thermophilus* mussels exhibit similar evidence for re-invasion from NEPR (or GAR) sources, with limited introgression of *B. antarcticus* genes from the PAR region (Johnson et al., 2013; Plouviez et al., 2013). Re-invasion by other annelids appears to be unidirectional, however. For example, very low levels of genetic diversity in SEPR populations of *Riftia pachyptila* might have resulted from unidirectional invasion and founder events involving NEPR or GAR immigrants (Coykendall et al., 2011). A PAR refugium may not exist for *R. pachyptila*. In contrast, SEPR populations of *Alvinella pompejana* are distinct from the NEPR and GAR populations (Hurtado et al., 2004), leading Plouviez et al. (2010) to hypothesize that the lineages split about 1–2 million years ago. SEPR populations of several gastropod limpets also exhibit varying degrees of differentiation that are consistent with secondary invasion and recent range expansions. Together, patterns of genetic diversity in SEPR populations of vent-restricted species support a hypothesis of secondary invasions by NEPR/GAR immigrants and recent range expansions (Plouviez et al., 2009, 2010).

The two cases that differ from this pattern involve admixture between NEPR and PAR sources. Higher genic diversity in SEPR populations of *T. jerichonana* resulted from re-invasion and admixture between NEPR and PAR lineages. Higher genic diversity in the southern SEPR populations of *B. thermophilus* also appears to be a consequence of hybridization and limited introgression of *B. antarcticus* alleles from the PAR region (Johnson et al., 2013; Plouviez et al., 2013). The time-scales for differentiation between the parental lineages differ greatly, however. Molecular evidence suggests that *B. thermophilus* and *B. antarcticus* probably split about 4–5 million years (Ma) ago roughly

coincident with orogeny of the Easter Microplate (Won et al., 2003a); whereas the *T. jerichonana* lineages appear to have split more recently. Divergence between the NEPR and PAR lineages was small and not indicative of species-level differentiation. Only 1–3 mutational steps separated the leading NEPR and PAR allelic haplotypes for each gene. Due to statistical uncertainties, we did not attempt to estimate a divergence time between the two *T. jerichonana* lineages. However, Plouviez et al. (2013; 2009) hypothesized that geotectonic activities in equatorial region during the last 0.5 Ma severed the connections between NEPR and SEPR populations of *A. pompejana*, *B. thermophilus*, and possibly other vent species. The confidence intervals for such estimates are large, however, ranging from 0 to 2 Ma. Thus, for now it is only safe to conclude that these events were more recent than the orogeny of the Easter Microplate.

5. Conclusions

A broad zone of intergradation was identified between lineages of hydrothermal vent tubeworm *Tevnia jerichonana*. The marginal signal for cytonuclear disequilibrium and the slight divergence between lineages indicated the separation might have happened more recently. We hypothesize that the SEPR region is a demographic sink for *T. jerichonana*. Like other weedy species, *T. jerichonana* are continuously subjected to local extinction and recolonization events. If local extinction rates exceed recolonization rates, a regional metapopulation will crash. Extinction and recolonization rates will differ among vent species due to their unique life histories, dispersal rates, and susceptibilities to local extinction. Thus, attempts to infer a 'shared' vicariance event (or events) from genetic data might be extremely difficult. Efforts to reconstruct the tectonic history of the SEPR axis and the history of ocean circulation in this region might provide better insights

into the factors that shaped the diverse population structures of these hydrothermal vent species.

Author's contributions

HZ, SBJ and VRF conducted DNA sequencing. HZ, RCV, and SBJ performed statistical analyses. RCV conceived of the project and led oceanographic expeditions that collected the samples. HZ and RCV prepared the manuscript.

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Figure Legends

Figure 1. *Tevnia jerichonana* collected from Alvin dive A4093. Photo by G.W. Rouse.

Figure 2. Allelic frequencies in *Tevnia jerichonana* samples. (A) Southeastern Pacific vent localities: present samples [blue, yellow and red dots]; records for *T. jerichonana* but no samples [gray triangles]; no records of *T. jerichonana* [white dots]. (B) Allelic frequencies at four loci. Black wedges represent frequencies of private alleles, and yellow wedges represent singletons.

Figure 3. Haplotype networks: (A) Cytochrome-*c*-oxidase subunit-I; (B) Cytochrome *b*; (C) Heat-shock protein; (D) ATP synthetase subunit α . Sizes of circles correspond to abundance of each sequence. Numbers in or adjacent to each circle denote the haplotypic allele. Each link indicates a single mutation, and the slash in panel B indicates a mutation that was not observed.

Figure 4. Clines and IBD analysis. (A) Frequencies of leading 'northern' alleles versus degrees N latitude. (B) Correlation of pairwise Φ_{ST} 's with geographic distances among the sample localities. Negative Φ_{ST} values (Supplementary Table 1) were set to zero. Coefficients of determination (R^2) and significance of correlations (P -values) are listed in boxes.

Figure 5A. STRUCTURE analysis of multilocus genotypes for the seven sample localities. Blue bars denote probability that an individual has an EPR genotype and red bars denote

probability that an individual has a PAR genotype. 5B. Posterior probabilities of BAYESASS assignments of migrant ancestries. Dark blue bars indicate non-migrant, medium blue indicate 1st generation, and light blue indicates 2nd generation EPR immigrants. Red bars indicate non-migrant, dark pink bars indicate 1st generation, and light pink bars indicate 2nd generation PAR genotypes.

Tables

Table 1. Coordinates and depths for *Tevnia jerichonana* samples.

Locality	Latitude ¹	Longitude ^a	Depths (m)	Alvin dive numbers ^b	Sampling dates
N13	12.82	-103.95	2630– 2636	A2227–A2229	Jun-90
N11	11.42	-103.78	2516– 2522	A2225–A2226	Jun-90
N9	9.28	-104.22	2505– 2583	A2351, A2365, A2498 A2499, A2502, A2852	Apr-91, Oct-94
S7	-7.43	-104.78	2737– 2747	A3320–A3322	Dec-98
S17	-17.43	-113.20	2582	A3328	Jan-99
S31	-31.15	-111.93	2334– 2338	A3337–A3339, A4094	Jan-99, Mar-05
S32	-31.87	-112.05	2235– 2336	A3341, A4092–A4093	Mar-05

^a Coordinates: negative numbers indicate south latitudes and west longitudes.

^b Dive logs: [http://www.marine.who.edu/divelog.nsf/By Dive Number/Date?OpenView](http://www.marine.who.edu/divelog.nsf/By%20Dive%20Number/Date?OpenView)

Table 2. DNA primers, coding information, and fragment lengths included in analyses.

Locus	Product	Length	Primer	Primer Sequence (5'–3')	Ref. ^a
<i>COI</i>	Cytochrome-c-oxidase subunit I	775 bp	COIF	TCMACTAATCAYAARGAYATTGGNAC	1
			COIR	CCDCTTAGWCCTARRAARTGTTGNGG	1
<i>Cytb</i>	Cytochrome B	320 bp	424F	GGWTAYGTWYTWCCWTGRGGWCARAT	2
			876R	GCRTAWGCRAAWARRAARTAYCAYTCWG G	2
<i>HSP</i>	Heat shock protein-70	173 bp	HSP242F1	GGGCGTCGCTTCGACGAACA	4
		75 exon-1	HSP922R1	CCTGGCGCTGGGAGTCGTTG	4
		98 intron	HSP_R2_tv	AAGTAGGCGGGCACGGTGAC	4
<i>ATPsa</i>	ATP synthetase subunit α	281 bp all intron	ATPsa_F1	GAGCCMATGCAGACTGGTATTAAGGCYGT	3
			ATPsa_R1	AACTTNGMGAAGACCACTACTACCACAG	3
			ATPsa_F2_tv	CGACAGTCTCGTGCCAATTG	4
			ATPsa_R2_tv	AATAGCAATGGCAGTCTTGC	4

^a References: 1. (Nelson and Fisher, 2000); 2. (Boore and Brown, 2000); 3. (Jarman et al., 2002); 4. present study.

Table 3. Genic diversity indices for *Tevnia jerichonana* samples from seven East Pacific Rise vent localities.

Indices ^a	Localities						
	N13	N11	N9	S7	S17	S31	S32
<i>COI</i> (<i>N</i>)	(28)	(14)	(24)	(31)	(24)	(40)	(20)
<i>S</i>	0	2	5	7	4	3	3
<i>H</i>	1	3	5	8	5	3	3
<i>h</i>	0	0.275	0.377	0.609	0.630	0.273	0.353
π	0	0.286	0.493	1.346	1.163	0.427	0.468
<i>a'</i>	1	2.143	2.565	3.548	2.991	1.996	2.207
<i>Pa</i>	0.000	1.143	1.232	1.361	0.848	0.071	0.301
<i>Cytb</i> (<i>N</i>)	(28)	(19)	(25)	(26)	(31)	(41)	(21)
<i>S</i>	3	1	6	5	4	4	4
<i>H</i>	4	2	5	4	3	3	3
<i>h</i>	0.458	0.351	0.637	0.588	0.551	0.342	0.267
π	0.492	0.351	0.920	1.702	1.634	0.722	0.467
<i>a'</i>	2.501	1.915	3.274	2.809	2.440	2.192	2.010
<i>Pa</i>	0.598	0.006	1.032	0.837	0.456	0.596	0.629
<i>HSP</i> (<i>N</i>)	(34)	(8)	(34)	(36)	(54)	(58)	(36)
<i>S</i>	2	3	3	8	7	9	6
<i>H</i>	3	4	4	10	10	14	7
<i>h</i>	0.398	0.750	0.620	0.710	0.670	0.839	0.786
π	0.424	1.036	0.733	1.411	1.152	1.466	1.316
<i>F_{IS}</i> ^b	0.416	0.700	0.150	0.459	0.173	0.139	0.010
<i>a'</i>	2.329	4.000	2.990	4.282	3.904	5.047	4.367
<i>Pa</i>	0.000	0.804	0.235	0.799	0.781	1.943	0.743
<i>ATPsa</i> (<i>N</i>)	(30)	(16)	(30)	(46)	(40)	(68)	(36)
<i>S</i>	1	1	2	1	1	5	4
<i>H</i>	2	2	3	2	2	5	4
<i>h</i>	0.370	0.500	0.570	0.348	0.409	0.403	0.211
π	0.370	0.500	0.644	0.348	0.409	0.737	0.376
<i>F_{IS}</i>	0.468	1.000	0.306	0.506	0.395	0.347	0.481
<i>a'</i>	1.916	1.997	2.461	1.884	1.944	2.524	1.844
<i>Pa</i>	0.000	0.000	0.469	0.000	0.000	1.177	0.498
Totals							
Haplotypes	10	11	17	24	20	25	17
Singletons ^c	2	2	4	6	3	1	1
Richness (<i>a'</i>) ^d	1.940	2.510	2.820	3.130	2.820	2.940	2.610
Privates(<i>Pa</i>) ^e	0.150	0.490	0.740	0.750	0.520	0.950	0.540

^a Diversity indices: *N*, number of sequences; *S*, number of polymorphic sites; *H*, number of haplotypes (i.e. alleles); *h*, haplotype diversity; π , mean number of differences between pairs of sequences;

^b Significant values indicated in bold.

^c Singletons (alleles observed only once).

^d Rarefaction estimate of allelic richness for the four genes, adjusted to minimum sample size of 8.

^e Privates allelic richness for the four genes, adjusted to minimum sample size of 8.

Fig. 1

Fig. 2

Fig. 3

FIGURE 4

Fig. 5