Isolation-by-distance in the eastern Pacific hydrothermal vent tubeworm *Tevnia jerichonana* (Jones, 1985)

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Abstract
Tevnia jerichonana is a deep-sea hydrothermal vent vestimentiferan tubeworm that is sessile as an adult, but with mobile larvae that are important in dispersal. Its geographic range is from 13°N to 32°S along the east Pacific rise (EPR). A major question is how *Tevnia* larvae disperse among these island-like, ephemeral habitats. To answer this, we carried out a population genetics study using two mitochondrial genes (COI and CytB) for seven populations along the EPR. Populations fall into three groups: Northern (13°N, 11°N, and 9°N), middle (7°S and 17°S), and southern (31°S and 32°S). DNA sequence variation among these three groups was significant, and a cline of haplotype frequency exists, with a northern-dominant haplotype and southern-dominant haplotype. Results suggest that *Tevnia* follows a stepping-stone dispersal model, which has led to isolation-by-distance for these populations.

Introduction
Tevnia jerichonana is a deep-sea hydrothermal vent vestimentiferan tubeworm in the phylum Annelida and family Siboglinidae (Black et al. 1997). As sessile adults, individuals build tubes that attach to rock or hard substrates. The juvenile larvae are mobile, and can potentially disperse between vent sites. Hydrothermal vents exist at plate divergent boundaries, where two of the Earth's tectonic plates spread apart from each other, along the global mid-ocean ridge (MOR) system (Vrijenhoek 2009b, Fig. 1). They are oases within the vast abyssal plain that support numerous microbes and invertebrate fauna.

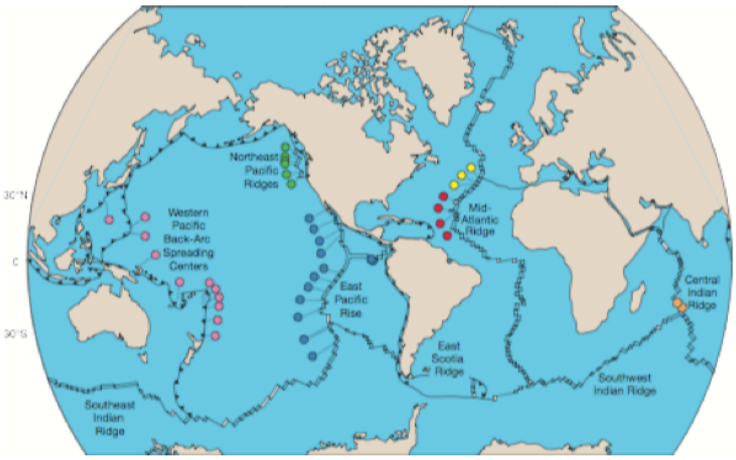

Figure 1. Global distribution of hydrothermal vents along the mid-ocean ridge system.

Hydrothermal vents arise due to the tectonic activity: Fissures will occur at various points along a spreading center, allowing seawater to interact with magma under the crust. The water is geothermally heated and when it resurfaces it can be as hot as 400°C, and contains chemicals such as reduced sulfides and methane (Vrijenhoek 2009b, Fig. 2). Since this only occurs at certain points along a MOR, an island-like system of discrete habitats results (Fig. 1).

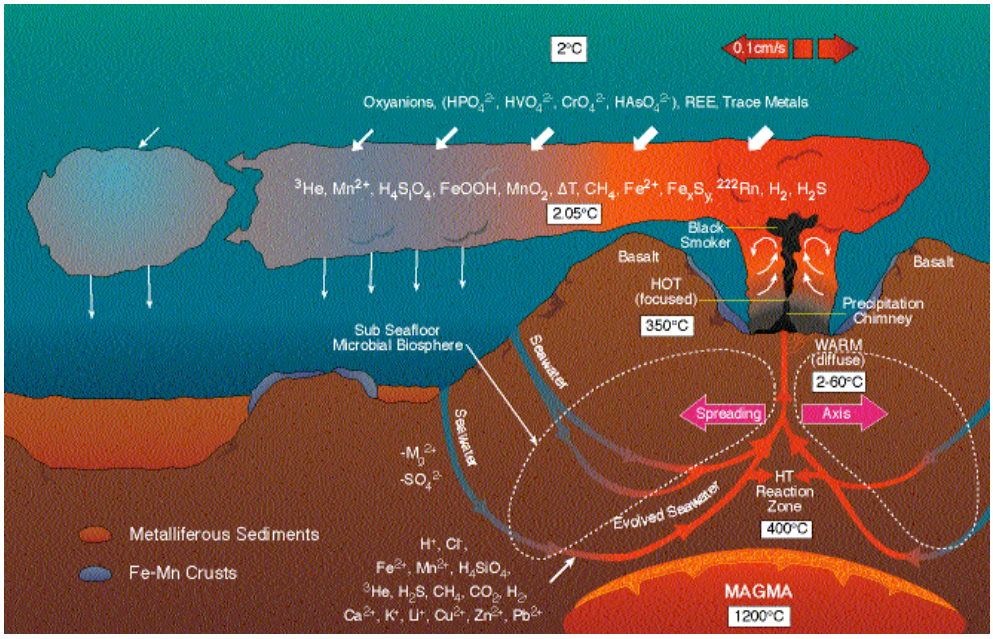

Figure 2. Model showing hydrothermal activity at a vent occurring along a spreading center. Chemicals within the magma become incorporated with interacting seawater, which returns to the surface and provides inorganic nutrients, supporting a whole ecosystem.

Tevnia and other vent invertebrates survive in this extreme environment with endosymbiotic chemotrophic bacteria that act as the primary producers in vent ecosystems (Van Dover et al. 2002). In vestimentiferans, the endosymbionts are housed in a specialized organ called the trophosome (Fig. 3). *Tevnia* can not survive without these microbes, which are in turn reliant on the inorganic nutrients available at vent sites (Nussbaumer et al. 2006). Consequently, when a hydrothermal vent declines, the fauna that lived there also perish.

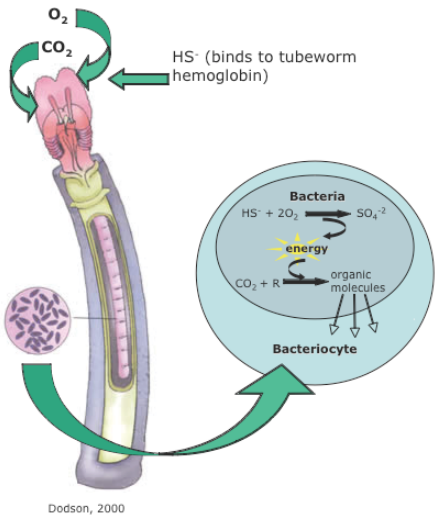

Figure 3. Model of nutrient uptake through endosymbiotic chemolithotrophic bacteria in vestimentiferan tubeworms, which are housed in the tubeworms trophosome.

Although we understand how these tubeworms survive as established adults, another major question is how populations persist and how larval dispersal occurs among these island-like habitats. This is especially intriguing due to the ephemeral nature of these habitats, which last only a few years to decades. Volcanic and tectonic activity frequently creates or destroys vent habitats (Vrijenhoek 2009b). The rate of habitat turnover is correlated with the spreading rate, which in turn affects the genetic diversity of a population. For example, it has been found that spreading rates in the southern eastern Pacific rise (SEPR) are significantly faster than in the northern EPR (NEPR), and consequently many organisms exhibit less genetic diversity in the SEPR, due to the frequent bottleneck events (Vrijenhoek 2009b). *Tevnia* has been observed to be one of the primary colonizers in newly formed habitats, but it is still not completely understood how larvae disperse among habitats. There are numerous factors involved, including hydrodynamics, organism life history, and geological structures such as transform faults.

The dispersal model that it is thought most sessile hydrothermal vent invertebrates follow is the stepping-stone dispersal model. This model applies to linear discrete metapopulations, such as in a hydrothermal vent system. It states that migrants are much more likely to only move as far as the next metapopulation in line, rather than making a large jump (Kimura and Weiss 1964). This makes sense for *Tevnia*, given what is known about larval dispersal in the most closely related tubeworm, *Riftia pachyptila* (Coykendall et al. 2011). Marsh et al. (2001) found that larvae of this tubeworm have a maximum survival time of about 38 days, and in that time can travel about 100 km. This would certainly restrict dispersal in these discrete populations. Depending on other factors, such as generation time and mutation rate, this model can lead to a pattern of isolation-by-distance, when genetic differentiation increases with geographic distance.

The only MOR where *Tevnia* has been found is the EPR, which is west of Central and South America (Hurtado et al. 2004, Fig. 4). Along the SEPR is the Easter Microplate. Studies have found limited gene flow across this feature, as well as across the equator (Hurtado et al. 2004). We can learn more about these things by studying the population genetics of an organism. Population genetic studies help us understand how individuals move around their environment by tracking gene flow. The questions we addressed in this study were: 1) How much variation in populations of *Tevnia*

jerichonana exists along the EPR from 13°N-32°S? 2) Are there points of restricted gene flow? And 3) What dispersal model does *Tevnia* follow?

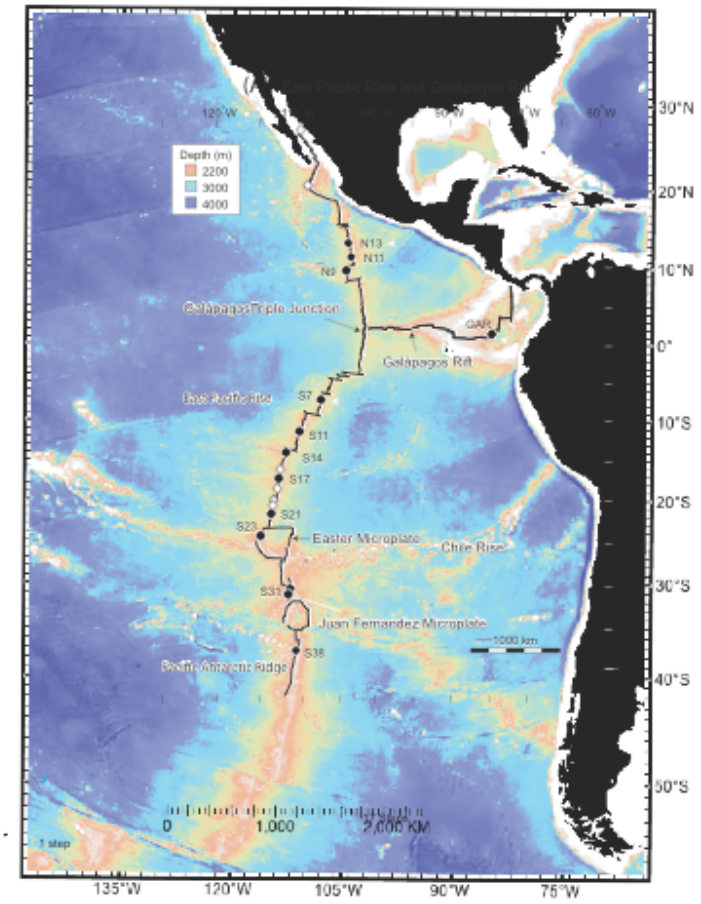

Figure 4. Map of the east Pacific rise between 30°N and 50°S, horizontal lines represent transform faults. The EPR intersects with the Galapogos Rift, and is interrupted by the Easter Microplate.

Methods

Sample collection

Expeditions were conducted between 1998-2005 as outlined in table 1, and specimens collected from hydrothermal vents along the NEPR and SEPR. Specimens were collected using the human occupied vehicle (HOV) *Alvin* (Woods Hole Oceanographic Institution, Massachusetts), and placed in insulated, ambient seawater until brought to the surface, where they were stored in 2˚C filtered seawater, and later frozen or preserved.

Table 1. Sampling sites and number of samples from each locality.

						Gene loci	
Region	Dive #	Latitude	Longitude	Depth(m)	Sample Date	COI	cytB
13N	A2227-A2229	12°49'N	103°57'W	2630-2636	06/1990	28	28
11N	A2225-A2226	11°25'N	103°47'W	2516-2522	06/1990	14	19
9N	A2365 A2498 A2499 A2502	09°17'-09°51N	104°13'-104°18'W	2516-2583	04/1991 - 03/1992	24	25
7S	A3320-A3322	07°26'-07°22' S	104°47'W	2737-2747	12/1998	31	26
17S	A3328	17°26' S	113°12'W	2582	01/1999	24	31
31S	A3337-A3341 A4094	31°09'-31°10'S	111°56'W	2334-2338	01/1999	49	54
32S	A4092-A4093	31°52'S	112°03'W	2235-2336	03/2005	11	8

DNA methods

Total genomic DNA was extracted using a microplate extraction method as described in Elphinstone et al. 2003, except that we used 100μL of lysed tissue, and 100μL of elution buffer. Two mitochondrial loci were used in this study: cytochrome oxidase subunit I (COI) and cytochrome B (CytB). Each region was polymorphic and suitable for population studies (Vrijenhoek 2009a). Previously published primers were used for both COI (Nelson and Fisher, 2000) and CytB (Boore and Brown, 2000). Polymerase chain reactions (PCR) were run using Amplitaq Gold® Fast PCR Master Mix, UP (Life Technologies Corp., Carlsbad, CA) in a Veriti® thermal cycler (Life Technologies Corp., Carlsbad, CA). Amplification for both genes used an initial denaturation of 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. PCR products were diluted in 40 μl sterile water and purified using Multiscreen HTS PCR 96 vacuum manifold system (Millipore Corp. Billerica, MA), then resuspended in 50 μl sterile water. Purified products were sequenced using the same primers used for the thermal cycling reactions in an ABI 3100 capillary sequencer (Life Technologies) using BigDye v. 3.1 termination chemistry. Sequences were proofread using CodonCode Aligner (v3.7.1.1, CodonCode Corp. Dedham, MA), aligned using MUSCLE (www.ebi.ac.uk/Tools/msa/muscle/) and edited by eye using MacClade v4.08 {Maddison, 2005 #40}.

Statistical analyses

We used Tcs v. 1.21 to construct statistical parsimony networks for each locus, which were edited using Adobe Illustrator. We used ARLEQUIN v. 3.5.1.2 {Excoffier, 2010 #44} to estimate *H*, the number of haplotypes; *k*, the number of segregating (polymorphic) sites; *h*, haplotype diversity {equation 8.4`, \Nei, 1987 #45}; and Fu's *Fs*. This program was also used to run Analysis of Molecular Variance (AMOVA) tests, and to obtain pairwise estimates of F_{ST}, which were used in a Mantel test of the isolation-by-distance model. The 'Isolation-with-Migration' program IMA2 {Hey, 2004 #57;Hey, 2007 #58;Hey, 2010 #67;Hey, 2010 #68} was used to determine directionality of migration.

Results

For COI, 15 haplotypes were found across 12 polymorphic sites, and for CytB 12 haplotypes were found across 13 polymorphic sites. Table 2 summarizes sample sizes and parameters of genetic diversity found across the known range of *Tevnia* for both loci. The greatest diversity was found in the mid-latitudes (7S and 17S) for both loci, as well as for 9N in CytB.

Table 2. Summary of genetic diversity found in populations of *Tevnia jerichonana* along the eastern Pacific rise.

Parameter*	13N	11N	9N	7S	17S	31S	32S
COI (775bp)							
N	28	14	24	31	24	49	11
K	0	2	5	7	4	3	0
H	1	3	5	8	5	3	1
h	0	0.2747	0.3768	0.6086	0.6304	0.3520	0
SD	0	0.1484	0.1224	0.0935	0.0652	0.0799	0
Fu's *Fs*	0	-1.475	-2.799	-2.667	-0.495	0.470	0
Fs P-value	N.A.	0.003*	0.003*	0.046*	0.328	0.540	N.A.
CytB (320bp)							
N	28	19	25	26	31	54	8
k	4	2	7	6	4	5	2
H	4	2	5	4	3	3	2
h	0.4577	0.3509	0.6367	0.5877	0.5505	0.2970	0.4286
SD	0.0960	0.1112	0.0833	0.0746	0.0577	0.0762	0.1687
Fu's *Fs*	-1.303	0.758	-1.054	1.694	3.186	1.020	0.536
Fs P-value	0.087	0.480	0.210	0.837	0.931	0.691	0.407

* *N* = sample size per locus; *H* = number of haplotypes; *k* = number of polymorphic sites; *h* = haplotype diversity; SD = one standard deviation.

Pairwise F_{ST} values, which measure genetic distance, were obtained for all possible site comparisons for both loci (Table 3). These values were used to determine population groupings. Sites that were not significantly different from each other were determined to be in the same group for AMOVA analyses. Three groups were determined: a northern group (13N, 11N, and 9N), a mid group (7S and 17S), and a southern group (31S and 32S).

Table 3. Mean percentage of sequence divergence (F_{ST} values) among populations of *Tevnia jerichonana*. Values with asterisks were found to be significantly different ($P<0.05$).

Locus	13N	11N	9N	7S	17S	31S	32S
COI							
13N	0						
11N	0.05332	0					
9N	0.02209*	0.00109	0				
7S	0.26844*	0.19034*	0.16248*	0			
17S	0.57155*	0.45155*	0.42268*	0.05314	0		
31S	0.83421*	0.78084*	0.75024*	0.41351*	0.20954*	0	
32S	1.00000*	0.92519*	0.84158*	0.42228*	0.24565*	0.00937	0
CytB							
13N	0						
11N	-0.03981	0					
9N	-0.00740	-0.01447	0				
7S	0.28711*	0.28088*	0.22622*	0			
17S	0.31941*	0.31372*	0.25968*	-0.02695	0		
31S	0.80311*	0.81253*	0.75862*	0.44852*	0.40937*	0	
32S	0.86236*	0.89188*	0.77793*	0.43366*	0.40759*	0.07206	0

For both genes, AMOVA tests determined approximately 60% of the sequence variation to be among groups, and approximately 40% within populations (Tables 4 and 5).

Table 4. AMOVA design and results for CytB (320 bp, 191 samples)

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P value
Among groups	2	83.868	0.65822 Va	59.00	0.0088+-0.0025
Among populations within groups	4	1.235	-0.00694 Vb	-0.62	0.5787+-0.0131
Within populations	184	85.441	0.46435 Vc	41.62	0.0000+-0.0000
Total	190	170.545	1.11563		

Table 5: AMOVA design and results for COI (775 bp, 181 samples).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P values
Among groups	2	53.547	0.43428 Va	57.62	0.0059+-0.0026
Among populations within groups	4	2.174	0.01074 Vb	1.43	0.0811+-0.0079
Within populations	174	53.705	0.30865 Vc	40.95	0.0000+-0.0000
Total	180	109.425	0.75367		

For both genes there were two dominant haplotypes found: one that was much more abundant in northern latitudes, and one more abundant in southern latitudes (Figures 5-6). More haplotypes in general, and more singletons (individuals with a haplotype not shared with anyone else) were found in the mid-latitudes (Figures 5-6).

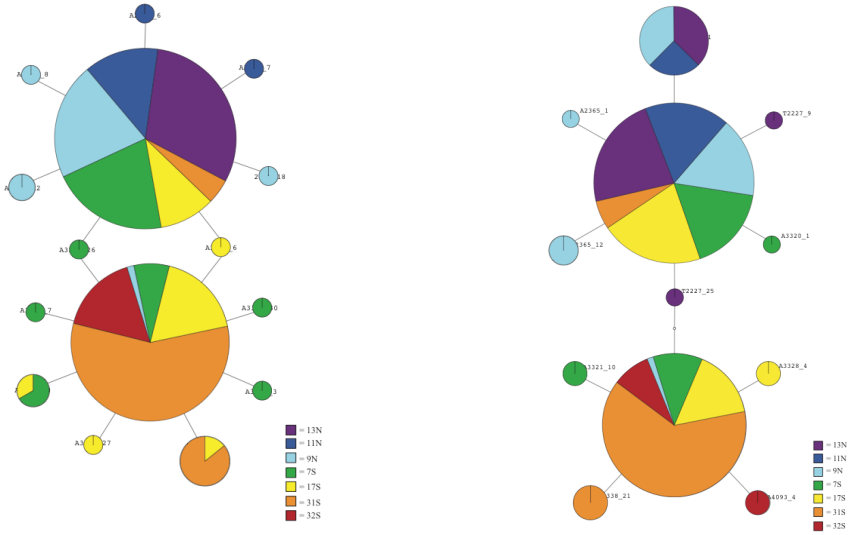

Figure 5. Parsimony networks for a) COI and b) CytB. Each pie represents a different haplotype, and one line is equivalent to one nucleotide change in the sequence. The slices within each pie represent localities, with cooler colors representing northern sites and warmer colors representing southern sites. Pie size corresponds to the number of individuals found with that haplotype.

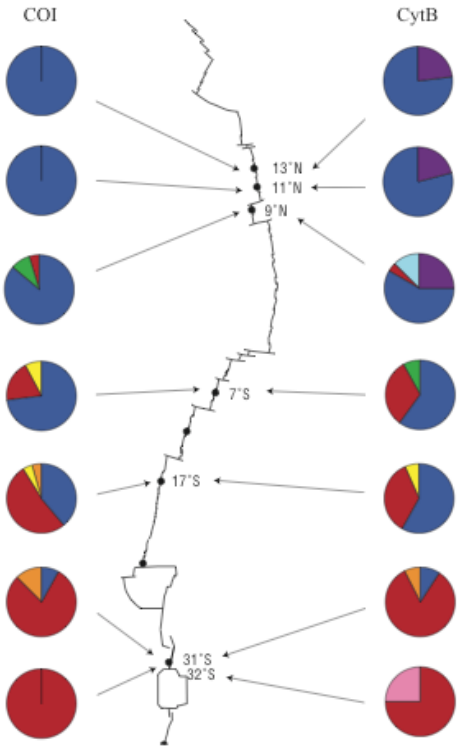

Figure 6. Map showing distribution of more common (two or more individuals) alleles found at each sampling site. Pies represent an individual sampling locale, and slices represent the different haplotypes found at that site.

The Mantel test of correlation between F_{ST} values and geographic distance resulted in a correlation coefficient of 0.980 and an R^2 value of 0.961, with a P-value <0.0001 (Figure 7). The R^2 value indicates that 96% of sequence variation found among these populations can be explained by the geographic distance between individuals.

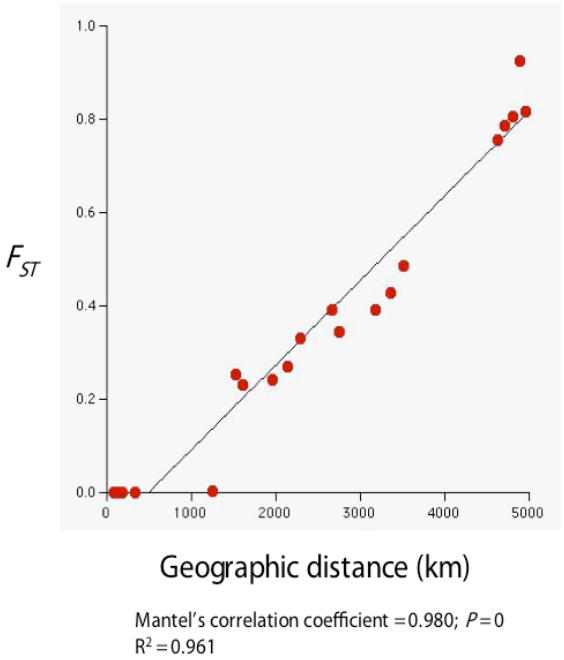

Figure 7. Results of a Mantel test showing linear correlation between geographic distance and genetic distance (as measured by F_{ST}) for populations of *Tevnia jerichonana* tubeworms from different hydrothermal vents between 13°N and 32°S on the east Pacific rise.

The preliminary IM tests gave a significant value for individuals migrating from southern populations into middle populations, and a nearly significant value for individuals migrating form northern populations into middle populations. No other directions of migrations were found to be significant (Figure 8).

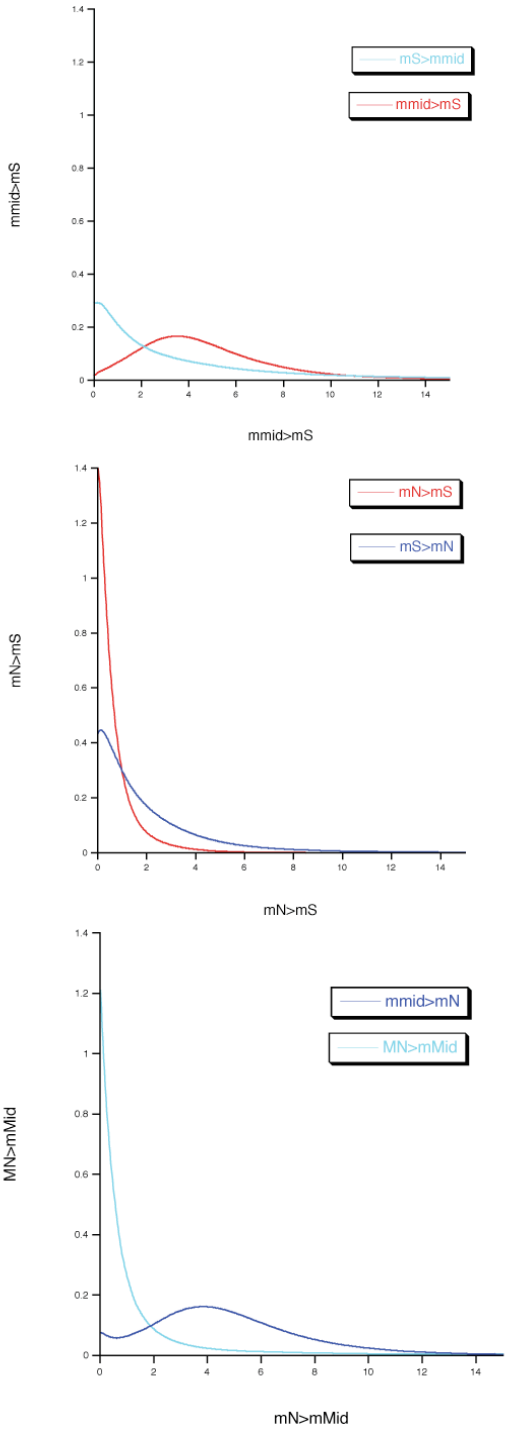

Figure 8. Results for IM analysis, testing migration patterns in *Tevnia jerichonana*.

Discussion and Conclusion

Results indicate that populations of *Tevnia jerichonana* follow the stepping-stone dispersal model. The large amount of variation among groups indicates limited dispersal capabilities for larvae of *Tevnia jerichonana*. This can be explained by different factors, including habitat, life history, and geology. Hydrothermal vent systems form linear, discrete habitats, so it makes sense that the most likely place an individual would move to is the next population over. A large jump is also unlikely because when a larva moves laterally away from a ridge it is not as likely to make it back to the ridge, and therefore other vents, due to deep-sea currents (Tyler and Young 2003). Closely related tubeworms have been found to only be able to disperse a short maximum distance within their lifespan (Marsh et al. 2001). This is likely to be true for *Tevnia*, as well, also explaining why they have limited dispersal. There are also geologic factors, such as transform faults, which offset a ridge. Larvae are less likely to be able to continue dispersing along a ridge if it offset, and it is much less likely to make it to an even further population that may have multiple transform faults in the way (Vrijenhoek 2009b).

The pattern of stepping-stone dispersal has led to isolation-by-distance in *Tevnia*. The further away individuals are from each other, the more genetically differentiated they tend to be. Due to the limited dispersal and the factors affecting it, there is not complete gene flow and alleles are not shared through out all the population because they do not make it as far as the other end of the distribution. They do seem to fall into three groups: Northern (above the equator), middle (between equator and Easter microplate), and southern (south of Easter microplate). These groups are statistically similar within, but different from the other groups, with about the same amount of variation whether genes were tested separately or combined. These groupings are consistent with previous findings that there may be some restriction to gene flow across the equator and the Easter Microplate. However the results from the IM analysis indicate that there is some migration occurring across these boundaries, although migration seems to be converging into the middle latitudes (7°S and 17°S). This could explain why the greatest diversity is seen at these latitudes. To really be able to confirm all these ideas, we need to include more loci, especially nuclear loci, in this study. Different genes have different rates of mutation, and can be affected differently by the environment (Vrijenhoek 2009a). To have more power in these studies and more fully understand how these populations interact, we need a greater sample size of loci.

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