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Testing biological control of colonization by vestimentiferan tubeworms at deep-sea hydrothermal vents (East Pacific Rise, 9°50′N)☆

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Abstract

Three species of vestimentiferans are found at hydrothermal vents on the East Pacific Rise (EPR). *Tevnia jerichonana* is an early colonist and *Riftia pachyptila* has the greatest biomass in established vent assemblages, but the role of *Oasisia alvinae*, a small species that occurs sporadically, is unknown. Anecdotal evidence suggests that *O. alvinae* may be abundant in the microhabitat underneath mussels. Previous studies have suggested that early *T. jerichonana* colonists may facilitate settlement of the late colonist *R. pachyptila*. To address potential mechanisms for the successional sequence and to explore the role of *O. alvinae*, we examined the effects of the presence of vestimentiferan (*R. pachyptila* and *T. jerichonana*) tubes and mussel (*Bathymodiolus thermophilis*) shell cover on recruitment of vestimentiferans on basalt blocks deployed at 9°50′N, 104°17′W on the EPR. A molecular assay was used to identify individuals to species since they were too small to be identified morphologically. Although colonists in both experiments belonged to all three species of vestimentiferans, only a few were *T. jerichonana.* Colonization of vestimentiferans did not increase in the presence of vestimentiferan tubes. The presence of mussel shell cover did not influence the proportions of *R. pachyptila* and *O. alvinae*, or the total number of colonists. Because the experimental blocks in this study were placed within dense clumps of *R. pachyptila*, we suggest that, while *T. jerichonana* may be an important cue for vestimentiferans settling at new vents, adult *R. pachyptila* also can act as a settlement cue for larvae. *O. alvinae* colonists were abundant in all of the treatments in our experiments, indicating that, although adults of this species are apparently rare at these sites, *O. alvinae* can settle in abundance if a suitable micro-environment is available.

Keywords: Hydrothermal fields; Colonization; Recruitment; Benthos; Mexico; East Pacific Rise

☆The first and second authors contributed equally to the research and data interpretation. Hunt is listed first because she took the lead in writing the paper.

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1. Introduction

Hydrothermal vents are patchy and ephemeral habitats. On fast-spreading ridges, individual vents can open and close within years to decades (Hessler et al., 1988; Haymon et al., 1993), and changes in hydrothermal fluid flow can result in increases in or mortality of vent fauna (e.g. Fustec et al., 1987). Consequently, patterns of colonization and succession likely play a crucial role in the ecology of communities at hydrothermal vents. Because of the strong physical and chemical gradients at hydrothermal vents, spatial and temporal patterns of distribution of vent fauna have been attributed mainly to their physiological tolerances and the requirements of their symbionts (e.g. Shank et al., 1998). However, there is recent evidence of the importance of biological interactions such as facilitation (Mullineaux et al., 2000), predation (Micheli et al., 2002), and adult-larval interactions (H. Lenihan, unpublished data) in organizing biological assemblages at hydrothermal vents.

Vestimentiferan tubeworms are the most visually striking sessile organisms at hydrothermal vents on the East Pacific Rise (EPR). Observations at newly formed vents on the EPR have shown a successional sequence of initial colonization by the tubeworm *Tevnia jerichonana*, followed by its replacement by the larger tubeworm *Riftia pachyptila* (Lutz et al., 1994; Shank et al., 1998). *R. pachyptila* itself may later be replaced by the mussel *Bathymodiolus thermophilus*. Vent communities also exhibit strong patterns of zonation along gradients of hydrothermal fluid flux. On the EPR, vestimentiferan tubeworms are found in areas with strong diffuse flow ($\leqslant 25^{\circ}$C), bivalves in moderate diffuse flow ($\leqslant 10^{\circ}$C), and suspension feeders in peripheral areas with weak diffuse flow ($\leqslant 2^{\circ}$C) (Hessler and Smithey, 1983; Van Dover and Hessler, 1990).

Recent work has begun to examine the interactions between the three species of vestimentiferans, *T. jerichonana, R. pachyptila*, and *Oasisia alvinae*, found at EPR sites. Shank et al. (1998) documented patterns of community development at 9–10°N after a volcanic eruption opened up new vents in 1991 (Haymon et al., 1993). Shank et al. (1998) suggested that the temporal change in species composition from *T. jerichonana* to *R. pachyptila* results from changes in hydrothermal fluid flux. In the same area in 1994–1995, Mullineaux et al. (2000) examined patterns of colonization of vestimentiferans on basalt blocks deployed among tubes of adult vestimentiferans for 5–13 months. They observed colonists of *R. pachyptila* and *O. alvinae* only on blocks that also were colonized by *T. jerichonana*. In contrast to Shank et al. (1998), they hypothesized that *T. jerichonana* facilitates settlement of the other species of tubeworms by providing a chemical cue. Such chemical facilitation has been observed for shallow-water polychaete species in which larvae exhibit settlement responses to chemical compounds produced by conspecific adults (reviewed by Pawlik, 1992).

The role of *O. alvinae*, the third vestimentiferan, in the successional sequence remains unclear. *O. alvinae* is a small vestimentiferan that is morphologically similar to *T. jerichonana*, and difficult to identify in the field. Observations during colonization experiments suggest that *O. alvinae* is numerous in microhabitats, such as those covered by mussels, where vent fluids are relatively undiluted by ambient seawater (personal communications, C. Fisher, S. Schaeffer, S. Simmons). Because vent fluids at the 9–10°N site are depleted in oxygen and enriched in H_2S, CO_2 and metals (e.g., Fe, Mn, Cu, Zn) relative to ambient deep seawater (von Damm, 1995), the mussel-covered habitats are likely to differ physically and chemically from typical vestimentiferan habitat.

In the present study, we examined the role of biological interactions in colonization patterns of vestimentiferan tubeworms in two experiments at the same vent field studied by Mullineaux et al. (2000) but 4–5 years later; thus, assemblages were most likely at a later successional stage. In one experiment, we investigated whether the presence of vestimentiferan tubes can provide a biochemical or structural cue and thus enhance settlement of vestimentiferan larvae, particularly *R. pachyptila*. In a second experiment, we investigated whether being covered by a mussel bed (as mimicked experimentally with empty *B. thermophilus* shells) enhances recruitment of *O. alvinae* relative to other vestimentiferan species.

2. Experimental design and methods

This study was conducted at hydrothermal vents near 9°50′N, 104°17′W on the EPR (2500 m depth, Fig. 1) from the submersible Alvin. We examined the recruitment of vestimentiferans on cubic basalt blocks (~10 cm on a side), because this colonization substrate was used successfully by Mullineaux et al. (2000). The microhabitat of each block was characterized on deployment and recovery by taking water temperature measurements beneath the block with the Alvin temperature probe. For information on longer-term temperature variation, measurements were made with an internally recording "Hobo" temperature probe every 6 (mussel experiment) or 7 (tube experiment) hours. Although water temperature does not necessarily correlate well with vent fluid chemistry on large (i.e., between vent field) scales, it does appear to be a reasonable proxy on smaller scales (i.e., within a vent field; Johnson et al., 1988). Upon recovery, blocks were examined at 4°C under a dissecting microscope, and individual vestimentiferan recruits were enumerated and removed for storage at −80°C prior to molecular identification.

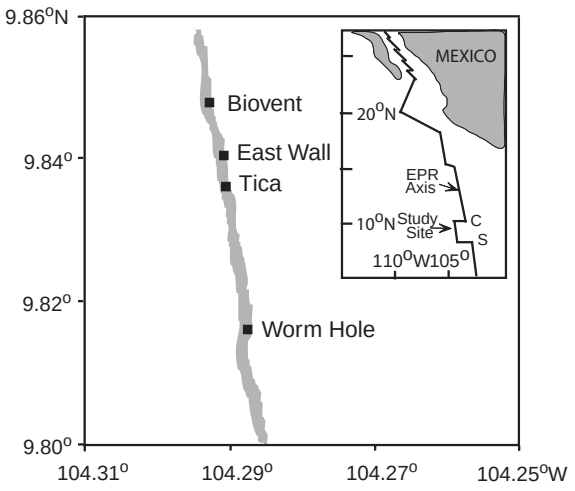

Fig. 1. Map of EPR axis (shaded corridor) near 9°50′N, showing the vent sites used in the tube experiment (East Wall), the mussel experiment (Tica), and the Mullineaux et al. (2000) experiments (Biovent, East Wall, and Worm Hole). The sites are located between the Clipperton (C) and Sequieros (S) fracture zones.

In the first experiment, we investigated whether the presence of the tubes of *T. jerichonana* increases (and thus potentially facilitates) settlement of vestimentiferan larvae, and, if so, whether the mechanism of facilitation is structural. Four treatments were set up in quadrants on each face of each of seven blocks: *T. jerichonana* tube, *R. pachyptila* tube (to test whether any effect is specific to *T. jerichonana*), tygon tube (to control for the effect of the gross physical structure of the tubes), and no tube (control). In each treatment quadrant, three 3 × 1 cm strips of the appropriate tube material were attached with a dab of marine epoxy (A-788 Splash Zone Compound; Z-Spar, Los Angeles, CA, USA). The vestimentiferan tubes were collected from the study site and the worm tissue was removed 1 day before the tubes were attached to the blocks. This randomized block arrangement of treatments was selected because previous experiments had shown that vestimentiferan recruitment varied substantially among the six faces of a block, but was relatively consistent across an individual face. Additional blocks ($n = 3$) were deployed to test for any effect of the epoxy used to attach the tubes. On these blocks, one half of each block face was unmanipulated and the other half contained 3 dabs (~0.5 cm diameter) of marine epoxy. The blocks were deployed for 7 months (May–December 1998) in clumps of *R. pachyptila* at the East Wall site (Fig. 1).

In the second experiment, blocks were deployed with and without a cover of mussel shells to test the effect of a physical barrier that restricted mixing of vent fluid with ambient seawater on vestimentiferan recruitment. Empty mussel shells were used to mimic mussel cover because it was not possible to bring mussels to the surface and return them to the bottom alive in an experimental manipulation and live mussels could not easily be confined in place on the bottom. Unconfined live mussels are likely to migrate away from a transplant site (C. Fisher unpublished data). Our shell cover treatment was designed to test the effect of physical structure of mussels, not replicate that of filter feeding of live mussels. Mussels were collected from the study site, shucked, and secured by cable ties with the two valves in 'life' position.

The shells were attached inside a Vexar mesh bag (ca. 30 cm diameter), and the resulting "blanket" of mussel shells was attached to the mussel treatment blocks, covering the top and sides. Control blocks had no attached mussels. Blocks ($n = 6$ replicates of each treatment) were deployed for 5 months (December 1999–May 2000) in clumps of *R. pachyptila* at the Tica vent site. Tica is approximately 200 m south of East Wall along the axial valley (Fig. 1). This site was chosen because *R. pachyptila* clumps at East Wall were becoming overgrown by mussels at this time, while those at Tica were growing vigorously and did not contain any mussels. Only vestimentiferans growing on the blocks and not those on the mussel shells or bag were included in the analysis.

Most of the juvenile vestimentiferans recruiting onto the blocks were <1 cm in length, and were identified using a molecular assay. The method is based on the polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLPs), and is capable of unambiguously distinguishing the three species of vestimentiferans (*T. jerichonana, R. pachyptila*, and *O. alvinae*) present at the vents at 9°50′N. This assay is similar to the one in Mullineaux et al. (2000) in its use of PCR-RFLP, but differs in the choice of gene (18S vs. 28S) and restriction enzyme (*HhaI* vs. *TaqI*). The new assay produces consistent results with small amounts of tissue. The program Sequencher was used to analyze several candidate genes (K. Halanych, unpublished data) for potential RFLP patterns. We selected a portion of the 18S (nuclear small ribosomal subunit or nSSU) gene, amplified by the PCR primers 18e and 18L (Halanych et al., 1998), because it contained restriction enzyme sites whose resulting fragment patterns were distinct and easily interpreted. Additionally, these primers sites are identical across vestimentiferan species, helping to eliminate species-specific variation in PCR amplification. DNA was extracted from individual vestimentiferan recruits with the DNeasy extraction kit (Promega), using overnight digestion of tissue and one-half the standard reagent volumes. PCR amplifications were conducted with *Taq* polymerase (Promega) under the following reaction conditions: initial denaturation, 2 min. at 94°C; 35 cycles of 30 s at 94°C, 30 s at 50°C, 45 s at 72°C; final extension 7 min at 72°C; final hold 4°C. The resultant 560 bp fragment was purified with the QiaQuick PCR purification kit (Qiagen) and digested at 37°C for 1.5 h with the restriction enzyme *HhaI* (New England Biosystems). Fragments were separated by electrophoresis in 2% agarose/TBE buffer gels containing ethidium bromide. When tested on adult tissues, this produced a distinct RFLP pattern: 209 bp, 169 bp, 128 bp, and 53 bp fragments for *T. jerichonana*, 261 bp, 169 bp, and 128 bp fragments for *O. alvinae*, and 430 bp, and 128 bp fragments for *R. pachyptila*. If a given individual did not amplify initially, a second attempt was made before scoring it a failure. No systematic biases were observed between samples that did and did not amplify the first time.

The relative abundance (percentage) of individuals identified as *R. pachyptila* and the total number of vestimentiferan colonists were compared among tube treatments by ANOVA for a randomized block design. However, post-hoc power analysis indicated that because only two blocks were colonized and there was a great deal of variability between the blocks, even large differences between treatments could not be detected statistically. Therefore, we have not presented any statistical results for the tube experiment. For the mussel experiment, the percentages of *R. pachyptila* and *O. alvinae* and the total number of vestimentiferans were compared between blocks with mussel cover and control blocks by *t*-tests. Data for the proportions were arcsin $(\sqrt{x})$-transformed to remove heterogeneity of variance, as detected by Cochran's test.

3. Results

3.1. Tube experiment

Water temperatures under individual blocks ranged from 2.6°C to 3.6°C (Table 1), compared to an ambient water temperature of 1.8°C. Continuous temperature measurements also indicated moderate diffuse flow, with temperatures between 1.9°C and 6.8°C over the 8-month period (Fig. 2). Two of the seven treatment blocks and

Table 1
Abundances of the three vestimentiferan species, *Tevnia jerichonana, Riftia pachyptila*, and *Oasisia alvinae*, colonizing basalt blocks in the tube and mussel-cover experiments

Treatment	Block no.		Temperature		Vestimentiferans				Total
			In (°C)	Out (°C)	*Tevnia*	*Riftia*	*Oasisia*	Unk	
Tube experiment									
Control	1	Control	2.6[a]	3.6	0	11	1	11	23
		Epoxy			0	1	0	2	3
Control	2		2.6[a]	3.4	0	0	0	0	0
Control	3		2.6[a]	3.0[b]	0	0	0	0	0
Tubes	1	*Riftia*	2.6[a]	3.0[b]	0	4	0	0	4
		Tevnia			0	4	1	2	7
		Tygon			0	0	0	0	0
		Control			0	3	0	1	4
Tubes	2	*Riftia*	2.6[a]	2.4	0	16	2	18	36
		Tevnia			0	5	2	4	11
		Tygon			1	11	0	9	21
		Control			0	9	1	2	12
Tubes	3		2.6[a]	3.0[b]	0	0	0	0	0
Tubes	4		2.6[a]	3.0[b]	0	0	0	0	0
Tubes	5		2.6[a]	3.0[b]	0	0	0	0	0
Tubes	6		2.6[a]	3.0[b]	0	0	0	0	0
Tubes	7		2.6[a]	3.0[b]	0	0	0	0	0
Mussel-cover experiment									
Control	1		⩾25	23.0	0	5	5	1	11
Control	2		5.6	12.8	2	5	9	2	18
Control	3		⩾25	13.5	1	0	5	2	8
Control	4		6.5	1.9	0	0	0	0	0
Control	5		12.5	26.0	0	0	1	0	1
Control	6		5.5	2.9	1	3	1	1	6
Mussel	1		15.0	18.0	3	6	16	1	26
Mussel	2		14.1	4.5	0	1	1	0	2
Mussel	3		⩾25	4.0	1	14	3	2	19
Mussel	4		11.1	3.6	0	0	0	0	0
Mussel	5		7.5	2.5	0	0	0	0	0
Mussel	6		⩾25	26.3	1	7	4	0	12

In the tube experiment, blocks were deployed for 8 months with controls for the epoxy (epoxy and control areas; $n = 3$ blocks) or with tube treatments (*R. pachyptila* tube, *T. jerichonana* tube, tygon tube, control; $n = 7$ blocks). In the mussel-cover experiment, blocks were deployed for 5 months with and without a cover of mussel shells ($n = 6$ blocks for each treatment). Temperature represents the maximum temperature recorded at the base of each block when it was placed into and removed from the habitat. Vestimentiferans were identified to species by molecular techniques. Individuals for whom molecular identification failed were unidentified (Unk).

[a] Mean temperature ($n = 3$) from measurements in the area where these blocks were deployed.

[b] Mean temperature ($n = 2$) from measurements in the area where these seven blocks were recovered.

one of the three epoxy-control blocks were colonized by vestimentiferans (15–79 individuals per block; Table 1). The PCR-RFLP assay successfully amplified and identified to species 72 (60%) of the 121 tubeworms collected on the three colonized blocks.

One block face on each of two treatment blocks was colonized by vestimentiferans. Only one individual of *T. jerichonana* was found on the blocks; most of the vestimentiferans were *R. pachyptila* (Fig. 3a). On average, treatment quadrants on the two colonized block faces had 9.0 ± 2.8 (mean ± 1 SD), 10.5 ± 14.8, 8.0 ± 5.7, and 20.0 ± 22.6 vestimentiferan colonists on *R. pachyptila* tube, *T. jerichonana* tube, tygon tube, and control quadrants, respectively (Fig. 3b).

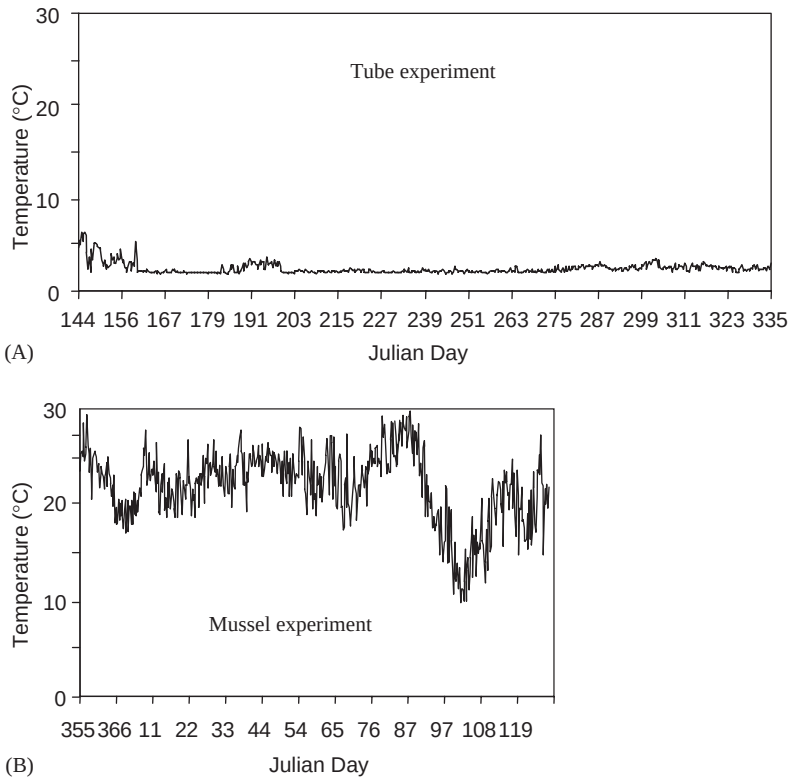

Fig. 2. Temperatures measured with internally recording "Hobo" probes during (A) the tube experiment (May–December 1998 at East Wall) and (B) the mussel-cover experiment (December 1999–May 2000 at Tica). Measurements were made within the same vestimentiferan clumps where blocks were deployed, but not at the precise position of any one block.

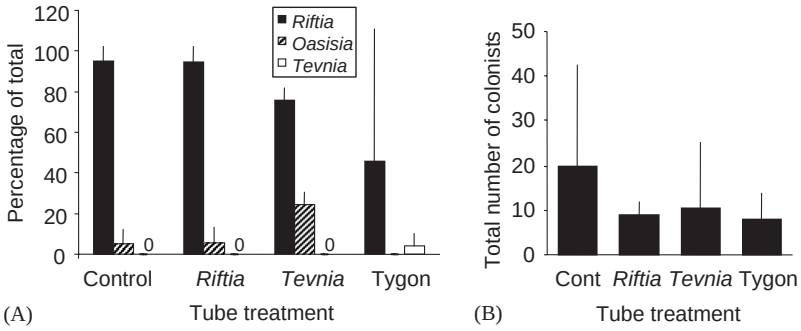

Fig. 3. (A) Mean (+1SD) proportion of *R. pachyptila, T. jerichonana*, and *O. alvinae*. (B) Mean (+1SD) number of vestimentiferans (including unidentified individuals) colonizing the 4 tube treatments (*R. pachyptila* tube, *T. jerichonana* tube, tygon tube, and control area). The treatments were randomly assigned to each of the four quarters on each block face. $n = 2$ colonized blocks (one face on each). Blocks were deployed at East Wall for 8 months (May–December 1998).

Vestimentiferans also settled on three faces of one of the epoxy-control blocks. On average, control sections had 4.0 ± 5.3 (mean ± 1 SD) and epoxy sections had 0.3 ± 0.6 vestimentiferan colonists. Pooling individuals across all treatments and blocks, the proportions of the three species of vestimentiferans in the tube experiment were 88.9% *R. pachyptila*, 9.7% *O. alvinae*, and 1.4% *T. jerichonana*.

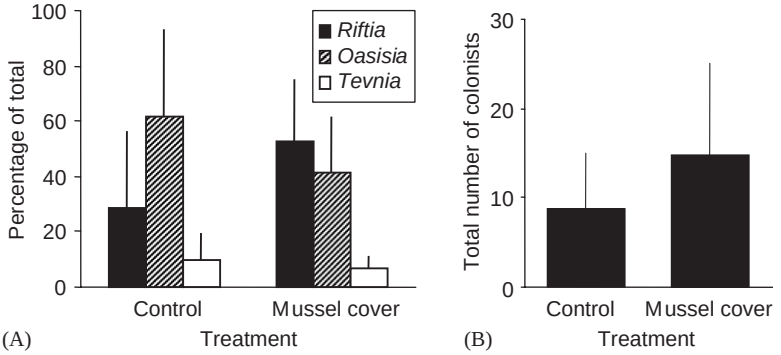

Fig. 4. (A) Mean (+1SD) proportion of *R. pachyptila, T. jerichonana*, and *O. alvinae*. (B) Mean (+1SD) number of vestimentiferans (including unidentified individuals) colonizing control blocks and those with mussel shell cover. $n = 5$ and 4 colonized blocks for controls and treatment blocks, respectively. Blocks were deployed at Tica for 5 months (December 1999–May 2000).

3.2. Mussel cover experiment

One control block and two mussel-covered blocks had no vestimentiferan recruits, while the other 9 blocks were colonized by at least one of the three species (Table 1). The uncolonized control block had rolled out of the tubeworm clump and was exposed to ambient temperatures (1.8°C), while the two uncolonized mussel-covered blocks had recovery temperatures of 2.5°C and 3.5°C, respectively. Recovery temperatures of the blocks with vestimentiferan colonists ranged from 2.9–26.0°C (Table 1). Continuous temperature measurements indicated strong diffuse flow, with temperatures of 10–30°C over the 4-month period of the experiment (Fig. 2). The PCR-RFLP assay successfully amplified and identified to species 95 (92%) of 103 tubeworm specimens collected in the mussel experiment.

All three vestimentiferan species colonized the blocks. *T. jerichonana* was the least common species (<10% of individuals), while *R. pachyptila* represented 28% and 53% of colonists on control and mussel-covered blocks, respectively, and *O. alvinae* represented 62% and 41% of individuals on the two treatments, respectively (Fig. 4a). There was no significant difference between control and mussel-covered blocks in the proportions of either *R. pachyptila* ($t_7 = 1.42, P = 0.20$) or *O. alvinae* ($t_7 = 1.16, P = 0.29$). On average, 8.8 (±6.3 SD) and 14.8 (±10.2 SD) juvenile vestimentiferans colonized control blocks and mussel-covered blocks, respectively (Fig. 4b). The total number of vestimentiferans (including unidentified individuals) colonizing the blocks also did not differ between the two treatments ($t_7 = 1.08$, $P = 0.32$). Given the observed variability, an $\alpha = 0.05$, and a specified power $(1-\beta)$ of 80%, analysis of statistical power indicated that the observed differences ranged from 31% (total number of vestimentiferans) to 39% (proportion of *R. pachyptila*) of the minimum detectable difference. Pooling individuals across blocks and treatments, the relative abundance of the three species of vestimentiferans was 43.2% *R. pachyptila*, 47.4% *O. alvinae*, and 9.5% *T. jerichonana*.

4. Discussion

4.1. Tube experiment

Mullineaux et al. (2000) hypothesized that *T. jerichonana* facilitates the colonization of the two other species of vestimentiferans on the EPR, *R. pachyptila* and *O. alvinae*. To test the hypothesis that the tube of *T. jerichonana* facilitates colonization via a chemical or physical settlement cue, we deployed blocks on which strips of *T. jerichonana, R. pachyptila* and tygon tubes were attached. Settlement of vestimentiferans was very sparse, perhaps because of the relatively low diffuse flow

and water temperatures. There was no trend towards increased colonization in the presence of tubes of either vestimentiferan species, and therefore no evidence that the tubes acted as cues for tubeworm colonization. The results of this experiment should be viewed with caution because tubeworms colonized only two of the treatment blocks and, consequently, we had no power to detect differences among treatments. Also, we have not tested for the effects of live vestimentiferans on subsequent colonization, only for the chemical or physical effects of tubes.

In both the tube and mussel-cover experiments, colonists of *R. pachyptila* and *O. alvinae* were found in the absence of any identified individuals of *T. jerichonana*. Although in some cases it is possible that *T. jerichonana* were among the individuals not identified, the failure rate is unlikely to be species-specific and it appears likely that vestimentiferan colonization in our experiments was not dependent on the close proximity (i.e., centimeters on the same block) of *T. jerichonana*. This result contrasts with the study by Mullineaux et al. (2000), in which *R. pachyptila* and *O. alvinae* colonists were found only on blocks that also were colonized by *T. jerichonana*, but is not inconsistent with possible mechanisms of facilitation. We propose that in well-developed vent communities, *R. pachyptila* and *O. alvinae* use established vestimentiferans (particularly *R. pachyptila*) as a cue to settle into appropriate habitats. Many adult *R. pachyptila* were present and in contact with the blocks. Because of their great abundance and biomass, these individuals may provide a larger-scale (10 s of centimeters) biochemical or structural cue that was not well-mimicked by the affixed strips of tube on the blocks. Alternatively, the microbial community on the blocks may have provided settlement cues for the recruiting vestimentiferans.

4.2. Mussel-cover experiment

In the second experiment, we were particularly interested in recruitment by *O. alvinae* and its potential preference for mussel-covered microhabitats. Adults of *O. alvinae* are believed to be relatively rare at 9°50′N, although they can be abundant at other sites (e.g. Hanging Garden at 21°N, where *T. jerichonana* does not occur; Van Dover and Hessler, 1990). In our study, 47% of colonists in the mussel cover experiment and 10% of those in the tube experiment were *O. alvinae* (Fig. 2). *O. alvinae* also comprised 25% of vestimentiferan colonists in prior experiments at 9°50′N (5 and 8 months blocks at East Wall, Mullineaux et al., 2000; Fig. 2). The successful colonization of blocks by *O. alvinae* at three different times and a variety of sites indicates that their rarity as adults in the field does not reflect a lack of larval supply or settlement when a suitable substrate is provided. Instead, *O. alvinae* may be excluded from resources by the faster-growing *R. pachyptila*, or unable to grow and survive to maturity in the physical and chemical environment in which the *R. pachyptila* clumps occur. Alternatively, it is possible that *O. alvinae* adults are more abundant than previously thought in a variety of EPR habitats, but remain undetected because of their small size and morphological similarity to *T. jerichonana*.

The absence of a mussel-cover effect on colonization by *O. alvinae* suggests that the physical effect of mussel cover (i.e., reduced dilution of low-oxygen vent fluids with oxygenated ambient seawater) may not be responsible for the prevalence of that species under mussel beds. However, the oxygen concentration under our mussel blankets may have been higher than under natural mussel beds because live mussels are tightly bound together by byssal threads and they constantly filter, respire, and excrete into the water immediately above the bed. Even if mussel shell cover does not produce a preferred microhabitat for colonization of *O. alvinae*, the possibility remains that cover by a live mussel bed does. Alternatively, differential mortality among the three vestimentiferan species, and the dominance of *O. alvinae* over the other two may require longer than 5 months to develop. In previous colonization experiments, blocks deployed for 29–42 months became covered by mussels and had higher proportions of *O. alvinae* than those deployed for 5–13 months (S. Simmons and S. Schaeffer unpublished data).

4.3. Settlement variability and changes in species composition of vestimentiferans

In both experiments and in Mullineaux et al. (2000), there was a great deal of variability in recruitment among faces of a block and between blocks. The sources of this small-scale variability in recruitment remain poorly understood, but may include small-scale differences in physical conditions such as temperature (e.g. between bottom and sides of block), in the development of the microbial community, and in gregarious settlement of tubeworms.

In our study, only 1% and 9% (in the tube and mussel experiments, respectively) of vestimentiferans were genetically identified as *T. jerichonana* (Fig. 5). In contrast, Mullineaux et al. (2000) found that 64% of genetically identified colonists on blocks deployed for 5–8 months at East Wall in 1994–1995 were *T. jerichonana* (Fig. 5). Since both studies used the same types of blocks placed within healthy clumps of *R. pachyptila* in diffuse hydrothermal flow, differences in colonists are most likely not due to experimental procedural differences. Differences in vent temperature and chemistry also cannot explain the decrease in abundance of *T. jerichonana* between the studies. Temperatures during the tube experiment were lower (Fig. 2) and those during the mussel experiment were higher (Fig. 2) than the temperatures (generally 5–10°C) recorded in Mullineaux et al. (2000). The difference in the proportion of *T. jerichonana* between the two studies may reflect a change in the regional pool of vestimentiferan larvae since *T. jerichonana*, an early successional species, were less abundant in 1998–2000, when our study was done, than in 1994–1995 (Shank et al., 1998; pers. obs.). Alternatively, because the 1998 and 2000 experiments were each conducted at only one site, differences between these experiments and that of Mullineaux et al. (2000) may result from site-specific temporal patterns (although the tube experiment was carried out at one of Mullineaux's sites).

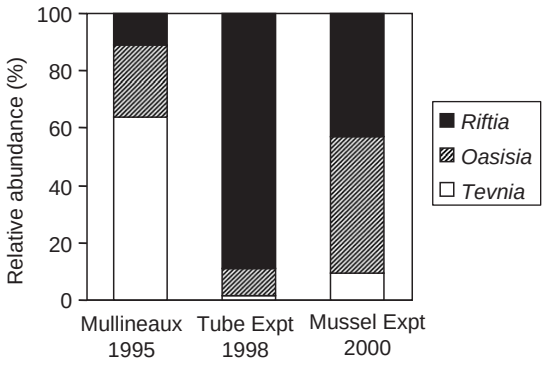

Fig. 5. Proportion of *R. pachyptila, T. jerichonana*, and *O. alvinae* in colonization experiments in 1994–1995 (5 and 8 months blocks at East Wall, from Mullineaux et al., 2000), 1998 (the tube experiment at East Wall, this study), and 2000 (the mussel-cover experiment at Tica, this study). Individuals were pooled across treatments. $n = 164$ (Mullineaux et al., 2000), 72 (tube experiment), and 95 (mussel-cover experiment) genetically identified individuals.

This study contributes to our understanding of the ecological relationships between vestimentiferan species on the EPR. Because of their high abundance and biomass, tubeworms play an important role in vent community dynamics at hydrothermal vents in the east Pacific Ocean (reviewed by Tunnicliffe, 1991). Our results extend previous work on the importance of cues for settlement for vestimentiferans: while *T. jerichonana* provides a cue in developing communities, we suggest that other vestimentiferan species (in particular *R. pachyptila*) can also provide a cue in more established communities. The high abundances of *O. alvinae* colonists in these and other colonization experiments at 9°50′N highlight the importance of uncovering the role of this species, which is apparently rare as an adult.

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References

Fustec, A., Desbruyères, D., Juniper, S.K., 1987. Deep-sea hydrothermal vent communities at 13°N on the East Pacific Rise: microdistribution and temporal variations. Biological Oceanography 4, 121–164.

Halanych, K.M., Lutz, R.A., Vrijenhoek, R.C., 1998. Evolutionary origins and age of vestimentiferan tube-worms. Cahiers de Biologie Marine 39, 355–358.

Haymon, R.M., Fornari, D.J., Von Damm, K.L., Lilley, M.D., Perfit, M.R., Edmond, J.M., Shanks, W.C., Lutz, R.A., Grebmeier, J.M., Carbotte, S., Wright, D., MacLaughlin, E., Smith, M., Beedle, N., Olson, E., 1993. Volcanic eruption of the mid-ocean ridge along the East Pacific Rise crest at 9°45–52′N: direct submersible observations of seafloor phenomena associated with an eruption event in April, 1991. Earth and Planetary Science Letters 119, 85–101.

Hessler, R.R., Smithey, W.M., 1983. The distribution and community structure of megafauna at the Galapagos Rift hydrothermal vents. In: Rona, P.A., Boström, K., Laubier, L., Smith, K.L. (Eds.), Hydrothermal Processes at Seafloor Spreading Centers. Plenum Press, New York, pp. 735–770.

Hessler, R.R., Smithey, W.M., Boudrias, M.A., Keller, C.H., Lutz, R.A., Childress, J.J., 1988. Temporal change in megafauna at the Rose Garden hydrothermal vent (Galapagos Rift; eastern tropical Pacific). Deep-Sea Research 35, 1681–1709.

Johnson, K.S., Childress, J.J., Hessler, R.R., Sakamoto-Arnold, C.M., Beehler, C.L., 1988. Chemical and biological interactions in the Rose Garden hydrothermal vent field, Galapagos spreading center. Deep-Sea Research 35, 1723–1744.

Lutz, R.A., Shank, T.M., Fornari, D.J., Haymon, R.M., Lilley, M.D., Von Damm, K.L., Desbruyères, D., 1994. Rapid growth at deep-sea vents. Nature 371, 663–664.

Micheli, F., Peterson, C.H., Mullineaux, L.S., Fisher, C., Mills, S.W., Sancho, G., Johnson, G.A., Lenihan, H.S, 2002. Species interactions at deep-sea hydrothermal vents: the role of predation in structuring communities in an extreme environment. Ecological Monographs 72, 365–382.

Mullineaux, L.S., Fisher, C.R., Peterson, C.H., Schaeffer, S.W., 2000. Tubeworm succession at hydrothermal vents: use of biogenic cues to reduce habitat selection error? Oecologia 123, 275–284.

Pawlik, J.R., 1992. Chemical ecology of the settlement of benthic marine invertebrates. Oceanography and Marine Biology Annual Review 30, 273–335.

Shank, T.M., Fornari, D.J., Von Damm, K.L., Lilley, M.D., Haymon, R.M., Lutz, R.A., 1998. Temporal and spatial patterns of biological community development at nascent deep-sea hydrothermal vents (9°50′N, East Pacific Rise). Deep-sea Research II 45, 465–515.

Tunnicliffe, V., 1991. The biology of hydrothermal vents: ecology and evolution. Oceanography and Marine Biology Annual Review 29, 319–407.

Van Dover, C.L., Hessler, R.R., 1990. Spatial variation in faunal composition on the East Pacific Rise and Galapagos Spreading Center. In: McMurray, G.R. (Ed.), Gorda Ridge A Seafloor Spreading Center in the United States Exclusive Economic Zone. Springer, New York, pp. 253–264.

von Damm, K.L., 1995. Controls on the chemistry and temporal variability of seafloor hydrothermal fluids. In: Humpris, S., Zierenberg, R.A., Mullineaux, L.S., Thomson, R.E. (Eds.), Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions. Geophysical Monograph 91. American Geophysical Union, Washington, DC, pp. 222–247.