Vol. 3: 79–87, 2008
doi: 10.3354/ab00060

AQUATIC BIOLOGY
Aquat Biol

Printed August 2008
Published online July 17, 2008

OPEN ACCESS

Juvenile and adult scale worms *Branchipolynoe seepensis* in Lucky Strike hydrothermal vent mussels are genetically unrelated

Sophie Plouviez[1,2], Claire Daguin-Thiébaut[1,2], Stéphane Hourdez[1,2], Didier Jollivet[1,2,*]

[1]Université Pierre et Marie Curie-Paris 6, Laboratoire Adaptation et Diversité en Milieu Marin, Roscoff cedex, France

[2]CNRS, UMR7144 Station Biologique de Roscoff, BP 74, Place Georges Teissier, 29682, Roscoff cedex, France

ABSTRACT: The deep-sea Mid-Atlantic Ridge hydrothermal vent mussel *Bathymodiolus azoricus* harbors the polynoid polychaete *Branchipolynoe seepensis* in its palleal cavity. The latter species possesses large, yolky oocytes (~400 µm) and has been hypothesized to brood its developing embryos. The present study used 6 microsatellite markers to assess kinship among adult and juvenile worms from the same host, among worms from mussels collected within a 1 m^2 quadrat, and among samples taken on 2 dates (July 1998 and July 2001) at 2 sites (Tour Eiffel, TE, and Bairro Alto, BA) separated by 900 m at Lucky Strike hydrothermal vents on the Mid-Atlantic Ridge (37° 16′ to 19′ N, 32° 15′ to 18′ W). The hypothesis of genetic relatedness between females and juveniles within a single mussel, or among worms from the same quadrat was rejected. Conversely, great heterozygote deficiencies have been detected at nearly all loci, suggesting possible local inbreeding. There was no apparent genetic differentiation among worms from the 2 spatially separated quadrats collected at the TE and BA sites in 2001. These results suggest that embryonic or juvenile *B. seepensis* are released from their host mussel at a relatively early stage of development. They may settle close to the point of release or be dispersed to nearby sites or beyond.

KEY WORDS: Adult/offspring kinship · Microsatellite · Dispersal · Polychaete · Mid-Atlantic Ridge

INTRODUCTION

Long-distance migration has been frequently invoked as an adaptation to ensure population persistence in highly fluctuating and fragmented environments, especially during range expansion (Nichols & Hewitt 1994). In marine environments, dispersal of invertebrates occurs mainly during the larval phase. More specifically in the deep sea, pelagic larval dispersal is considered extremely unfavorable (because of the need to move through the water column) and may be replaced by direct development (Thorson 1936). This idea was however challenged by Young (1994), who reviewed the literature on dispersal in the deep sea, and argued that lecithotrophic and planktotrophic larval stages may be more widespread than previously thought, especially in unstable environments such as hydrothermal vents and cold seeps, where most species possess pelagic larvae (Tyler & Young 1999). Given the level of the vent instability and the oligotrophic nature of bottom seawater surrounding the vents, long-distance dispersal and lecithotrophic larval development are therefore expected to be favored.

The hydrothermal vent scale worms *Branchipolynoe* spp. Pettibone live inside the mantle cavity of mussels belonging to the genus *Bathymodiolus*, which form dense mussel beds at the base of vent chimneys. This association is widespread, being found in vent ecosystems and cold seep areas around the world (Chevaldonné et al. 1998). *Branchipolynoe seepensis*, which lives in the mussel *Bathymodiolus azoricus* at the Azorean triple junction zone on the Mid-Atlantic

*Corresponding author. Email: jollivet@sb-roscoff.fr

Ridge, displays unusual reproductive behavior (Jollivet et al. 2000). Females possess a reproductive system with an ovisac containing large yolky oocytes (about 400 µm in diameter) and a pair of spermathecae (Jollivet 1996), indicating that reproduction involves pairing and internal fertilization. Mussels usually harbor only a single, large, sedentary female scale worm. However, for a limited number of observations, the female was associated with either a few juveniles or a male (much smaller in size). This pronounced size dimorphism in favor of females was found in all species of the genus, probably as the result of differing predatory rates between genders or intra-specific competition rather than dwarfism (Jollivet et al. 2000).

These findings raise an intriguing question regarding the dispersal mode of the scale worm and the filial relationships between individuals found inside the same mussel. Mating and offspring-caring behaviors are frequent occurrences in coastal polychaetes that produce large yolky eggs. The terebellids *Ramex californiensis* (maximum oocyte size: 410 µm) and *Thelepus crispus* (maximum oocyte size: 400 µm), brood their larvae inside the maternal tube (Schroeder & Hermans 1975). Brooding inside the host has also been well documented in other commensal polynoids (e.g. *Polynoe antarctica* Kinberg by Fauvel 1916 and *Halosydna brevisetosa* Kinberg by MacGinitie & MacGinitie 1949).

The reproductive features of the scale worm *Branchipolynoe seepensis* are typical of species that brood their offspring. Large lecithotrophic eggs can, however, be viewed either as the result of phylogenetic constraints towards direct development and limited dispersal capabilities or as an advantage for long-distance dispersal in species subjected to recurrent population bottlenecks, especially when the surrounding abyssal waters are poor in food sources for larvae (Young 1994). While previous genetic analyses based on ribosomal and mitochondrial sequences found no genetic differentiation over large spatial scales for either the Atlantic or Pacific *Branchipolynoe* species (Jollivet et al. 1998, Hurtado et al. 2004), suggesting either possible long-distance dispersal or a recent colonization of the sites, Daguin & Jollivet (2005), using highly polymorphic microsatellite markers, revealed that *B. seepensis* displayed genetic breaks along the Mid-Atlantic Ridge between populations ranging from 14 to 35° N. Dispersal of the worm could thus be more limited than previously thought.

The aim of the present study was to perform kinship analyses with the microsatellite markers previously used by Daguin & Jollivet (2005) to determine if the female and juveniles are genetically related inside the same host. Two alternative hypothetical reproductive strategies are possible: (1) females brood the juveniles inside the host; then, the female and juveniles are genetically related as mother/offspring; or (2) eggs and/or juveniles are released from the host and are able to colonize new mussels. In the latter case, the female and juveniles are not expected to show genetic relatedness as mother/offspring. In addition, for the second strategy, 2 alternative scenarios can also be discriminated using kinship analyses: (1) the offspring hatch inside the host, but the juveniles tend to leave their mussel of origin to settle into a new host, and, then, a relationship between juvenile age and kinship is expected within the host with a possible kin structure within the mussel bed at a small spatial scale, or (2) fertilized eggs or larvae are released into the water column to disperse passively with the (deep) bottom currents, and, then, no kinship/size relationship or small-scale spatial structure is expected.

MATERIALS AND METHODS

Sampling. The hydrothermal vent field Lucky Strike is located on the Mid-Atlantic Ridge at the triple junction of the Eurasian, American and African tectonic plates (between 37° 16′ and 37° 19′ N, 32° 15′ and 32° 18′ W, 1700 m deep). A 300 m diameter lava lake governs the hydrothermal system, and the evolution of the associated communities (Desbruyères et al. 2000). The vent sites Tour Eiffel (TE; 37° 17.22′ N, 32° 16.30′ W) and Bairro Alto (BA; 37° 17.37′ N, 32° 17.00′ W) are located on opposite sides of this lake, 900 m apart. Mussel beds *Bathymodiolus azoricus* were sampled on 2 oceanographic cruises: PICO in 1998 and ATOS in 2001. The TE site was sampled twice, once in July 1998 and once in July 2001; and the BA site was sampled once in July 2001. At each site, mussels were collected within a 1 m^2 quadrat using the telemanipulator arms of the submersible 'Nautile' (PICO98 cruise) or the remotely operated vehicle (ROV) 'Victor6000' (ATOS01 cruise), and brought back on board the ship inside a temperature-insulated box. Mussels were opened and carefully examined to collect all *Branchipolynoe seepensis* from the mantle cavity. Scale-worm specimens from a given mussel were then preserved together in 96% ethanol. A total of 299 scale worms was finally obtained out of 117 mussels from the 3 samples (Tour Eiffel 1998 [TE98], Tour Eiffel 2001 [TE01] and Bairro Alto 2001 [BA01]; see Table 1).

Scale-worm biometry. The total length of each scale worm was measured ventrally to the nearest 0.03 mm from the anterior part of the prostomium (evaginated proboscis excluded) to the anus (pygidial appendages excluded) using an ocular micrometer on a binocular microscope (magnification ×25). In addition, chaetigers were counted on each individual in order to determine

Table 1. *Branchipolynoe seepensis* and its host *Bathymodiolus azoricus*. Number of sampled mussels and of female, male and juvenile scale worms at Tour Eiffel (TE98 and TE01) and Bairro Alto (BA01) sites during PICO98 and ATOS01 oceanographic cruises

	B. azoricus	*B. seepensis*			
Site	Total	Females	Males	Juveniles	Total
TE98	36	35	9	24	68
TE01	36	34	8	62	104
BA01	45	50	4	73	127

its developmental stage relative to a fully developed scale worm, which has 20 chaetigerous segments plus 1 achaetous segment. In the present study we report the number of segments as a measure of worm size. The sex of each individual was also determined when possible following the morphological criteria of Jollivet et al. (2000).

DNA extraction and genotyping. Genomic DNA was extracted from the 299 scale worms using a Chelex protocol (Walsh et al. 1991). A small piece of ethanol-preserved tissue (equal to 1 mature female parapodium) was digested in 400 µl of a 5% Chelex100 solution containing 10 mM Tris-HCl, 1 mM EDTA and 25 µl of Proteinase K (20 mg ml^{-1}) and was incubated at 55°C overnight. DNA extracts were vortexed (5 min), centrifuged (10 min at 15 000 × *g*), heated at 95°C for 10 min, and finally stored at –20°C until PCR amplification. These amplifications were carried out for 6 highly polymorphic microsatellite loci (Bs 3E4, Bs 6C3, Bs 3D5, Bs 5H11b, Bs 6D7, Bs 3C8) following the protocol of Daguin & Jollivet (2005). PCR products were then electrophoresed on a denaturing acrylamide 41 cm gel in a Li-Cor NEN Global IR2 DNA analyzer. Individuals that did not amplify at all loci (i.e. 53 ind.) were excluded from the genetic analysis, but scored for estimating null allele frequencies.

Population analysis. The genetic structure of *Branchipolynoe seepensis* was analyzed based on allele frequencies at 6 microsatellite loci using GENETIX 4.05 (Belkhir et al. 2004). Departures from Hardy-Weinberg equilibrium were assessed using the Weir & Cockerham (1984) estimators *F* and *f* at each locus for the whole collection and for each sample, respectively, with and without discriminating adults from juveniles. Differentiation tests were performed using the Weir & Cockerham (1984) estimator θ for each locus: (1) between juveniles and adults within samples and (2) between samples without separating individuals into age categories. Departures of *F*, *f* and θ from zero were tested by permuting either alleles between genotypes within the whole collection, genotypes within samples, or genotypes between samples, respectively, following the recommendations of Belkhir et al. (2004). Allelic richness (El Mousadik & Petit 1996) was calculated for each locus and site based on the smallest sample size (i.e. at TE98: 54 ind.) with FSTAT 2.9.3 (Goudet 2001). Frequencies of putative null alleles were estimated using both frequencies of individuals that did not yield an amplification (counted as null homozygotes) and the CERVUS 2.0 (Marshall et al. 1998) method, which derives null allele frequencies from heterozygote deficiencies using a Markov-chain Monte Carlo process.

Kinship analysis. In order to establish the marker efficiency in ruling out false parentage, the probability of exclusion was calculated for the 6 microsatellite loci using FSTAT 2.9.3 (Goudet 2001). First, based on allele frequencies characterizing populations, the average level of individual relatedness was estimated at increasing spatial scales within the Lucky Strike hydrothermal vent field from multilocus assignments for: (1) individuals living together within a mussel, (2) individuals from the same quadrat, and (3) the whole sample. The aim of this analysis was to determine whether a kinship structure could be detected. The relatedness coefficient was calculated for each pair of scale worms using KINSHIP 1.2 (Queller & Goodnight 1989). Mean kinship coefficients between the 2 former and the 2 latter spatial scales were tested using Student's *t*-tests.

Second, several hypotheses of kinship based on probabilities that individuals share alleles by descent through maternal (R_m) or paternal (R_p) inheritance were tested. These hypotheses are: (1) juveniles are direct descendants of the female(s) when located inside the same mussel (R_m = 1, R_p = 0), (2) juveniles living in the same mussel are full siblings (R_m = 0.5, R_p = 0.5). Relative likelihood ratio tests with 1 degree of freedom (Sokal & Rohlf 1981), in which the null hypothesis H_0 (individuals are not related) and the alternative hypothesis H_1 (individuals are related), were used. The r correlation between female/juvenile kinship coefficients and the number of segments of juveniles were tested according to Sokal & Rohlf (1981) to address the age of leaving the mussel of origin.

Finally, the most probable mother was determined for each juvenile from the whole female set using CERVUS 2.0 (Marshall et al. 1998). CERVUS searches only for the most probable mother by excluding the other females using relative likelihood tests (Delta criterion value), similar to those of KINSHIP, at a given threshold value. Parameters used to define the Delta criterion were: 10 000 cycles (number of offspring), 1.000 the proportion of loci typed and 0.010 the proportion of loci mistyped. A search for the most probable mother was first performed inside each 1 m^2 quadrat (TE01 and BA01) with the proportion of candidate parents sampled set to 1.000 under the hypothesis that

juveniles dispersed only to a nearby mussel. A second search for the most probable mother was then performed over the whole female 2001 collection with the proportion of candidate parents set to 0.010 (75 candidate females) under the alternative hypothesis that larva/juvenile dispersal is not limited to the quadrat. The multilocus genotype of a given female was thus compared to that of each juvenile. A female was considered as a potential mother when its genotype was compatible with those of the tested juveniles at all loci (with a relaxed confidence level of 80 and 95%). A relaxed confidence level of 80% was preferred in the global parentage analysis because the alternative 95% confidence is known to be highly stringent and often excludes true kinship in studies where parent and offspring are already known (e.g. in presence of relatives; Marshall et al. 1998). The Poisson likelihood of descent (LOD) distribution was normalized to check whether significant positive assignments fall out of the 95% range of the values, and thus to have more confidence with 'true' kinship.

RESULTS

Biometric analysis

The number of *Branchipolynoe seepensis* per mussel varied from 0 to 13 ind. (Fig. 1), with a median of 1, 2 and 3 ind. mussel^{-1} for TE98, TE01 and BA01, respectively. The lengths of the collected mussels *Bathymodiolus azoricus* ranged from 38.5 to 105 mm. The number of worms per mussel was exactly the same in the TE01 and BA01 quadrats (2.9 worms mussel^{-1}), despite a noticeable difference in the length-frequency distributions of the host. In general, large mussels yielded more scale worms than small ones (data not shown).

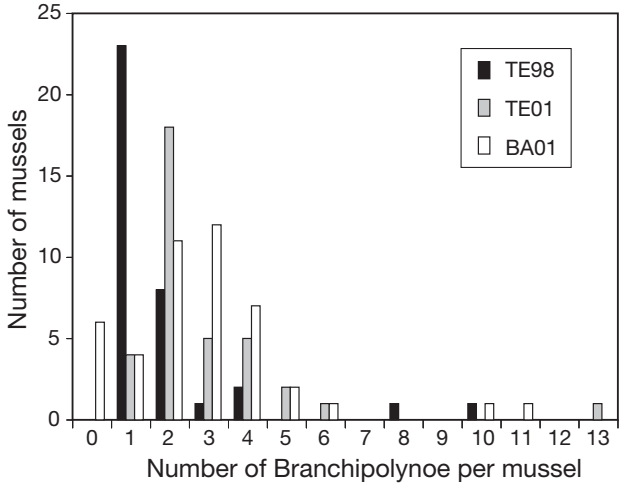

Fig. 1. *Branchipolynoe seepensis* and its host *Bathymodiolus azoricus*. Distribution of the number of individuals per mussel for the sites Tour Eiffel (TE98 and TE01) and Bairro Alto (BA01) during PICO98 and ATOS01 oceanographic cruises

Juveniles, males and females displayed significantly different mean sizes in the 3 samples (Mann-Whitney *U*-test, $p < 0.001$), with the exception of BA01, for which males and females exhibited similar sizes ($p = 0.118$). When genders were compared between samples, the only significant difference was found for females from TE98, which were larger than females from TE01 and BA01 ($p < 0.002$). The number of segments of juveniles ranged from 12 to 20 in our collection. Although there was no difference in the number of segments in juveniles between samples, juveniles from TE01 were significantly larger than those from BA01 ($p = 0.002$).

Population analysis

Multilocus genotypes revealed that both males and females were diploid, with an average number of 3.5 (out of 6) heterozygous loci ind.$^{-1}$. The number of alleles per locus ranged from 4 to 75 alleles for Bs 6D7 in TE01 and Bs 3E4 in BA01, respectively (Table 2). Expected heterozygosities (H_e) varied from 0.460 for Bs 3E4 in TE98 to 0.969 for Bs 3D5 in TE98. The fixation indices F, f and θ, together with the frequency of the most frequent allele, are presented in Table 2 for each locus. Multilocus f-estimates were significantly different from zero in the 3 samples (TE98, $f = 0.193$; TE01, $f = 0.155$; BA01, $f = 0.171$). Only 4 of the 18 single-locus f values were not indicative of significant heterozygote deficiencies (Table 2). F estimates were significantly different from zero for all loci except Bs 6C3, and θ did not depart from zero for all loci except Bs 3D5. Putative null allele frequency estimates (Table 2) ranged from 0.240 for Bs 3C8 and 0.000 for Bs 6C3, and were very similar with the 2 calculation methods used. The greatest heterozygote deficiencies corresponded to the putative null allele frequencies, but were not correlated with increasing gene diversities.

To avoid bias in their estimation, allele frequencies were checked to see whether they differed between adults and juveniles within samples. If individuals inside a mussel were to be related, some alleles may be more frequent in juveniles than in adults. In our case, frequencies were not significantly different between juveniles and adults ($-0.008 \leq \theta \leq 0.004$). Juveniles, males and females were thus grouped together for the tests of differentiation between samples.

The overall multilocus θ estimate (0.003) was not significantly different from zero ($p > 0.05$ with 1000 permutations), indicating that there was no differentiation among individuals from the 3 quadrats.

Table 2. *Branchipolynoe seepensis*. Number of individuals in samples used for kinship analyses (N), observed number of alleles (N_a), frequency of the most frequent allele (f_a), expected heterozygosity (H_e), allelic richness (R_s) and fixation index (f), at the Tour Eiffel (TE98 and TE01) and Bairro Alto (BA01) sites during PICO98 and ATOS01 oceanographic cruises. Null allele frequencies estimated for each locus from the non-amplified homozygous individual method (A) and the CERVUS 2.0 method (B). Fixation indices (F and θ) for each locus were estimated according to Weir & Cockerham (1984). NS: not significantly different from zero; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

Parameter	Microsatellite locus					
	Bs 5H11b	Bs 3E4	Bs 3D5	Bs 6C3	Bs 6D7	Bs 3C8
TE98 (N = 54)						
N_a	6	55	11	20	5	5
f_a	0.401	0.086	0.727	0.140	0.673	0.629
H_e	0.667	0.969	0.460	0.920	0.487	0.544
R_s	6.000	46.616	8.999	17.945	3.982	5.000
f	0.301***	0.139***	0.050^{NS}	0.096***	0.169*	0.464***
TE01 (N = 78)						
N_a	5	63	13	24	4	5
f_a	0.505	0.097	0.564	0.138	0.670	0.680
H_e	0.604	0.965	0.645	0.927	0.488	0.499
R_s	4.679	48.049	11.126	20.729	3.992	4.692
f	0.352***	0.054***	0.181***	−0.019^{NS}	0.172**	0.384***
BA01 (N = 114)						
N_a	5	75	13	20	8	5
f_a	0.500	0.073	0.488	0.112	0.673	0.609
H_e	0.591	0.974	0.711	0.927	0.491	0.562
R_s	4.330	50.870	11.086	17.514	5.597	4.997
f	0.348***	0.019^{NS}	0.346***	−0.014^{NS}	0.117*	0.376***
All pop.						
A	0.160	0.066	0.197	0.066	0.208	0.208
B	0.190	0.017	0.151	0.000	0.081	0.235
F	0.314***	0.051***	0.284***	−0.001^{NS}	0.146***	0.409***
θ	−0.001^{NS}	0.000^{NS}	0.018**	0.000^{NS}	−0.005^{NS}	−0.003^{NS}

Kinship analysis

The probability of exclusion calculated over the 6 loci was 0.989, providing robust assignments of juveniles for our kinship study. Mean kinship coefficients (R), calculated at different spatial scales (within mussels, within sites and between sites) between females and juveniles, were low, indicating that most scale worms were not related (Fig. 2). These means were not statistically different at the intra-mussel versus intra-site spatial scales (p = 0.458), and at the intra- versus inter-site spatial scales (p = 0.064).

A few pairs of individuals, however, displayed significant kinship coefficients. Out of these related pairs of individuals, the likelihood-ratio test obtained from 1000 simulated pairs indicated that 'true' mother/offspring relationships (R_m = 1, R_p = 0) represented only 5.9% (1/17), 0% (0/34) and 1.5% (1/66) of the individual pairings in TE98, TE01 and BA01, respectively (Table 3). In nearly all cases, the female was not the mother of juveniles living in the same mussel. Moreover, no correlation was found between the female/juvenile kinship coefficient R and the juvenile's number of segments (p = 0.582, Fig. 3). However, 2 juveniles appeared to be related to the female living inside the same mussel. Similarly, none of the males were related to a juvenile inside the same mussel (Table 3). The percentages of full siblings between juveniles represented 0% (0/15), 3.7% (2/54) and 1.6% (1/61) in TE98, TE01 and BA01, respectively (Table 3). These percentages were low (3 pairs) and close to those found for the mother/offspring relationships. Consequently, juveniles were generally not full siblings, and related individuals within mussels were very rare.

Because parents and offspring were apparently not found in the same mussel, we then used the CERVUS software to search for the most probable mother among females living in the same quadrat as the juveniles. Within the BA01 sample, 10 juveniles out of 61 had a multilocus genotype compatible with a female from the same quadrat with a confidence level of 80%. Within the TE01 sample, 7 juveniles out of the 44 displayed a similar degree of kinship with a female from the same quadrat (α = 80%). However, the number of juveniles correctly assigned to a female

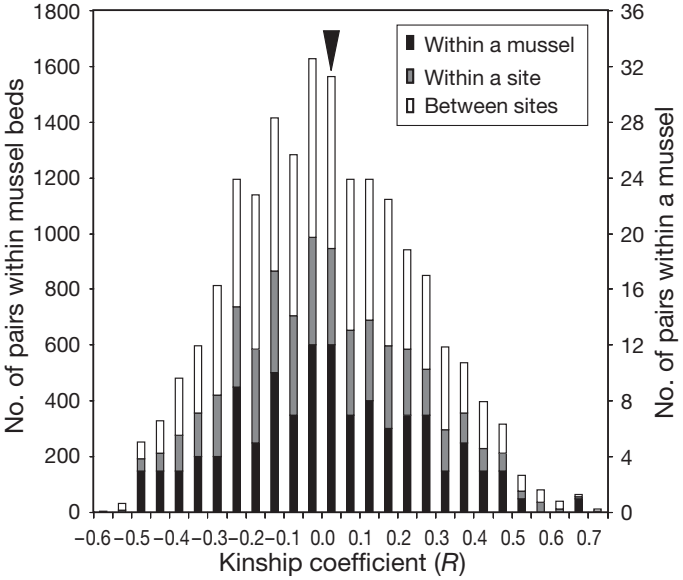

Fig. 2. *Branchipolynoe seepensis*. Superimposed kinship coefficients histograms showing the relatedness distributions at different levels of a spatial hierarchy: within a mussel (right axis), within a site and between sites (left axis). Average kinship coefficients (triangle) belong to the same size class and are 0.016, 0.001 and 0.009, respectively, for the 3 spatial scales

Table 3. *Branchipolynoe seepensis*. Proportion of individuals for which relative likelihood ratios favor the null hypothesis of relatedness (**bold**), relative likelihood ratios and Type II error (*italics*) at the exclusion threshold α = 0.01 for 1000 simulated pairs

Site	Mother/ descendant	Father/ descendant	Full-sibling juveniles
TE98	**1/17 (5.9%)**	**0/4 (0%)**	**0/15 (0%)**
	1.370	1.370	1.130
	0.081	*0.081*	*0.280*
TE01	**0/34 (0%)**	**0/4 (0%)**	**2/54 (3.7%)**
	0.932	0.932	1.060
	0.017	*0.017*	*0.280*
BA01	**1/66 (1.5%)**	**0/4 (0%)**	**1/61 (1.6%)**
	1.220	1.220	1.100
	0.035	*0.035*	*0.290*
Total	**2/117 (1.7%)**	**0/12 (0%)**	**3/130 (2.3%)**

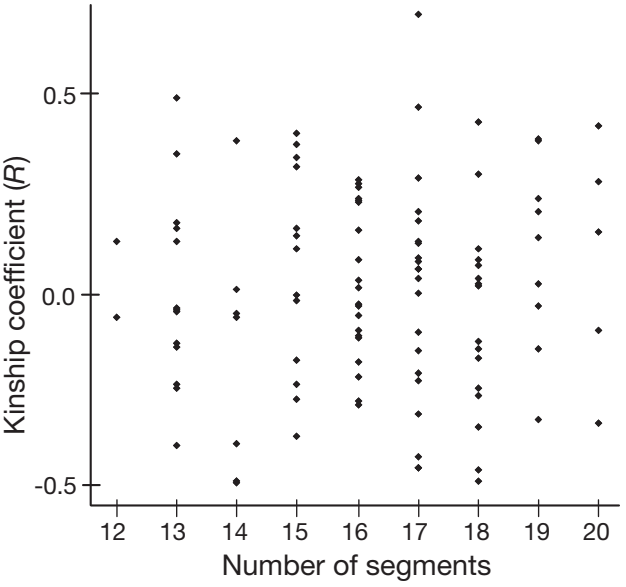

Fig. 3. *Branchipolynoe seepensis*. Relationship between the kinship coefficient (*R*) and the number of segments of juveniles showing an absence of correlation between the age of juveniles and the kinship structure

from the same quadrat was not very different from the number of juveniles correctly assigned to a female from the other quadrat. This number fell to only 2 (1 per quadrat) at a confidence level of 95% and did not correspond to the 'true' intra-mussel offspring/mother associations found in the kinship analyses. Among these 17 assigned juveniles, the 2 juveniles assigned at a 95% threshold and 8 juveniles assigned at a 80% threshold (also containing the juvenile from BA01 significantly related to a female within the same mussel using KINSHIP 1.2) were out of the 95% normalized LOD distribution and represent the most extreme Delta criterion values (Fig. 4). We then performed a search for the most probable mother for our set of juveniles among the whole female 2001 collection, taking into account that we only sampled a very small proportion of females from the entire population. This second analysis did not reveal any significant maternal relationship between juveniles and females within and between the 2 sites and could not exclude the hypothesis that the assigned juveniles may be the offspring of un-sampled females.

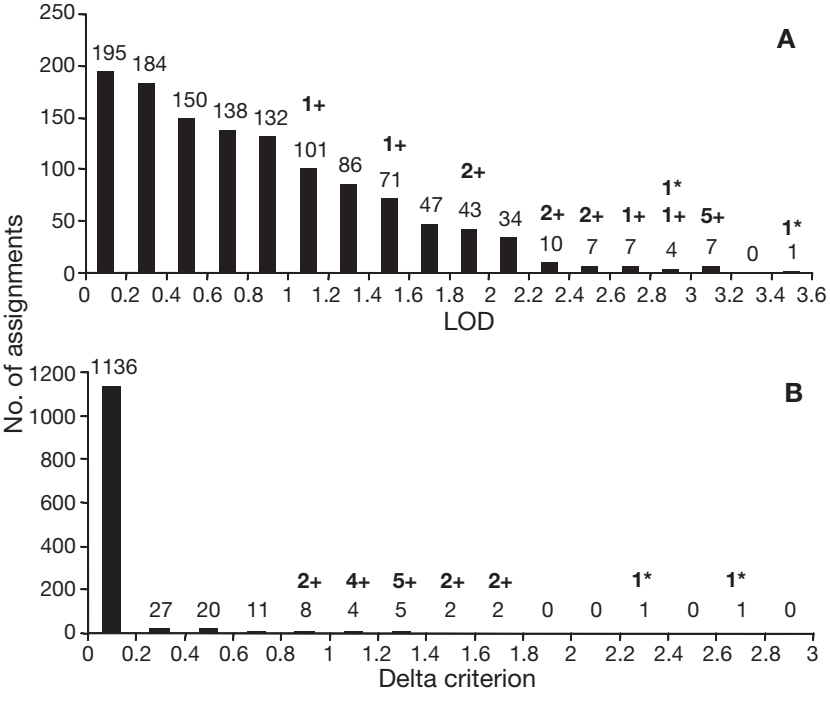

Fig. 4. *Branchipolynoe seepensis*. Distributions of the (A) LOD (likelihood of descent) and (B) Delta criterion values from all juvenile/female pairs obtained within each quadrat. The number of female/juvenile assignments performed for each class is presented above the class. **Bold**: significant assignments obtained from CERVUS (*95%; + 80% threshold). Individuals with a LOD >2.43 are out of the 95% distribution and are the most extreme Delta criterion values, which strengthens their probability to represent 'true' identity by descent. LOD: the best likelihood value that a juvenile is descended from a given female; Delta criterion: the difference between the 2 best LOD obtained from the set of females examined

DISCUSSION

Dispersal strategies of interacting species are influenced both by the proportion of suitable patches and the

level of patch connectivity in a fragmented landscape (Hiebeler 2007). The hydrothermal vent environment constitutes a very unusual landscape in which there are few suitable patches (very few fields), but in which connectivity is very high (numerous vent emissions within a field). Moreover, the rate of local extinction within a field is very high and may lead to bursts of colonization (Slatkin 1977). It is thus interesting to note that, in this kind of landscape, coevolving species are likely to coexist if they adopt a flexible dispersal strategy. This study represents the first attempt to perform parentage analyses in a deep-sea hydrothermal vent commensal species and to test whether brooding inside the host affects dispersal.

If *Branchipolynoe seepensis* females brood their juveniles inside the host, then their progeny must develop inside the host mussel's mantle cavity. The female and juveniles found inside the same host should then be related. However, our relatedness coefficients between juveniles and females were very low. In addition, although significant pairs of 'true' mother/offspring or full siblings inside the same mussel have been detected, these cases were very rare. In the light of our parentage analysis, we can reject the hypothesis of maternal caring and young brooding. The stage at which the offspring are released from the host, however, remains unclear. Tracking the stage at which a propagule is dispersing is not an easy task. Several indirect observations about developmental stage and relatedness or juvenile population structure could, however, help to shed some light on this issue. Juveniles displayed a number of segments ranging from 12 to 20, and this number was not correlated with the level of kinship. Consequently, offspring may be released prior to reaching the minimum developmental stage found in our worm collection. Offspring are thus most likely to be released at a very early stage, as fertilized eggs, larvae (if they exist, down to 10 segments for polychaetes), or very young juvenile stages. This is in agreement with previous studies on polynoid commensals, which, in most cases, have pelagic larvae (Britayev 1991) and tend to be aggressive and territorial towards conspecific individuals (Britayev 1991, Ruff 1991, Britayev & Zamishliak 1996). Dimock (1974) observed a similar pattern *in vivo* in the symbiotic polychaete *Arctonoe pulchra*, which forces congeners to leave the host limpet and move elsewhere in the vicinity. This may explain both genetic unrelatedness, small males and the observed worm distribution inside hosts.

Interestingly, our results also indicated a lack of genetic differentiation between the TE01 and BA01 quadrats. This is in agreement with the low number of juveniles assigned to females from the same quadrat (15.90% for TE01 and 16.39% for BA01), suggesting that a great proportion of juveniles are dispersed outside their quadrat of origin. The absence of apparent differentiation is not surprising given the relatively short distance between the 2 mussel beds (900 m) and because only 1 migrant generation^{-1} is enough to counteract genetic drift (Lewontin 1974). In addition, using the same microsatellite markers, Daguin & Jollivet (2005) suggested that *Branchipolynoe seepensis* are genetically differentiated over large portions of the Mid-Atlantic Ridge. *B. seepensis* may therefore not be able to disperse very far.

Although gene flow does not seem restricted to a given mussel bed but to a rift portion, leading to a certain flexibility in the dispersal distance, both the occurrence of heterozygote deficiencies at all loci and the presence of juveniles significantly related to a nearby female requires explanation. Two alternative hypotheses will be discussed: (1) the very few significant relatedness assignments represent a true mother/offspring kinship, and thus at least a small proportion of juveniles settle near their host of origin (nearby colonization), or (2) the very few significant relatedness assignments are only the result of chance or from a lack of power of the microsatellite markers, and thus *Branchipolynoe seepensis* is not likely to re-enter its host of origin (larval export).

(1) Significant offspring/mother pairs represent a 'true' kinship. On the one hand, based on external positions of nearly all the significant pairs in the LOD and Delta criterion value distributions, one can accept the hypothesis that at least a few juveniles are truly related to a nearby female. The 2 significant offspring/mother pairs found inside the same host indeed fall well outside the 95% LOD distribution together with pairs of individuals sampled in close proximity (same quadrat). Finding 2 significantly related pairs inside a given host does not seem easily explainable from a re-colonization process. Generally speaking, positively buoyant embryos or larvae would be entrained by bottom currents and subsequently passively carried away from the mussel bed. Even if a small proportion of lecithotrophic larvae was trapped within the hydrothermal plume by convective heat fluxes, ca. 200 m above the sea floor (Kim et al. 1994, Kim & Mullineaux 1998, Mullineaux et al. 2005), the chance that larvae would re-enter their mother's mussel or quadrat is close to zero, given the millions of mussels in the several hundred square meters of the Lucky Strike vent field. This may be a strong indication that a small proportion of juveniles stays close to their parents. Although we cannot rule out the hypotheses of null alleles or Wahlund effect, heterozygote deficiencies could also be due to local inbreeding, which would lead to such a within-population structure and also reinforce the nearby settlement hypothesis. As a consequence, offspring may be dispersed over variable

distances depending on such factors as the prevalence of worms in mussels (intra-specific competition) or nutritional limitation (cessation of vent activity). Such flexibility in dispersal distance depends on the distribution of suitable habitats for settlement (Gaines & Roughgarden 1985) and could result in a small mean dispersal distance (e.g. within a single mussel bed) with a long tail of much greater distances (e.g. a whole vent sector), the shape of the distribution being strongly dependent on the selective pressures associated with habitat fragmentation.

(2) Significant kinship coefficients are due to chance. On the other hand, despite a few significant kinship coefficients between individuals from the same host, the CERVUS software failed to find significant pairs under the hypothesis that genotyped individuals represent a very small proportion of the sampled worms in the whole population. Although the probability of exclusion was comparable to those in previous kinship studies (e.g. Avise et al. 2002), Marshall et al. (1998) demonstrated that some unrelated individuals may share at least 1 identical allele locus^{-1} just by chance or homoplasy. In the present study, the number of significant pairs was very low, so the hypothesis that larvae settle near the maternal host was only weakly supported. The high heterozygote deficiencies observed could also be attributable to null alleles, which is not an uncommon phenomenon when using highly polymorphic microsatellite markers (e.g. Taberlet et al. 1996). In our case, the frequencies of null alleles calculated using 2 different methods were almost identical. Consequently, heterozygote deficiencies may be, at least in part, explained by the non-amplification of some alleles. Consequently, if none of the juveniles are related to a nearby female, then the most likely explanation of the lack of differentiation between quadrats is to consider that *Branchipolynoe* sp. is able to disperse sufficiently far away from its host to prevent any re-colonization stage at the scale of the Lucky Strike vent field. Such a strategy contrasts with previous findings about the reproductive biology of this worm (large yolky eggs, pairing, internal fertilization of the oocytes), which usually typifies direct developers in polychaetes (see Jollivet et al. 2000). However, as stated by Young (1994), large yolky eggs may be greatly advantageous for long-distance dispersers that develop slowly in cold oligotrophic zones. From these results, most juveniles were not the offspring of females found within the same mussel, but at least a few individuals were related. The most probable explanation is, therefore, that *B. seepensis* must display a nearby dispersal strategy in which eggs/larvae/juveniles disperse into their immediate vicinity (on a square meter scale), with some kind of dispersal flexibility to colonize new empty patches of host mussels.

Acknowledgements. We are particularly indebted to the captains, chief scientists Daniel Desbruyères (PICO98) and Pierre-Marie Sarradin (ATOS01) and 'Nautile' and 'Victor6000' crews of the PICO98 and ATOS01 oceanographic diving cruises. We are also grateful to Fabienne Chausson, who sampled material for us during PICO98. We thank Carolyn Engel-Gautier, Denis Roze, Myriam Valero and anonymous referees for valuable comments and editorial suggestions on the manuscript. This work was supported by the French programme DORSALES and both the European AMORES (coordinator Daniel Desbruyères: MAST III-CT95-0040) project of the Marine Science and Technology programme and the EU-funded VENTOX project (coordinator David R. Dixon: EVK3 CT-1999-00003).

LITERATURE CITED

Avise JC, Fiumera AC, Jones AG, Walker D, DeWoody JA (2002) Genetic mating systems and reproductive natural histories of fishes: lesson for ecology and evolution. Annu Rev Genet 36:19–45

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) Genetix 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, Adaptations, UMR 5000, Université de Montpellier 2. Available at: www.genetix.univ-montp2.fr/genetix/genetix.htm

Britayev TA (1991) Life cycle of symbiotic scaleworm *Artonoe vittata* (Polychaete: Polynoidae). Ophelia 5:305–312

Britayev TA, Zamishliak EA (1996) Association of the commensal scaleworm *Gastrolepidia clavigera* (Polychaeta: Polynoidae) with holothurians near the coast of South Vietnam. Ophelia 45:175–190

Chevaldonné P, Jollivet D, Feldman RA, Desbruyères D, Lutz RA, Vrijenhoek RC (1998) Commensal scale-worms of the genus *Branchipolynoe* (Polychaeta: Polynoidae) at deep-sea hydrothermal vents and cold seeps. Cah Biol Mar 39:347–350

Daguin C, Jollivet D (2005) Development and cross-amplification of nine polymorphic microsatellite markers in the deep-sea hydrothermal vent polychaete *Branchipolynoe seepensis.* Mol Ecol Notes 5:780–783

Desbruyères D, Almeida A, Biscoito M, Comtet T and others (2000) Distribution of hydrothermal vent communities along the northern Mid-Atlantic Ridge. Dispersal vs. environmental control: a review. Hydrobiologia 440:201–216

Dimock RV Jr (1974) Intraspecific aggression and the distribution of a symbiotic polychaete on its host. In: Vernberg WB (ed) Symbiosis in the sea. South Carolina University Press, Columbia, p 29–44

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. Theor Appl Genet 92:832–839

Fauvel P (1916) Annélides Polychètes des Iles Falkland recueillies par M. Rupert Vallentin Esq. (1902-1910). Arch Zool Exp Gén 5:417–482

Gaines S, Roughgarden J (1985) Larval settlement rate: a leading determinant of structure in an ecological community of the marine intertidal zone. Proc Natl Acad Sci USA 82:3707–3711

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3). Update from Goudet, 1995. FSTAT (Version 1.2): a computer program to calculate *F*-statistics. J Hered 86:485–486. Available at: www2.unil.ch/popgen/softwares/fstat.htm

Hiebeler DE (2007) Competing populations on fragmented landscapes with spatially structured heterogeneities: improved landscape generation and mixed dispersal strategies. J Math Biol 54:337–356

Hurtado LA, Lutz RA, Vrijenhoek RC (2004) Distinct patterns of genetic differentiation among annelids of eastern Pacific hydrothermal vents. Mol Ecol 13:2603–2615

Jollivet D (1996) Genetic diversity and biodiversity at deep-sea hydrothermal vents: an overview. Biodivers Conserv 5:1619–1653

Jollivet D, Comtet T, Chevaldonné P, Hourdez S, Desbruyères D, Dixon DR (1998) Unexpected relationship between dispersal strategies and speciation within the association *Bathymodiolus* (Bivalvia)—*Branchipolynoe* (Polychaeta) inferred from the rDNA neutral ITS2 marker. Cah Biol Mar 39:359–362

Jollivet D, Empis A, Baker MC, Hourdez S and others (2000) Reproductive biology, sexual dimorphism, and population structure of the deep sea hydrothermal vent scale-worm, *Branchipolynoe seepensis* (Polychaete: Polynoidae). J Mar Biol Assoc UK 80:55–68

Kim SL, Mullineaux LS (1998) Distribution and near-bottom transport of larvae and other plankton at hydrothermal vents. Deep-Sea Res II 45:423–440

Kim SL, Mullineaux LS, Helfrich KR (1994) Larval dispersal via entrainment into hydrothermal vent plumes. J Geophys Res 99:12655–12665

Lewontin RC (1974) The genetic basis of evolutionary change. Columbia University Press, New York

MacGinitie GE, MacGinitie N (1949) Natural history of marine animals. McGraw-Hill Book Company, New York

Marshall TC, Slate J, Kruuk L, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations, CERVUS 2.0. Mol Ecol 7:639–655. Available at: http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.html

Mullineaux LS, Mills SW, Sweetman AK, Beaudreau AH, Metaxas A, Hunt HL (2005) Vertical, lateral and temporal structure in larval distributions at hydrothermal vents. Mar Ecol Prog Ser 293:1–16

Nichols RA, Hewitt GM (1994) The genetic consequences of long-distance dispersal during colonization. Heredity 72: 312–317

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275

Ruff RE (1991) A new species of *Bathynoe* (Polychaeta: Polynoidae) from the Northeast Pacific Ocean commensal with two species of deep-water asteroids. Ophelia 5:219–230

Schroeder PC, Hermans CO (1975) Annelida: Polychaeta. In: Giese AC, Pearse JS (eds) Reproduction of marine invertebrates. Academic Press, New York, p 1–214

Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinctions. Theor Popul Biol 12: 253–262

Sokal RR, Rohlf FJ (1981) Biometry. The principles and practice of statistics in biological research. Freeman, New York

Taberlet P, Friffin S, Goossens B, Questiau S, Manceau V (1996) Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Res 24:3189–3194

Thorson G (1936) The larval development, growth and metabolism of Arctic marine invertebrates, compared with those of other seas. Medd Gronl 100:1–155

Tyler PA, Young CM (1999) Reproduction and dispersal at vents and cold seeps. J Mar Biol Assoc UK 79:193–208

Walsh PS, Petzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370

Young CM (1994) A tale of two dogmas: the early history of deep-sea reproductive biology. In: Young CM, Eckelberger KJ (eds) Reproduction, larval biology and recruitment of the deep-sea benthos. Columbia University Press, New York, p 1–25

Editorial responsibility: Judith Grassle, New Brunswick, New Jersey, USA

Submitted: November 23, 2007; Accepted: May 14, 2008
Proofs received from author(s): July 4, 2008