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Colonization patterns of Nematoda on decomposing algae in the estuarine environment: Community assembly and genetic structure of the dominant species *Pellioditis marina*

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Abstract

We performed a field experiment in the Westerschelde Estuary (The Netherlands) to characterize the colonization dynamics of nematodes in relation to the proximity of a source population and to local environmental conditions. The effects of colonization on the population genetic structure of the dominant species, *Pellioditis marina*, were simultaneously investigated. Two contrasting sites, each containing four patches with defaunated algae, were sampled seven times during 1 month. Site A was situated amidst *Fucus* stands, which permanently harbor *P. marina*, while site B was approximately 100 m from any source population and experienced more stressful environmental conditions. We hypothesized that (1) colonization in site A would proceed faster than in site B and that (2) founder events and genetic bottlenecks would affect population genetic structure and differentiation at site B more than at site A. We screened 992 individuals for variation in 426 base pairs of the cytochrome oxidase c subunit 1 gene with the single-strand conformation polymorphism method. The algal deposits at site A were indeed more rapidly colonized and reached fivefold higher densities of nematodes than those in site B. Haplotype composition in site A was very similar to that of the source population, while rare haplotypes were abundant and genetic diversity was lower in site B. We conclude that founder effects and genetic bottlenecks structured the populations in site B. The genetic differences between patches in each site further indicate that effective migration in *P. marina* is low and that priority effects influence the genetic structure of *P. marina* populations.

A variety of marine meiobenthic organisms, such as gastrotrichs and nematodes, have a seemingly cosmopolitan distribution despite a lack of spawning, pelagic larvae and/or other dispersal stages (Coomans 2000). Nevertheless, passive dispersal of meiofauna can be substantial. Nematodes, for instance, are frequently observed in the water column, especially in areas with high tidal activity (Ullberg and Olafsson 2003), and appear to be among the most abundant rafting organisms (Thiel and Gutow 2005). Moreover, several meiofaunal taxa have relatively low abundances in the sediment but abound in epiphytic habitats or on macroalgal and other wrack deposits. In these microhabitats, these taxa are more prone to resuspension and, consequently, to passive transport (Bouwman et al. 1984; Warwick 1987; Alkemade et al. 1994). Based on these observations, it can be hypothesized that species living on wrack deposits have high dispersal abilities and should consequently show little isolation among populations.

Within the Nematoda, typical representatives on stranded algae belong to the families Monhysteridae and Rhabditidae (Warwick 1987). One of the first colonizers of new macroalgal deposits is the rhabditid nematode *Pellioditis marina*. This sexually reproducing, oviparous to ovoviviparous species has a high reproductive output, a short generation time (down to 3 d under optimal conditions; Moens and Vincx 2000), and can raft on floating algae (Derycke unpubl. data), indicating effective dispersal. Nevertheless, a pronounced population genetic structure has been found in *P. marina* on a local scale (40 km; Derycke et al. 2005). Such lack of correlation between dispersal capacity and genetic differentiation has also been observed in small crustaceans in lenthic habitats and has been explained by limited effective long-distance

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dispersal (Brendonck et al. 2000), persistent founder effects (Boileau et al. 1992), and/or local adaptation (De Meester et al. 2002). We have also observed temporal differences in haplotype frequencies, indicating that population genetic structure in *P. marina* is caused by metapopulation dynamics (Derycke et al. 2006). Indeed, macroalgal wrack is irregularly deposited near the high water line and therefore provides a patchy, fragmented habitat. These deposits break down as a result of dehydration, aging, and fragmentation, the rates of which are highly variable and are influenced by site- and time-specific environmental conditions (Colombini and Chelazzi 2003). For example, an in situ litterbag experiment at the sites and environmental conditions of the present study showed that *Fucus* thalli lost approximately 50% of their carbon and 60% of their nitrogen within 30 d; the associated fauna was dominated by nematodes even after 85 d in situ (Moens unpubl. data). Furthermore, interspecific interactions and priority effects may influence the nematode community composition once the algae are stranded. Finally, the colonization of stranded deposits is also largely influenced by stochastic factors (Ekschmitt and Griffiths 1998).

Although there is a solid theoretical framework explaining the ecological and evolutionary consequences of local colonization and extinction in metapopulations (e.g., Harrison and Hastings 1996; Hanski and Gilpin 1997), including the effect on genetic differentiation among and within demes within a metapopulation (Slatkin 1977; Wade and McCauley 1988; Whitlock and McCauley 1990), empirical studies in natural environments are still quite scarce (Whitlock 1992; Saccheri et al. 1998; Aars et al. 2006), especially in the marine environment. Theory indicates that the amount of genetic differentiation among demes within a metapopulation is largely dependent upon the mode of founding of new demes within that metapopulation (Wade and McCauley 1988): large propagules with individuals from many demes will cause little genetic differentiation within the metapopulation. In contrast, a small number of colonizers originating from one or a few demes will enhance genetic differentiation within the metapopulation as a result of genetic bottlenecks. Since ephemeral habitats are short-lived, allele frequencies within the demes of the metapopulation are not expected to be homogenized by gene flow (Whitlock and McCauley 1990), especially if priority effects are involved.

The main focus of the present article is to investigate the colonization dynamics of *P. marina* on empty algal patches and to characterize the genetic structure of the founder populations at two sites in the Paulina salt marsh (Westerschelde Estuary, The Netherlands). Patches of defaunated algae were incubated amidst (site A) and at least 100 m away (site B) from the nearest source population of nematodes. The source population is characterized by the permanent availability of *Fucus* stands, which can act as a 'mainland' population. Distance from the source population was not, however, the only major difference between both experimental sites: environmental conditions at site B were more variable and stressful because of the higher elevation above sea level and, consequently, because of the longer exposure time during low tide. We expected that (1) patches in site A would be colonized more quickly than patches in site B as a result of their closer proximity to a source population; (2) genetic variation within patches in site B would be smaller than genetic variation within patches in site A as a result of genetic bottlenecks due to the larger distance to the source population and the more stressful conditions at site B; (3) on average, genetic differentiation between patches in site B and the source population would be higher than between patches in site A and the source population as a result of founder events; and (4) genetic differences among patches in site B would be larger than among patches in site A because priority effects would prevent additional colonization after the arrival of the first colonizers.

Material and methods

Experimental setup and sampling—Colonization of defaunated algal deposits was investigated at two different sites (A and B) along the edges of the Paulina salt marsh, which is in the polyhaline reach of the Westerschelde Estuary (The Netherlands). Site A was in close proximity (<5 m) to living *Fucus* thalli, in which *P. marina* occurs year-round, while site B lacked *Fucus* stands or other suitable substrata for *P. marina*. The *Fucus* stand nearest site B was ca. 100 m downstream and was less elevated than site A. Because of their relative position, passive transport of algae from the nearby stand to site B was mainly dependent on wind direction and was probably very limited during our experiment. Other 'permanent' *P. marina* populations were ca. 1 km or more from site B. *Fucus* and other organic material, however, are episodically washed ashore, the amounts and frequency largely depending on wind force and direction. Therefore, passive dispersal from such sources could also occur in site B.

Fucus thalli, collected from another location in the Westerschelde Estuary (Kruispolderhaven), were defaunated by submerging the thalli in tap water and incubating them overnight at 35°C. The combination of freshwater and high temperature is lethal to marine and brackish-water nematodes within minutes to hours (Moens unpubl. data). After treatment, all thalli were additionally rinsed with tap water to remove dead meiofauna.

Four algal deposits (1 m × 0.6 m, containing algae up to a volume of 10 liters) were fixed at each site with agricultural fencing wire (mesh diameter: 1 mm) during low tide on 01 October 2004. The defaunated algae were distributed equally among and within these fencing wires. Distance between algal deposits within a site averaged 2 m, while sites A and B were approximately 1 km apart. In this way, the four algal deposits within a site were considered replicates (A1–A4 and B1–B4).

Samples were taken after 2 d and 5 d in situ and then subsequently every fifth day for 1 month (30 d). Site B was also sampled after 35 d because colonization started later than in site A (*see Results*). This period was long enough for *P. marina* to produce up to seven generations, as its generation time is <5 d under the prevailing climatic conditions of our experiment (Moens and Vincx 2000). On each sampling occasion, *Fucus* fragments were haphazardly

collected from different parts and layers within each patch and pooled until a volume of 100 mL (ca. 30 g wet algae) was obtained.

Processing of Fucus *thalli*—On each sampling occasion, a small amount of *Fucus* (ca. 4 g wet weight) was rinsed with tap water, dried, homogenized, and weighed for carbon and nitrogen analysis on a Carlo-Erba elemental analyzer (type NA-1500) to document the decomposition process of the algae. The remaining algal fragments (ca. 26 g wet weight) were incubated on agar slants (salinity of 25, 0.7%, and 10 : 1 Bacto : Nutrient agar; Moens and Vincx 1998). Nematodes were subsequently allowed to colonize the agar for 2 d, which is less than the shortest generation time of *P. marina* under optimal conditions (Vranken and Heip 1983; Moens and Vincx 2000). This procedure greatly facilitates identification and isolation of *P. marina* under a dissecting microscope. For the first two sampling periods, all specimens observed on the agar were isolated (day 2: average $n = 2.7$ for site A and $n = 0$ for site B, and day 5: average $n = 17$ for site A and $n = 4.6$ for site B). From day 10 onward, approximately 30 mature *P. marina* were handpicked from each sample, transferred to sterile water, and preserved in acetone for molecular analyses.

Nematode community—From day 10 onward, nematodes became abundant on the algae in the field, and additional *Fucus* thalli (ca. 5 g wet weight) were collected from each patch to analyze the nematode community composition. These fragments were thoroughly rinsed with tap water over a 38-μm sieve, and nematodes were preserved in 4% buffered formaldehyde, stained with Rose Bengal, and then counted under a dissecting microscope. *P. marina* was counted in three patches from each site for each date. Other nematode genera were identified by randomly handpicking 100 specimens from the preserved samples and mounting them on Cobb slides according to the method of Vincx (1996). This community analysis was performed on three patches from each site on days 10, 20, and 30 of the experiment. When less than 100 specimens were present in a sample, all nematodes were handpicked (mainly site B). The *Fucus* fragments from which nematodes were counted and identified were subsequently dried at 60°C and weighed until a stable dry weight was obtained (usually in >4 d). This dry weight was used to standardize nematode counts among samples.

Molecular analysis of P. marina—To investigate the dynamics of the genetic composition of *P. marina* populations at both sites, genetic variation was assessed in approximately 30 *P. marina* individuals (except on days 2 and 5; *see above*) from each of three patches per site and date. Genetic diversity in a 426–base pair–long fragment of the mitochondrial deoxyribonucleic acid cytochrome oxidase c subunit 1 (COI) gene was screened using the single-strand conformation polymorphism (SSCP) method. Because of the high variability among patches in site B, we decided to also analyze a fourth replicate from site B. For a detailed description of the DNA extraction protocol, polymerase chain reaction (PCR) amplification, SSCP conditions, and sequencing strategy, see Derycke et al. (2005). PCR products were amplified with primers JB3 and JB5. Different SSCP profiles were sequenced with the aforementioned primers, and the most abundant haplotypes in each site were sequenced several times to confirm the SSCP band mobility and sequence variability. Sequences can be found in GenBank under accession numbers AJ867447-AJ867457 and AJ867477-AJ867478 (Derycke et al. 2005).

Data analysis—*Fucus* quality and nematode abundance: A univariate repeated-measures analysis of variance (ANOVA) was performed to evaluate whether carbon and nitrogen content of the *Fucus* thalli, absolute nematode abundances, and the relative abundance (%) of *P. marina* changed over time between sites A and B. When the data did not fulfill the assumptions for parametric tests (normality, homogeneity of variances), they were transformed (absolute nematode abundances per gram of dry weight were log transformed, carbon content was arcsine transformed). All univariate tests were performed with the Statistica 6.0 program (2001; Statsoft).

Nematode community: Differences in taxon composition between sites A and B were analyzed with the software package Primer v. 5.2.9 (Clarke and Gorley 2001). Absolute abundance data were double root-transformed to down-weight the importance of the highly abundant genera *Pellioditis* and *Geomonhystera*. Similarity among all pairs of patches was assessed by calculating the Bray–Curtis coefficient and was visualized in a nonmetric multidimensional scaling plot. Differences among sites A and B were analyzed by a two-way crossed ANOSIM test, allowing for the fact that there were changes over time. Significance levels were assessed by a permutation procedure (Clarke and Gorley 2001). Similarity percentage (SIMPER) analysis was performed to identify the taxa that account for similarity within, and dissimilarity among, sites A and B.

Genetic patterns and diversity of *P. marina*: Haplotype diversity (*h*; Nei 1987) was calculated for each patch and compared among sites and times by means of a repeated-measures ANOVA in Statistica 6.0. The data were arcsine transformed, but variances were not homogeneous for one time variable. Nevertheless, we used the repeated-measures ANOVA because there is no nonparametric alternative for the repeated-measures ANOVA and because the nonhomogeneity of variances was only borderline significant ($p = 0.032$).

Genetic differentiation within sites A and B was assessed by means of a hierarchical AMOVA (analysis of molecular variance) for each site, as implemented in the Arlequin v. 2.0 software package (Schneider et al. 2000). Absolute haplotype frequencies from each date were grouped according to patch. This resulted in three and four groups for sites A and B, respectively.

Subsequently, we used the absolute haplotype frequencies of *P. marina* in site A obtained from a previous study covering four consecutive seasons (Derycke et al. 2006) to

characterize the source population. Genetic differences between this source population and sites A and B were investigated by calculating pairwise Fst values in Arlequin v. 2.0 using the haplotype frequencies of the last sampling day (day 30 for site A, day 35 for site B) in each patch. All Fst values calculated between the source population and patches from site A, and between the source population and patches from site B, were subsequently grouped. We then performed a nonparametric Mann–Whitney *U*-test to address differences between groups. We also calculated Fst values between each patch within sites by summing all haplotype frequencies over time in each patch. Because the data per patch are not independent (cf. repeated sampling from the same unit), we used a permutation test ($n = 1{,}000$) in Fstat v. 2.9.3. (Goudet 2001) to assess whether Fst values among patches differed significantly between sites.

Colonization dynamics of haplotypes: The colonization dynamics of haplotypes over time were investigated in two ways. First, we calculated Bray–Curtis similarity coefficients of absolute haplotype frequencies between the onset of the experiment (days 2, 5, and 10) and day 30 for site A and between days 5, 10, 15, and 20 and day 35 for site B for each patch separately. Frequencies were double root-transformed to down-weight the importance of the highly abundant haplotypes (A, D, and F) and were standardized for each sampling date within each patch. Second, we investigated whether the relative abundances of haplotypes at the start (i.e., day 2 for A1 and A3, day 5 for A2 and B1, day 10 for B4, day 15 for B3, and day 20 for B2) were positively correlated with their relative abundances at day 30 (or day 35). The nonparametric Spearman rank correlation analysis (Statistica 6.0) was performed for each patch within each site. A positive correlation indicates that the first haplotypes arriving at each site constitute a major part of the population build-up by the end of the experiment.

Results

Fucus *quality*—Carbon and nitrogen content of *Fucus* thalli varied between 29.9% and 37.1% and between 1.44% and 1.74%, respectively. After an initial decrease, carbon and nitrogen remained fairly constant at both sites (carbon: $F_{7,42} = 2.28$, $p = 0.045$; nitrogen: $F_{7,42} = 0.48$, $p = 0.84$). The borderline significance of the carbon dynamics reflected fluctuations at site B during days 10 to 20 rather than differences in carbon dynamics among sites (data not shown). Nitrogen content was significantly higher at site A (site effect: $F_{1,6} = 7.84$, $p = 0.03$), indicating that *Fucus* thalli were less decomposed than at site B because nitrogen content correlates well with the decomposition of organic material (De Mesel 2004). This was, however, at odds with our observations in the field: *Fucus* thalli incubated in site A changed color more rapidly, were clearly covered by microbial films, and were more fragmented; the latter result was likely caused by the activity of crabs. The differences in nitrogen content and decomposition dynamics between both sites are most likely caused by abiotic factors such as humidity (Newell et al. 1985) and tidal differences (Halupa and Howes 1995), since site B was located higher above sea level and was consequently inundated for shorter periods than site A.

Nematode abundances—Nematode counts were standardized per gram of dry weight of *Fucus*. *Fucus* incubated in the proximity of algae (site A) yielded a fivefold higher nematode density than *Fucus* incubated at site B (site effect: $F_{1,4} = 12.14$, $p = 0.025$; Fig. 1). Nematode abundances increased during the first 20–25 d of the experiment (time effect: $F_{4,16} = 17.42$, $p < 0.001$) and subsequently stabilized (Fig. 1). This did not, however, result in a significant time × site interaction ($F_{4,16} = 0.48$, $p = 0.74$), indicating that the pattern of increase was similar in both sites.

Nematode community—In total, 25 genera were found on the incubated algae during the course of the experiment, 16 of which were present after 10 d of incubation. Of these 16 genera, 15 were present at site A and 10 occurred at site B (Table 1). The average number of genera observed during the course of the experiment was similar in both sites (14 in site A and 13 in site B). The developing nematode communities were dominated by the genera *Pellioditis* and *Geomonhystera* (Fig. 2a; Table 1). This dominance was already apparent at site A from day 10 onward. The abundance of *Pellioditis* increased over time at both sites to more than 60% of total nematode abundance after 30 d. The other colonizing genera are listed in Table 1. *Monhystera* and *Daptonema* were frequently found on the *Fucus* thalli in both sites, whereas *Syringolaimus* was only encountered in site B. Community composition between both sites was not significantly different (two-way crossed ANOSIM, $R = 0.16$, $p = 0.09$). In contrast, time effects were significant ($R = 0.31$, $p = 0.02$; Fig. 3). Pairwise comparisons showed that after 10 d samples were clearly separated from those after 30 d of incubation ($R = 0.49$, $p = 0.04$), while samples taken after 20 d were not significantly different from those taken after 30 d ($R = 0.20$, $p = 0.2$) and after 10 d ($R = 0.32$, $p = 0.08$). This implies that the nematode community converged over time across sites. SIMPER analysis indicated that the genera

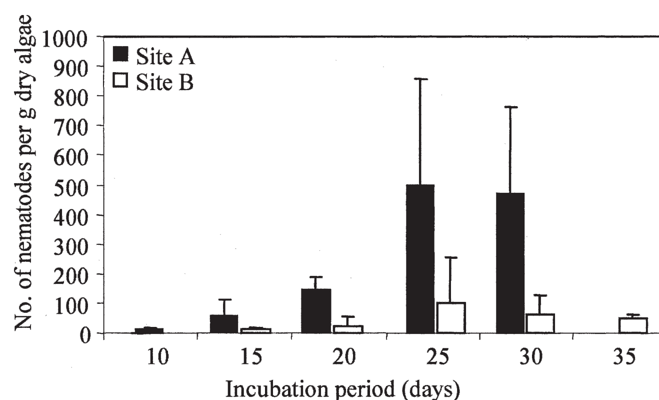

Fig. 1. Total number of nematodes per gram of dried *Fucus* thalli from day 10 onward. Columns are averages of three patches, and error flags represent the standard deviation across three patches.

Table 1. Nematode genera occurring at sites A and B after 10, 20, and 30 d of incubation. Densities are calculated as the number of individuals per gram dry weight of *Fucus* and are averages of three replicates. Feeding group refers to the dominant feeding type in each genus, which include (1) bacterial feeders, (2) unicellular eukaryote feeders, (3) carnivores, and (4) plant feeders. The genera that contributed more than 50% to the differences among sites A and B are indicated in bold. Rhabditid nematodes other than *P. marina* were identified to the family level. SD indicates standard deviation.

		Site A		Site B		Site A		Site B		Site A		Site B	
		10 d incubation				20 d incubation				30 d incubation			
Feeding group	Genus	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
1	*Desmolaimus*	—	—	—	—	—	—	0.3	0.5	—	—	—	—
1	*Diplolaimella*	—	—	—	—	—	—	—	—	—	—	0.5	0.9
1	***Geomonhystera***	**5.6**	**1.3**	**0.1**	**0.2**	**43.0**	**18.8**	**0.8**	**0.1**	**88.6**	**21.0**	**9.8**	**6.5**
1	*Gnomoxyala*	—	—	—	—	0.7	1.2	—	—	—	—	—	—
1	***Monhystera***	**0.3**	**0.3**	**0.3**	**0.4**	**4.5**	**3.0**	**1.4**	**0.5**	**11.5**	**6.7**	**0.7**	**1.2**
1	*Panagrolaimus*	0.3	0.3	—	—	0.7	1.2	—	—	1.8	3.1	0.7	0.8
1	***Pellioditis***	**1.5**	**2.6**	**0.1**	**0.2**	**76.8**	**19.2**	**9.8**	**16.4**	**346.6**	**294.0**	**37.8**	**44.7**
1	*Rhabditidae*	—	—	—	—	—	—	—	—	—	—	0.6	1.0
Total bacterial feeders		7.7	4.5	0.5	0.9	125.7	43.4	12.3	17.5	448.5	324.8	50.0	55.1
2	*Chromadora*	0.2	0.3	0.1	0.2	—	—	0.9	1.5	—	—	2.9	4.1
2	*Chromadorina*	0.3	0.2	0.1	0.2	—	—	0.6	1.0	—	—	3.1	5.3
2	***Daptonema***	**0.9**	**0.3**	**—**	**—**	**4.7**	**0.7**	**2.1**	**1.9**	**10.5**	**11.8**	**—**	**—**
2	***Metachromadora***	**0.6**	**0.7**	**0.1**	**0.2**	**1.6**	**0.5**	**0.7**	**1.3**	**—**	**—**	**—**	**—**
2	*Microlaimus*	0.3	0.2	0.1	0.2	1.1	1.9	0.3	0.5	—	—	—	—
2	*Neochromadora*	0.5	0.5	—	—	1.0	1.0	—	—	0.9	1.6	0.5	0.9
2	*Paracanthonchus*	—	—	—	—	0.4	0.6	0.3	0.5	—	—	—	—
2	*Paracyatholaimus*	—	—	—	—	0.4	0.6	1.5	2.5	—	—	1.0	1.8
2	*Ptycholaimellus*	—	—	—	—	1.3	2.3	—	—	1.1	1.9	0.3	0.5
2	*Spilophorella*	0.5	0.5	—	—	—	—	—	—	—	—	—	—
2	*Theristus*	—	—	—	—	1.4	1.7	2.0	3.5	6.5	8.9	1.3	1.6
2	*Tripyloides*	0.1	0.2	—	—	1.1	1.8	1.5	2.5	0.9	1.6	—	0.0
Total unicellular eukaryote feeders		3.3	3.1	0.4	0.8	13.0	11.1	9.9	15.2	19.9	25.9	9.1	14.1
3	*Calyptronema*	0.2	0.3	0.1	0.2	1.6	2.9	0.6	1.0	—	—	—	—
3	*Eurystomina*	0.2	0.3	—	—	—	—	—	—	—	—	—	—
3	*Oncholaimus*	—	—	—	—	0.7	1.2	0.3	0.5	1.1	1.9	—	—
3	*Syringolaimus*	—	—	0.1	0.2	—	—	1.2	2.0	—	—	2.5	4.4
Total carnivores		0.3	0.5	0.2	0.3	2.3	4.0	2.0	3.5	1.1	1.9	2.5	4.4
4	*Hirschmanniella*	0.5	0.5	0.1	0.2	2.1	1.1	—	—	—	—	—	—

Pellioditis (21.69%), *Geomonhystera* (14.89%), *Monhystera* (8.94%), *Daptonema* (8.24%), and *Metachromadora* (5.42%) contributed most to the observed differences between day 10 and day 30.

When all genera were assigned to the feeding type classification of Moens et al. (2004), both sites were dominated by bacterial feeders. Because this is mainly a reflection of the high abundances of *P. marina* and

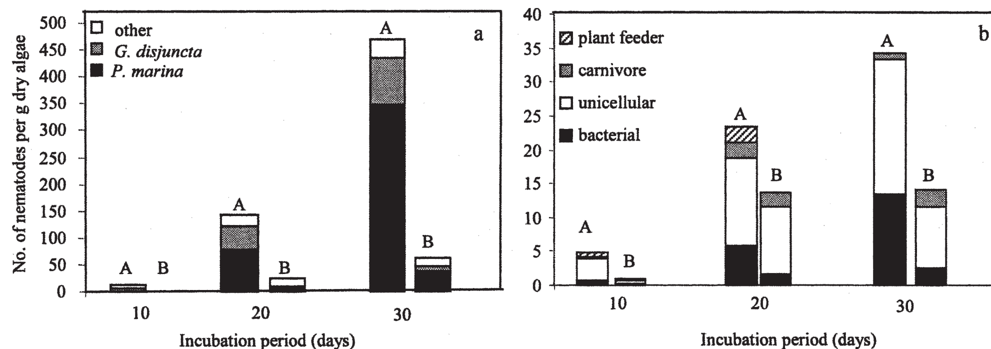

Fig. 2. (a) Absolute abundances of the dominant genera at sites A and B during the experiment. (b) Absolute abundances of the feeding types according to Moens et al. (2004), with feeding type referring to the dominant type within each genus. The genera *Pellioditis* and *Geomonhystera* were omitted from the data set.

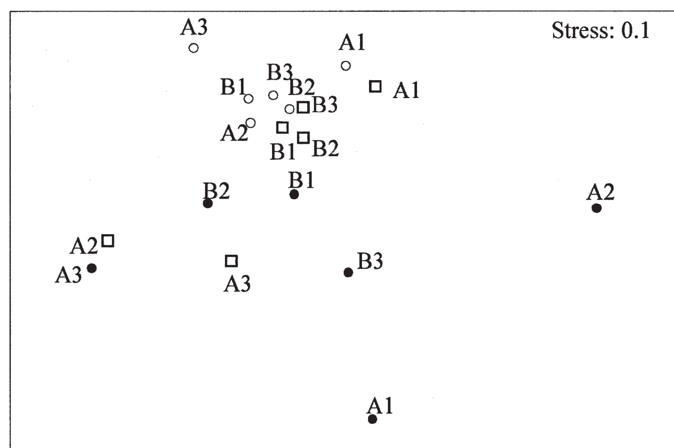

Fig. 3. Nonmetric multidimensional scaling plot of the generic composition of the patches at each sampling occasion. Closed circles are replicates after 10 d, open squares after 20 d, and open circles after 30 d of incubation. Sample names refer to sites (A or B) and patches (1, 2, and 3).

Geomonhystera disjuncta, these two species were omitted from the data set. This resulted in a dominance of unicellular eukaryote feeders in both sites (Fig. 2b).

Colonization of Fucus *by* P. marina—Although absolute nematode abundances were fivefold higher at site A than at site B, the percentage of *P. marina* in both sites was comparable and ranged between 25% and 70% (Fig. 4). This was confirmed by the repeated-measures ANOVA, which showed no significant differences between sites ($F_{1,4}$ = 0.63; p = 0.47). The relative abundance of *P. marina* gradually increased with time ($F_{4,16}$ = 7.92, p = 0.001), and this increase followed a similar pattern in both sites (site × time effect: $F_{4,16}$ = 1.5, p = 0.25).

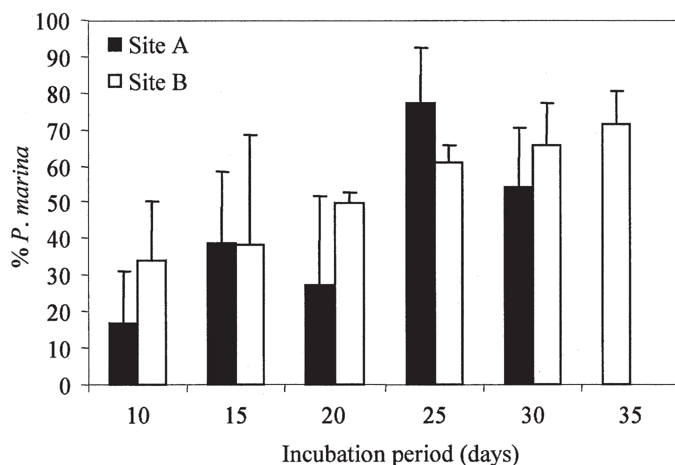

Fig. 4. *Pellioditis marina.* Relative abundance at site A and site B during the experiment. Each column is the average of three patches, and error flags represent the standard deviation across three patches.

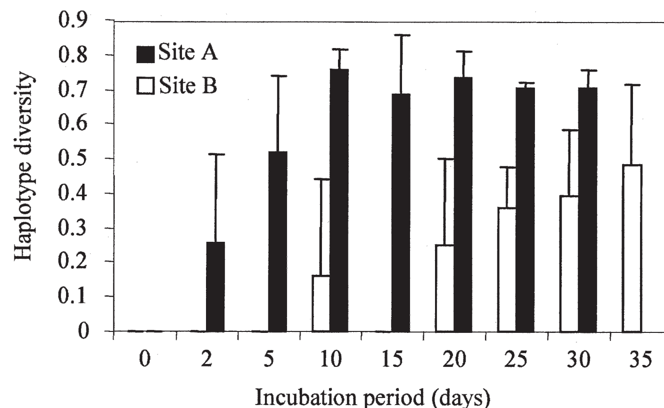

Fig. 5. *Pellioditis marina.* Haplotype diversity (h) at site A and site B during the experiment. Values are averages of three patches, and error flags represent the standard deviation across patches.

Genetic patterns and diversity of P. marina—Genetic variation was assessed from 478 *P. marina* specimens from site A and 514 specimens from site B. Figure 5 shows the patterns of haplotype diversity (h) for both sites. h was clearly higher in site A ($F_{1,4}$ = 176.76, p = 0.0002) and increased over time in both sites ($F_{6,24}$ = 5.49, p = 0.001). The pattern of this increase did not differ significantly between sites (site × time effect, $F_{6,24}$ = 1.47, p = 0.23).

In total, 11 haplotypes were found, 10 of which were present in site A and 8 of which were found in site B (Fig. 6). Divergences among these haplotypes ranged between 0.25% and 2.02% (one to eight substitutions). Differences in haplotype composition and in the type and number of dominant haplotypes were noticeable between both sites and between patches in a site (Fig. 6). Patches in site B typically had a lower number of haplotypes than those in site A (on average, four and seven haplotypes, respectively; Fig. 6). The haplotype composition was clearly different between both sites, with patches of site A closely resembling those of the presumed source population (Pa) (data not shown). One new haplotype was observed (G) in comparison to our earlier studies. In addition, haplotype W was not encountered previously in Paulina but has been observed elsewhere in the Westerschelde Estuary (Derycke et al. 2006).

AMOVA indicated that genetic differences were detectable between both sites, and genetic differentiation was 10-fold higher among patches in site B than among patches in site A. Fstat analysis indicated that this 10-fold difference in genetic differentiation between the two sites was significant (Fstat: p = 0.04). On the other hand, AMOVA indicated that genetic differentiation over time was small in both sites (Table 2). Fst values between site A and the source population were small (0.0–0.03), while Fst values between site B and the source population ranged between 0.02 and 0.39. However, this difference among the Fst values of both sites was not significant (U = 2.0, p = 0.15).

Colonization dynamics of haplotypes—Bray–Curtis similarity coefficients increased during the first 10 d (site A) and 20 d (site B) (data not shown). At days 10 (site A) and

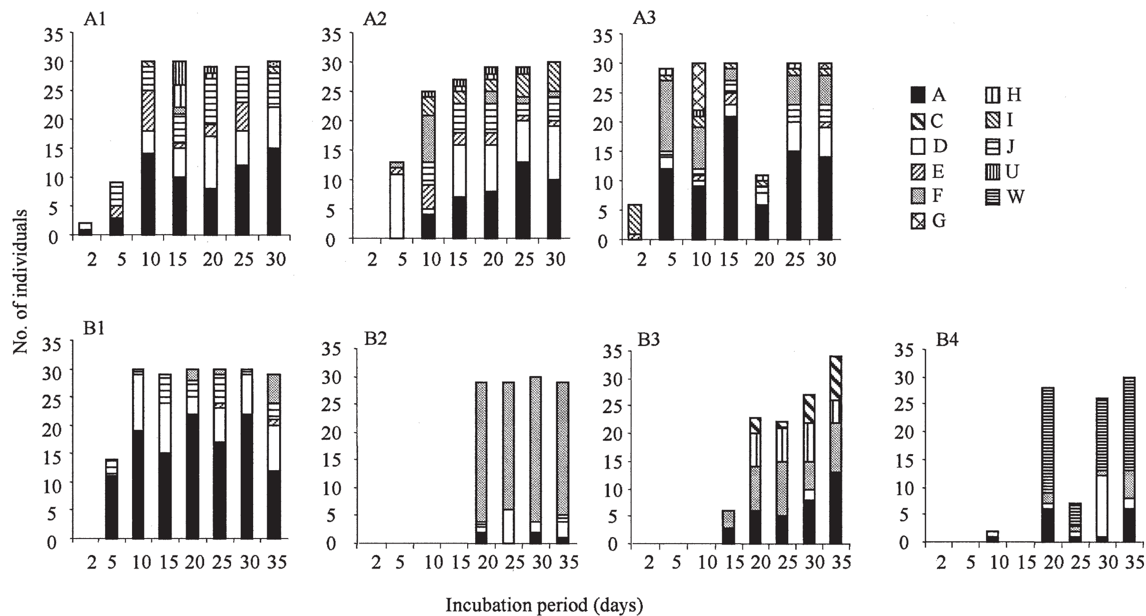

Fig. 6. *Pellioditis marina*. Haplotype composition of the developing *P. marina* populations for each patch separately. A1–A3 refer to the three patches in site A; B1–B4 refer to the four patches in site B. A–W are codes for haplotypes.

20 (site B), similarity was very high and ranged between 79.21 and 84.51 in site A and between 85.03 and 95.27 in site B. This indicates that new haplotypes were mainly added during the first 10 d for site A and during the first 20 d for site B (Fig. 6).

When we compared the relative abundances of the first haplotypes colonizing the patches with their abundances at the end of the experiment, a significant positive correlation was observed only at site B ($R = 0.83$, $p = 0.0028$; site A: $R = 0.56$, $p = 0.19$). Hence, at this site, haplotypes colonizing the patches at the onset of the experiment constituted an important part of the population after 35 d.

Discussion

Nematode community structure and abundance—The litter in the present study was colonized by a variety of typically benthic nematode genera, such as *Daptonema* and *Theristus*, which are not specifically associated with *Fucus* detritus but which opportunistically take advantage of the available resources (De Mesel 2004). Of the two most abundant species in our experiment, *P. marina* and *G. disjuncta*, the latter is a generalist that occurs on all sorts of litter as well as in sediment in and along the edges of the Paulina marsh, where our experiment was conducted (Moens unpubl. data). *P. marina*, by contrast, is more restricted to seaweeds, both live and dead, and does not live in the sediment (Bouwman et al. 1984; Moens unpubl. data). Both species colonized the experimental *Fucus* patches during the first days of the experiment and showed a rapid population growth. This may influence the population development of the other colonizing taxa and may prevent the settlement of new taxa (Jenkins and Buikema 1998; De Meester et al. 2002). This is not in contradiction with the five new taxa that were found after 30 d of incubation at site B, because abundances of *P. marina* and *G. disjuncta* were much lower at site B than at site A, which left more available space for other nematodes. Several nematode species have been observed to raft (Thiel and Gutow 2005), and consequently, drifting algae

Table 2. *Pellioditis marina*. Results of the hierarchical AMOVA to infer genetic differentiation within sites A and B.*

	df	*F*-statistics	% variation	*p*
Site A				
Among patches	2	Fct=0.031	3.13	<0.001
Among samplings within patches	17	Fsc=0.056	5.42	<0.0001
Within samplings	458	Fst=0.085	91.45	<0.0001
Site B				
Among patches	3	Fct=0.35	34.84	<0.0001
Among samplings within patches	17	Fsc=0.026	1.71	<0.0001
Within samplings	493	Fst=0.37	63.46	<0.0001

* %, % of the variation explained by; *p*, significance level of the *F*-statistics; Fct, correlation among haplotypes within patches relative to the correlation of random pairs drawn from the site (A or B); Fsc, correlation among haplotypes within time samples relative to the correlation of random pairs drawn from the patch; Fst, correlation among haplotypes within time samples relative to the correlation of random pairs drawn from the site (A or B).

typically contain nematode assemblages upon deposition on the shore. The presence of a dispersal propagule may influence subsequent colonization by other nematodes through interspecific interactions. The presence of other species would probably have only very limited consequences for the results of our study, however, because *P. marina* is the most successful colonizer species on freshly decomposing macroalgae, and its population dynamics do not seem to be affected by the presence of other microbivorous nematodes (Moens et al. 1996; dos Santos et al. unpubl. data).

The fivefold lower nematode abundance at site B was apparently caused by a slower colonization of the patches, a slower population development, or a combination of both. A slower rate of colonization may have been caused by the larger distance of site B with reference to a source population. In addition, site B is a more variable and stressful environment, because it is slightly higher up in the littoral than site A, and, consequently, it experiences longer low-tide exposure. Visual inspection indicated, for instance, that algae at site B were episodically subject to drying through exposure to wind, a factor known to hamper population development (Moens and Vincx 2000). The lower abundances in the single patch at site B that was colonized as rapidly as the patches at site A strengthen the assumption of suboptimal conditions for population development in most patches at site B.

Colonization of Fucus *by* P. marina—In spite of the lower absolute abundances of total nematodes and of *P. marina* at site B, the relative abundances of *P. marina* were similar in both sites and increased over time at the expense of the other genera. As expected, colonization and population development at site A occurred more quickly. This is probably because on the permanent algal stands in site A *P. marina* acted as a source population from which both active and passive dispersal could occur. In fact, colonization of *P. marina* at site A was slower than expected considering its high reproductive output (up to 600 eggs female^{-1}; Vranken and Heip 1983), its short generation time (less than 5 d under the field conditions; Moens and Vincx 2000), and its potential for active and passive dispersal. Considering the biology of *P. marina* and the very short distance between patches within a site (ca. 2 m), the significant genetic differentiation among patches within a site (*see also next section*) indicates that gene flow among these patches was limited and, hence, that effective migration (=migration followed by successful reproduction) in *P. marina* is low.

Genetic patterns and diversity of colonizing P. marina—As expected, we found a larger genetic differentiation between *P. marina* populations at site B than between those at site A. Several observations indicate that patches at site B acted as nonequilibrium demes (i.e., a set of populations among which little or no recolonization occurs; Harrison and Hastings 1996). First of all, the lower haplotype diversity and lower absolute *P. marina* densities at site B early in colonization indicate that the propagule initially colonizing site B was smaller than that colonizing site A. This can be explained by a combination of distance from the source population, which has a diluting effect, and the suboptimal conditions at site B, which hampered population development. Secondly, the nonequilibrium characteristics of site B are reflected in the haplotype composition of the colonizing nematodes. Haplotypes that first colonized patches at site B determined the population genetic composition after 35 d. Furthermore, three of the four patches at site B were dominated by haplotypes (C, F, and W), which are rare at Paulina and in the polyhaline part of the Westerschelde (Derycke et al. 2005, 2006). Rare haplotypes that become abundant in a population are typical for founder events and genetic bottlenecks (De Meester et al. 2002; Haag et al. 2006). The lower number of haplotypes at site B compared with the source population supports the occurrence of a genetic bottleneck. Thirdly, AMOVA demonstrated that there was a very high genetic differentiation between patches in site B, indicating that little gene flow among patches occurred.

Genetic differences among patches at site A were small, but nevertheless still significant, and indicated that founder events also occurred at site A. This result is striking because patches at site A lay within the source population and because colonization rates at site A were high. Interestingly, according to the Bray–Curtis analyses, changes in the haplotype composition over time were small at both sites, especially after 10 d at site A and after 20 d at site B. Several scenarios may produce such a pattern. First, individuals arriving at empty patches at each site may establish a population, with no new individuals added during the remainder of the experiment. Considering that up to 10% of endobenthic nematodes and an even larger percentage of nematodes on phytal substrata can be suspended during a single tidal cycle (Fegley 1987; Alkemade et al. 1994), it is very unlikely that no new individuals arrived at our patches after initial colonization. This is especially true for site A, at which *P. marina* should be able to actively migrate from the source population to the patches. A second explanation would be that our mitochondrial marker, which is maternally inherited, was not able to detect newly arriving individuals. This is in fact partly true: the COI marker overlooks the effect of newly arriving males, and in this way can miss new haplotypes. However, this effect should be of minor importance, because the ratio of males to females in *P. marina* is slightly biased in favor of females (~67% females at the temperatures during our experiment; Moens and Vincx 2000). Third, if individuals that are added during the experiment have identical haplotypes to the 'founders,' we would also not detect temporal differences. This scenario is unlikely, however, as all patches were clearly different from each other. Thus, new dispersal propagules, each originating from a homogeneous source population, are unlikely to have a haplotype composition similar to that of the populations already present on the patches, especially at site B. Alternatively, individuals that are added during the experiment after initial colonization (i.e., after 10 d at site A and after 20 d at site B) may have different haplotypes than the founders, but they remain in very low frequency in the patches. This pattern is characteristic of the 'persistent

founder effect,' in which new haplotypes are not established as a result of the strong population growth of the first colonizers (Boileau et al. 1992; De Meester et al. 2002). Such priority effects are most likely the best explanation for the low variability in haplotype composition at both sites and are in agreement with the low effective migration that was observed among patches at site B and to a lesser extent among patches at site A.

Our field experiment shows that empty patches are rapidly colonized by nematodes. Although nematode communities were initially different between both sites, they converged over time. In contrast, genetic patterns in the dominant species, *P. marina*, remained clearly different between both sites over time. *P. marina* exhibits strong colonization dynamics within the Westerschelde Estuary, and both regional and local factors influence the population genetic structure of this species. The observed patterns of genetic variation and differentiation were in agreement with expectations based on the proximity of a source population. Our results also indicate that founder effects play an important role in determining genetic differentiation in *P. marina* populations. The observed genetic differentiation among sites and among patches within sites further indicates that effective dispersal of *P. marina* is lower than previously thought and that priority effects best explained the observed patterns in the genetic structure of this species.

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