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Sibling species or poecilogony in the polychaete *Scoloplos armiger*?

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Abstract In marine invertebrates multiple modes of development, or poecilogony, may occur in a single species. However, after close examination, many of such putative cases turned out to be sibling species. A case in point may be the cosmopolitan orbiniid polychaete *Scoloplos armiger*, which inhabits marine shallow sediments. In addition to the well-known direct, holobenthic development from egg cocoons, pelagic larvae have also been described. Our culture experiments revealed a spatially segregated source of the two developmental modes. All females of an intertidal population produced egg cocoons and no pelagic larvae. All but 2 out of 15 females of an adjacent subtidal population produced pelagic larvae and no egg cocoons. Based on these results we performed a molecular genetic analysis (RAPD-PCR) on three intertidal and four subtidal populations in the North Sea. Selected samples from all sites were analysed also by the AFLP method. We found significantly higher genetic diversity within subtidal than within intertidal populations. This is consistent with a wider dispersal by pelagic larvae and a smaller effective population size when development is holobenthic. Total genetic divergence is not related to distance but to the intertidal/subtidal division. We suggest that *S. armiger* actually represents two sibling species.

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Introduction

For benthic marine invertebrates different modes of development within one species, or poecilogony, have been reported several times (Giard 1905). In many cases, however, a re-examination of supposed poecilogonous species revealed misidentification or laboratory disturbance and later a split-up into sibling species was necessary (Hoagland and Robertson 1988; Bouchet 1989). Shifts in developmental mode play an important role in speciation processes in the marine habitat. Shifts may be rapid and complete within local populations and affect dispersal, and this may lead to reproductive isolation and speciation (Hoagland and Robertson 1988). Currently, marine species represent a challenge to the idea of allopatric speciation. Generally, rates of dispersal and gene flow are assumed to be high (Palumbi 1994).

In *Scoloplos armiger*, one of the most common macrofauna species in sediments of eastern North Atlantic coastal seas, two distinct modes of development were identified recently. Spawning of this polychaete has usually been described to be benthic: conspicuous egg cocoons are laid on the sediment surface of intertidal mud flats, fixed with a stalk. Through this stalk juvenile worms crawl directly into the sediment when they have developed to a nine-setiger stage (Anderson 1959). Only a decade ago an additional mode of reproduction was attributed explicitly to *S. armiger* by Plate and Husemann (1991). They described pelagic larvae occurring around the offshore island of Helgoland, North Sea. Pelagic larvae are distinct from egg cocoon larvae by: (1) showing more ciliary bands, which gives them the ability to swim actively, and (2) by their smaller size, when comparing larvae of similar setiger numbers (Anderson 1959; Plate and Husemann 1991). This mode of development is herein called pelagobenthic, whereas development in egg cocoons is designated holobenthic (Jägersten 1972). Both pelagic and benthic larvae are lecithotrophic (Plate and Husemann 1991).

S. armiger is reported to have a cosmopolitan distribution (Hartmann-Schröder 1996) and is present from the intertidal down to the deep subtidal (Gibbs 1968; Holte 1998). Reports of egg cocoon occurrence of *S. armiger* in the literature, however, are restricted mainly to the soft bottom intertidal of the North Sea region (e.g. Schultze 1855; De Groot 1907; Thamdrup 1935; Gibbs 1968) and adjacent waters (Hornell 1891; Cabioch et al. 1968; Rasmussen 1973). To our knowledge, there are no reports on spawning behaviour of *S. armiger* from other regions of the world. In spite of the occurrence of two very distinct developmental types in the North Sea, no systematic comparison between these has been undertaken so far. *S. armiger* is purported to be the only representative of the genus *Scoloplos* (Blainville, 1828) (Hartmann-Schröder 1996) in the North Sea region. However, Blake (1980) suspects sibling species in *S. armiger* after comparing drawings of holobenthic *S. armiger* from the North Sea and of pelagic larvae from the White Sea.

The local origin of pelagic larvae has, however, remained unknown. Although *S. armiger* is very common in subtidal sediments of the North Sea, egg cocoons have neither been reported nor seen by staff of local research vessels nor by divers (personal communications). Recently, pelagic larvae of *S. armiger* have also been recorded close to the island of Sylt, at an even higher density than near Helgoland (up to 30 larvae per 10 l water sample; Kruse, unpublished data). In contrast to Helgoland, Sylt is located within the sedimentary Wadden Sea and, therefore, surrounded by suitable intertidal sediments for *S. armiger*. In the present study, we first hypothesise that pelagic larvae near the island of Sylt originate from subtidal sites. In the laboratory we cultured adult worms ready to spawn to test if pelagic larvae of *S. armiger* emerge from subtidal adults and if intertidal adults produce egg cocoons only.

We further hypothesise that the two developmental types represent sibling species. In a genetic analysis we compared the assumed geographic pattern of developmental modes with those of genetic divergence. We found no character available in *S. armiger*, which would allow prediction of which type of reproduction will be performed by an individual. Thus, examination of this hypothesis was based on separation of developmental modes by habitat type, according to our assumptions and the results of our laboratory experiment. We tested the hypothesis of genetic isolation by habitat type against isolation by distance. If the genetic diversity of *S. armiger* is mainly attributable to habitat type and not to distance, the hypothesis of sibling species would be supported. The alternative hypothesis would apply if isolation by distance contributes most to genetic population divergence. In our study, we used the RAPD (randomly amplified polymorphic DNA) (Williams et al. 1990) method to analyse genetic diversity of *S. armiger* within the Sylt area. AFLP (amplified fragment-length polymorphism) (Vos et al. 1995) markers were used to validate the results of the RAPD analysis. Samples from one location at Helgoland with postulated pelago-benthic development were used for comparison with Sylt locations and also as a distant reference population.

Materials and methods

Aquaria culture experiment on the origin of different developmental modes

To test the initial hypotheses that pelagic larvae originate from the subtidal habitat and that intertidal adults exclusively produce egg cocoons, *Scoloplos armiger* from both habitats were cultured separately in the laboratory until spawning in February/March 1998. These data were prerequisite for the genetic analyses on differences between subtidal and intertidal *S. armiger* populations.

Study area

This study was conducted on *S. armiger* in the Sylt–Rømø Bight, an enclosed tidal basin in the northern Wadden Sea, and near the island of Helgoland, further offshore in the North Sea (Fig. 1). In both areas tides are semidiurnal, with a range of 2–2.4 m. The Sylt–Rømø Bight covers about 400 km^2, of which 33% belong to the intertidal zone, 57% to the shallow subtidal (< 5 m depth) and 10% to deeper tidal channels (max. depth ~40 m). Water

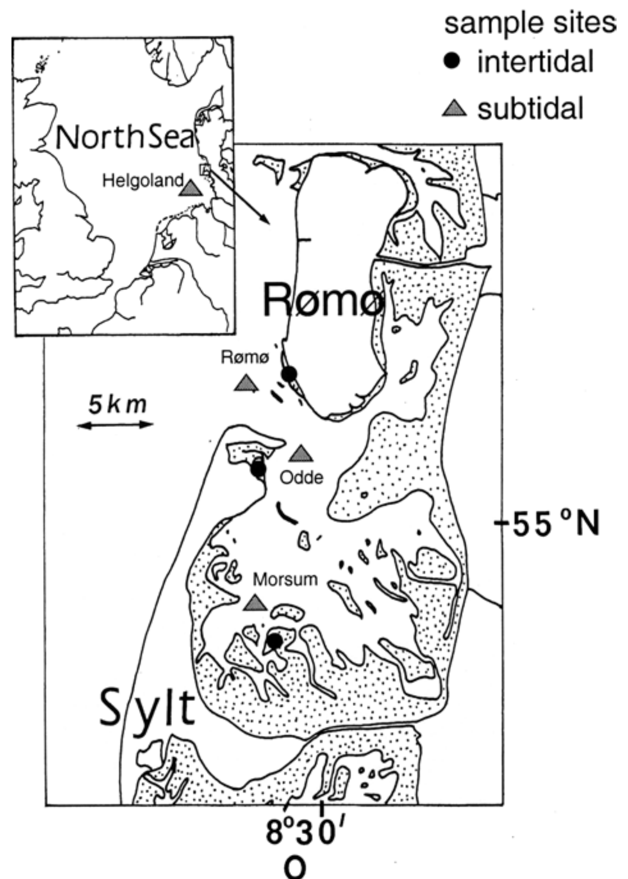

Fig. 1 Sampling design to test genetic differentiation according to geographic locations (Odde, Morsum, Rømø, Helgoland) against differentiation according to habitat (subtidal, intertidal)

exchange between the Sylt–Rømø Bight and the open North Sea takes place through a 2.8-km-wide tidal channel called the Lister Deep. More information about the area is given in Gätje and Reise (1998).

Origin-of-larvae experiment

Specimens of *S. armiger* from the subtidal and from the intertidal were collected at locality "Odde" (shown in Fig. 1) in January/February 1998, i.e. before spawning when males and females could be distinguished easily by their well-developed gonads. Individuals from the same habitat, five females and three males each, were put together in one aquarium. Both habitats were replicated three times. All six aquaria (55 × 40 × 30 cm) contained 7 cm of sediment from the subtidal habitat, sieved through 1 mm, covered with 20 cm of seawater. Placed in a culture room, aquaria were aerated and were subjected to artificial light in a day–night rhythm. Temperature was adjusted to the concurrent water temperature in the field (5–7°C). Each day we recorded deposition of egg cocoons by eye and occurrence of pelagic larvae and eggs by sampling the water column. The latter was done using a tube to take a representative 10 l water sample from each aquarium and to filter it through a 80 µm mesh.

Sampling for genetic study

Worms for DNA analysis were collected in July/August 2000 around the island of Sylt within each of three localities ("Odde", "Morsum", "Rømø") at one intertidal and one subtidal site (Fig. 1). This sampling was designed to test if larger differences exist between sites within localities (i.e. between intertidal and its adjacent subtidal habitat) than between different localities (within subtidal and intertidal habitats) situated further away from each other. In other words, the hypothesis of isolation by distance of localities was tested against isolation by type of habitat. Additionally, one site near the offshore island of Helgoland was sampled as a distant subtidal site where the *S. armiger* population was assumed to exhibit the pelago-benthic mode of reproduction. In this context it was tested whether individuals from the locality "Helgoland" were more similar to subtidal Sylt specimens than to intertidal ones. Positions of all sites are given in Table 1. Distances between the sampling areas Helgoland and Sylt are about 95 km, and within the Sylt area between the localities Morsum and Odde 9 km, Odde and Rømø 7.3 km and Rømø and Morsum 16.1 km. Habitats within localities (intertidal and subtidal sites) were 2–2.5 km apart from each other. Subtidal sites were sampled using a boxcorer from a boat. Intertidal worms were collected during low tide as single specimens sampled randomly at least 2 m apart from each other from 100 m^2 areas. *S. armiger* was sieved out of the sediment, sorted alive and isolated in seawater for at least 24 h, to allow the gut contents to be digested. Then worms were frozen at −80°C.

DNA extraction

DNA was extracted from frozen adult worms with the spin-column method (DNeasy tissue kit, Quiagen), using 20–25 mg fresh tissue from the front ends. Following the DNeasy protocol for animal tissues, a RNAse-A digestion step was included, and the elution was done once with 100 µl buffer AE.

RAPD procedure

A total of 30 arbitrary decamer primers from the kits A (OPA-01 to OPA-10) and B (OPB-01 to OPB-20) (Operon Technology, Calif., USA) and 5 from RAPD primer set 4 (Biotechnology Laboratory, University of British Columbia, Canada) were tested. The codes of the four primers that provided satisfactory results (several bright, clear bands for most of the samples) were: OPA-02, -03, -09 and -10. For PCR (polymerase chain reaction), vials were placed in a Biometra T gradient thermocycler for an initial 3 min at 94°C; then cycled 10 times through 1 min at 94°C, 1 min at 35°C and 1 min at 72°C; followed by another cycle of 30 times through 30 s at 94°C, 30 s at 35°C and 1:30 min at 72°C; and finally paused at 72°C. The reaction volume (20 µl) contained 2 µl of each 10x dNTP (Roche), 5 µM primer, 1% BSA, 25 mM $MgCl_2$, storage buffer B (20 mM Tris-HCL, pH 8.0; 100 mM KCL; 0.1 mM EDTA; 1 mM DTT; 50% glycerol; 0.5% Nonidet-P40; 0.5% Tween 20) and 0.15 µl *Taq* DNA polymerase (size = 100 U, 5 U µl^{-1}) (the latter three chemicals by Promega, Madison, USA), 1 µl of DNA template solution and 8.85 µl HPLC-water. Each PCR was run with all samples for one primer, placing two samples each of subtidal and intertidal polychaetes in alternate order in 96-well microtiter dishes and adding one replicate and one blank. The replicates were samples from one worm divided into two pieces prior to DNA extraction. All replicates indicated reproducibility. Amplified RAPD products (8 µl) were loaded on 2.5% agarose gels with two samples each of subtidal and intertidal polychaetes in alternate order. They were analysed by gel electrophoresis (200 V for 2 h 20 min to 3 h) in 1x TAE buffer. A 100 base pair (bp) ladder (Amersham Pharmacia Biotech), thinned to 0.1x, was used as a size standard. Gels were stained in an ethidium bromide bath (0.5 µg ml^{-1}) and recorded using a CCD video camera system with an image-processing workstation (GeneSnap by Syngene, Synoptics, Cambridge, England). In gel images printed from GeneTools (Syngene), the presence and absence of bands were scored visually. Only bands >400 bp size were considered in the analysis.

Data analysis

Presence/absence matrices of RAPD bands were obtained for each primer and entered first into TREECON (Van de Peer and De Wachter 1994) to calculate genetic distances using the Nei–Li distance coefficient. The advantage of Nei–Li distance estimates is that

Table 1 Position of sites, water depths (metres below spring low tide level) and number of sampled *Scoloplos armiger* (*intertidal* mid-intertidalzone, sampled randomly in 10×10 m^2; *waterdepth* in metres below spring low tide level)

Site	Abbreviation	Position	Water depth	Number of individuals
Sylt, intertidal				
Morsum	EM	54°56.26 N; 08°26.90°E	0	11
Odde	EO	55°01.09 N; 08°26.00°E	0	12
Rømø	ER	55°05.65 N; 08°27.75°E	0	12
Sylt, subtidal				
Morsum	SM	54°57.13 N; 08°26.40°E	5	12
Odde	SO	55°01.47 N; 08°27.98°E	10	12
Rømø	SR	55°05.90 N; 08°26.01°E	6	11
Helgoland, subtidal	SH	54°17.00 N; 7°48.00°E–54°16.20 N; 7°47.20°E	20	21
Total no. of individuals				91

they use only the shared presence of a band (assuming homology) (Nei and Li 1979; Harris 1999), which is appropriate for RAPD data (Lamboy 1994; Wolfe and Liston 1998). That is, since RAPD bands are scored as dominant markers, the presence of a band implies that the priming sites on either side of the fragment are present. In contrast, the absence of a band can have several causes (substitution, deletion, insertion, restriction site absent, etc.). UPGMA (unweighted pair-group method using arithmetic averages) and NJ (neighbour joining) trees were constructed based on genetic distances in TREECON (Sneath and Sokal 1973). Means of Nei–Li genetic distances were also calculated for each single site. Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was further employed to test differentiation between localities against differentiation between habitats. From the frequency of RAPD products, patterns of diversity were calculated for a pre-defined group structure. Data at the first level were grouped according to localities, and at the second level, according to habitat. Φ_{st}-statistics (analogous to Wright's F_{st}) and tests of significance (1023 permutations) were performed in ARLEQUIN version 2.0 (Schneider et al. 2000). To confirm above results independently, the dataset was also analysed using a different software, "Tools for Population Genetic Analysis" (TFPGA) (Miller 1997). To do so, it was converted to pseudohaploid input values, due to the dominant nature of RAPD. In TFPGA, *F*-statistics using the methods of Weir and Cockerham (1984) were calculated with the same two-level hierarchy structure as in AMOVA, habitats grouped within locality; 95% confidence intervals (CI) for Φ (analogous to Φ in AMOVA and Wright's F_{st}) were achieved by bootstrapping across loci with 1000 replications.

However, AMOVA assumes Hardy–Weinberg equilibrium, which cannot be determined by the dominant RAPD data. For this reason, an additional frequency measure, Shannon's index of diversity, was determined, since it does not rely on Hardy–Weinberg equilibrium (Bussell 1999). It was calculated as described in Engelen et al. (2001) and according to Bussell (1999) following the equation: $H'_j = -\Sigma p_i \times \log_{2\times} p_i$, where p_i is the frequency of the presence or absence of an RAPD band (i.e. locus) at site *i* (altogether six: subtidal and intertidal habitat, each at three locations). H'_j values were averaged per primer across sites $[H'_{pop} = 1/(n\sigma H'_j)]$ and per site across primers. Shannon's index served as estimation of: (1) diversity components within and between sites, (2) diversity detected by different primers within sites, (3) site-related differences in genetic diversity and (4) differences in total genetic diversity within sites between intertidal and subtidal *S. armiger* (Shannon indices compared by *t*-test using STATISTICA 5.1) (Chalmers et al. 1992; Engelen et al. 2001).

Genetic diversity among and within Sylt sites was also compared between habitats using Nei–Li genetic distances out of TREECON. For each individual, the mean of genetic distances (1) to each of all other individuals from the same site and (2) to each of the individuals from different sites of the same habitat type were calculated. These means (35 within sites for each habitat and 70 between sites for each habitat) were compared between habitats using ANOVA from STATISTICA 5.1.

AFLP analysis

The AFLP technique (Vos et al. 1995) was performed with selected samples of the same DNA extracts used in RAPD, to test for consistency of results by both methods. AFLP is considered a reliable and powerful tool for the evaluation of genetic variability, with better reproducibility than RAPD (Jones et al. 1997). The AFLP procedure involved three main steps. (1) Restriction of the DNA and ligation of adapters was carried out. For each sample, approximately 250 ng of DNA was digested with 4 U of *Mse*/*Eco*RI restriction enzyme in a reaction volume of 20 μl. In the same reaction, ligation was done with 6 U of DNA ligase and 100 ng μl^{-1} adapters. After incubation at 37°C for 2 h, samples were transferred to a 65°C water bath for 5 min, in order to inactivate the DNA ligase. Incomplete digestion of genomic DNA can lead to false polymorphism signals in AFLP profiles. Therefore, complete digestion was confirmed by running each sample on an agarose gel. We also checked whether each enzyme in the absence of the other led to complete digestion. (2) Pre-amplification was performed for PCR; 2.5 μl of the restriction–ligation product was combined with 17.5 μl of the pre-amplification primer solution, using the AFLP core mix (Perkin Elmer). The primers used were Eco + A and Mse + C. PCR consisted of 20 cycles of 94°C for 1 s, 56°C for 30 s and 72°C for 2 min, terminated by a single step of 60°C for 30 min using an PCR thermocycler (MJ Research, Waltham, Mass.). After checking for the presence of a smear by agarose electrophoresis, the pre-amplification mixture was diluted 1:19 with TE buffer. (3) Selective amplification was then carried out; primers that match the known adapter sequence plus three selective nucleotides were used to reduce the number of amplified fragments. Two primer combinations were identified to yield many polymorphic fragments, Mse-CAC and the fluorescently labelled *Eco*RI primers Ned-CAT and Joe-AGG. A touchdown PCR reaction was used with one cycle of 94°C for 2 min, 65°C for 30 s and 72°C for 2 min; followed by 23 cycles in which the annealing temperature was reduced in 1°C steps to 56°C; and again followed by a single step of 60°C for 30 min.

For gel electrophoresis 2 μl of the selective amplification product was added to a 3 μl mixture of formamide, loading buffer and a size standard (GeneScan 1000 Rox, Perkin Elmer). The amplified, labelled fragments were analysed on 5% Long Ranger polyacrylamide gels, using an ABI Prism 377 automated genetic analysis system (Perkin Elmer). Data were processed using ABI GeneScan Analysis 3.1 software (Perkin Elmer). Each sample was manually checked for correct alignment of the size standard and, when necessary, aligned by hand. Data were subsequently imported into Genographer (http://hordeum.oscs.montana.edu/genographer), and AFLP profiles were scored for the presence/absence of fragments between 50 and 500 bp. Reproducibility was tested for one individual by repeating the DNA extraction and AFLP procedure, and was found to be high (98%). In Genographer we used the Thumbnail option to visually score the presence and absence of bands and obtain 0/1 matrices. As described for RAPD analysis, a UPGMA tree was constructed in TREECON.

Results

Origin of pelagic larvae

The culturing experiment revealed that pelagic larvae were only produced by *Scoloplos armiger* of subtidal origin. From the 15 intertidal females, placed in three aquaria, 14 egg cocoons were formed, while this was the case in only 2 of the 15 subtidal females. In all three aquaria with subtidal specimens, free-floating eggs appeared first in the water column, and several days later trochophora and pelagic larvae as described by Plate and Husemann (1991) were found. These larvae emerged in calculated total maximum numbers of 255, 493 and 1540, respectively, per five females and aquarium. Specimens from both habitats spawned during a period of 2 weeks in 1998: intertidals from 21 February to 7 March and subtidals from 25 February to 10 March. In conclusion, habitat affiliation of adult individuals (subtidal/intertidal) was a good predictor of the two modes of development. Thus, in the genetic study, habitats are operationally defined to differentiate between the two population types of *S. armiger*. This was done in awareness that some females producing egg cocoons are obviously mixed up with the subtidal material, representing a mistake transmitted into further results.

Genetic structure using RAPD

With four decamer primers, 116 polymorphic bands were scored in total, none were monomorphic. An example of RAPD patterns is given in Fig. 2.

Between 28 and 32 bands, ranging from 300 to 2300 bp, were amplified by each primer (Table 2). Each of the 91 individuals showed a unique RAPD band profile. There were 48 bands specific for the subtidal habitat, and none, for the intertidal. Due to PCR failure, for each primer some samples (individuals) had to be omitted; thus, 9.6% of the data was missing.

AMOVA and Shannon's index analyses showed consistent results in that most of the variation occurred within sites (81% respectively 89%) and less between sites (22% respectively 11%). Primers varied in their power to detect variation within and between sites, as indicated by Shannon's index for diversity (H'_{pop}) calculated per primer per site and per primer across all sites (Table 3). Average multi-locus diversity per site was 0.889, ranging from 0.37 to 1.59. Only primer OPA-3 detected more diversity between than within sites.

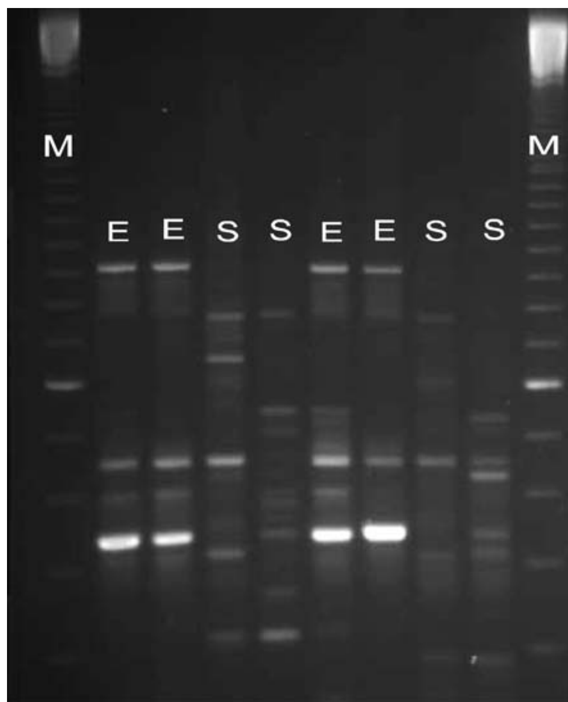

Fig. 2 *Scoloplos armiger*. RAPD marker pattern of eight individual *S. armiger* using primer OPA-2 from location Rømø (*S* subtidal specimen; *E* intertidal specimen; *M* molecular weight marker, 100 bp)

Table 2 RAPD primers used

Primer	Sequence 5′–3′	Total no. of bands
OPA-2	TGC CGA GCT G	28
OPA-3	AGT CAG CCA C	32
OPA-9	GGG TAA CGC C	29
OPA-10	GTG ATC GCA G	27
Avg. bands per primer		29
Total bands		116

Population structure was analysed by several statistical methods. Distance values obtained according to Nei and Li (1979) are illustrated by the tree based on cluster analyses with the UPGMA method (Fig. 3), where each sample is clustered individually.

The tree shows that intertidal and subtidal *S. armiger* each cluster together, indicating that the genetic distances are smaller within the same type of habitat (intertidal, subtidal) than within localities (Odde, Morsum, Rømø) across habitats. As an exception, five subtidal individuals from Morsum are among the intertidal group. Helgoland specimens group closer together with the subtidals than with the intertidals from Sylt, and also closer together than Sylt subtidals with their neighbouring intertidals. Within habitats a slight grouping according to locality is shown (Fig. 3). Cluster analysis of the same dataset by the NJ method gave similar results. In trees of both clustering methods bootstrap values are quite low, but are counterbalanced by the clear pattern resolved. In conclusion, Nei–Li distances show a clear grouping of individuals according to habitat (subtidal, intertidal) and none according to geographic origin.

F-statistics from AMOVA also revealed significant difference between subtidal and intertidal habitat and no differentiation between localities (Table 4); 81% of the variance was attributable to individual variation ($P<0.00001$), 22% was attributable to habitats ($\Phi_{between\ habitats}=0.21225$, $P<0.00001$), and no variance was due to geographic site, i.e. locality ($\Phi_{between\ sites}=-0.03068$). The negative variance between sites (Table 4) can be taken as zero (Weir 1996). Analysis with the software TFPGA confirmed these results with the same partitioning of variance: $\Phi_{between\ habitats}=0.1961$ (CI = 0.2395–0.1560) and $\Phi_{between\ sites}=0.0166$ (CI = 0.0288–0.0624).

Shannon's index of diversity reflected higher genetic diversity within subtidal sites than within intertidal ones (Table 5; *t*-test of H'_j values of Sylt sites for all four primers, $P<0.05$). Nei–Li genetic distances confirmed these results (AMOVA of mean distances for individual *S. armiger*, $P<0.01$).

Genetic diversity among sites, reflected in Nei–Li genetic distances as well, was also higher in the

Table 3 *Scoloplos armiger*. Shannon's indices for genetic diversity between and within sites for four random oligonucleotide primers

Primer	H'_{pop}	H'_{sp}	H'_{pop}/H'_{sp} (within sites)	$1-(H'_{pop}/H'_{sp})$ (between sites)
OPA-2	1.598	1.621	0.986	0.014
OPA-3	0.727	1.936	0.375	0.625
OPA-9	1.018	1.694	0.601	0.399
OPA-10	2.086	1.309	1.594	−0.594
Mean	2.854	1.245	0.889	0.111
Total	19.976	6.559	3.557	0.443

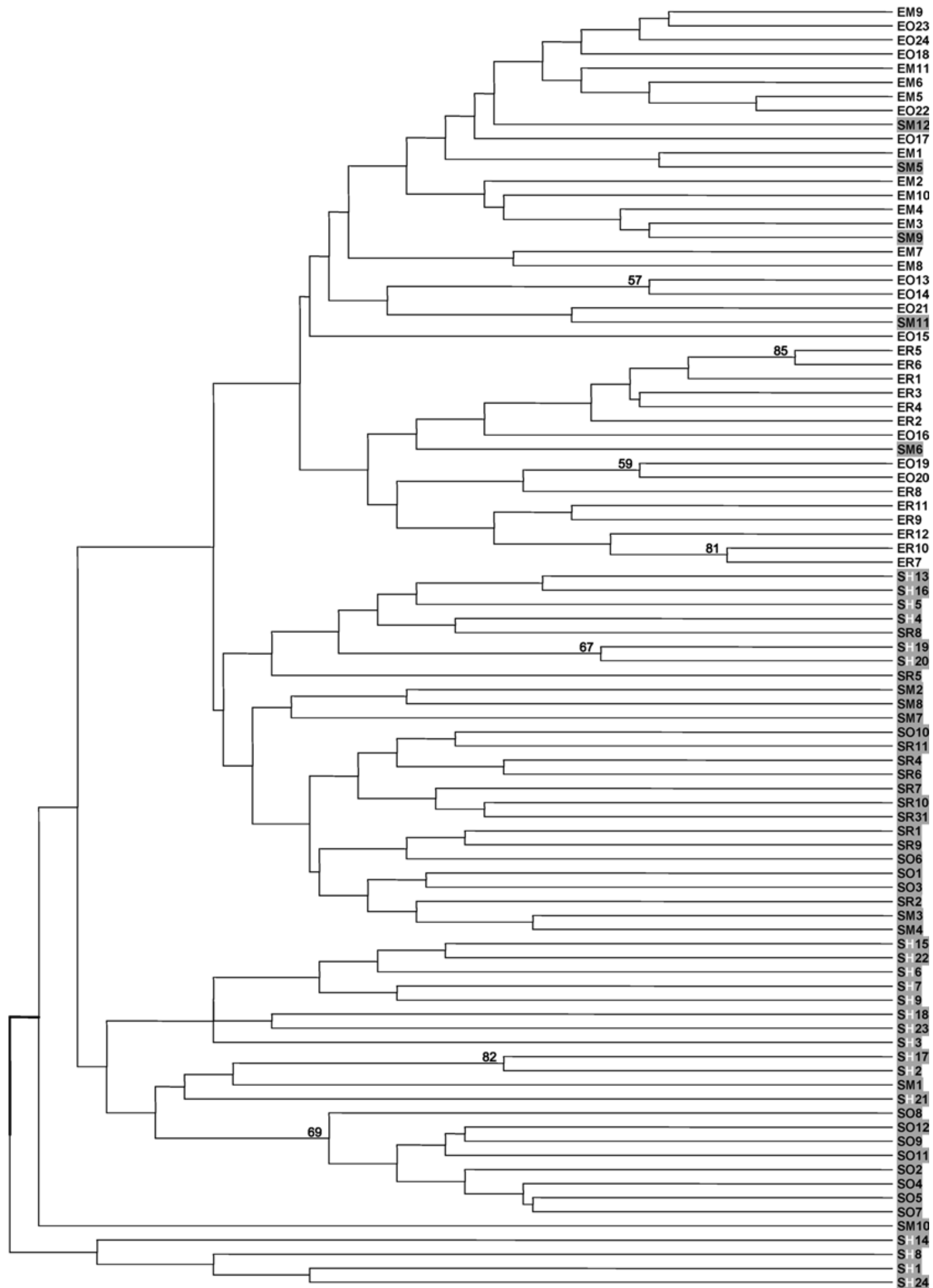

Fig. 3 *Scoloplos armiger*. RAPD genetic distances between subtidal and intertidal *S. armiger* from four different locations. Unrooted tree generated by the cluster analysis of UPGMA using Nei–Li distances. Bootstrap values >50 are indicated at the nodes (*E* intertidal, *S* $\text{subtidal}_{\text{habitat}}$, *O*, *M*, *R* Sylt locations Odde, Morsum, Rømø, respectively; *H* Helgoland location; *numbers* indicate individuals)

subtidal: overall mean distance within the subtidal was 78.1% (SD = 13.1) and within the intertidal was 57.2% (SD = 10.8) (Table 6; AMOVA of mean distances within sites for individual *S. armiger*, $P < 0.01$).

Table 4 *Scoloplos armiger*. Analysis of molecular variance for population structure at six sites around Sylt, grouped first according to location (Odde, Morsum, Rømø) and second according to habitat (subtidal, intertidal)

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation	*P*-value	Fixation indices	Interpretation
Between locations	2	52.878	−0.30591	−3.07	0.52297	$F_{ct} = -0.03068$	No differentiation between locations
Between habitats	3	100.552	2.18160	21.88	< 0.00001	$F_{sc} = 0.21225$	Significant difference between habitats (subtidal, intertidal)
Within all sites	64	518.212	8.09706	81.19	< 0.00001	$F_{st} = 0.18808$	

Table 5 *Scoloplos armiger*. Shannon's index (H'_j) as estimates of genetic diversity within sites, modified after Bussell (1999) (site abbreviations, see Table 1)

Primer	Intertidal sites			Subtidal sites				Average H'_j per primer across sites or H'_{pop}
	EM	EO	ER	SH	SM	SO	SR	
OPA-2	1.460	1.603	0.669	1.840	1.758	2.032	1.823	1.598
OPA-3	0.292	0.335	0.694	1.312	0.825	0.816	0.814	0.727
OPA-9	0.850	1.314	0.809	1.216	0.921	1.201	0.818	1.018
OPA -0	1.742	1.554	1.143	3.030	2.387	2.595	2.154	2.086
Multi-locus H'per site	3.232	3.480	2.696	2.739	2.772	2.419	2.638	2.854
Sum H'_j	4.344	4.806	3.315	7.397	5.890	6.644	5.608	5.610

Table 6 *Scoloplos armiger*. Genetic distances after Nei and Li (1979) between sites in percent; mean values and standard deviation (*parentheses*) (site abbreviations, see Table 1)

	Intertidal sites			Subtidal sites		
	EM	EO	ER	SM	SO	SR
EO	53.7 (13.2)					
ER	58.1 (8.3)	59.7 (9.6)				
SM	67.8 (17.0)	69.8 (14.7)	67.8 (17.0)			
SO	84.2 (10.4)	81.9 (10.6)	84.2 (10.4)	80.8 (12.4)		
SR	69.6 (10.0)	72.1 (8.3)	65.6 (8.1)	70.4 (10.8)	72.9 (12.7)	
SH	81.6 (11.8)	84.6 (11.9)	79.4 (12.8)	82.3 (10.6)	83.5 (9.9)	76.9 (12.3)

Genetic differentiation using AFLP

UPGMA clustering of AFLP haplotypes revealed the same genetic divergence among subtidal and intertidal habitats as did the RAPD analysis. As an example, the tree generated by the primer Ned-CAT is shown in Fig. 4. The independent primer combination Mse-CAC and Joe-AGG run with subsamples yielded the same pattern. In contrast to the RAPD tree there was a notable separated cluster of subtidal individuals from the locality Odde.

Discussion and conclusions

Our data support a genetic divergence of subtidal and intertidal *Scoloplos armiger*. We also confirm our initial assumption that pelagic larvae of *S. armiger* originate from the subtidal habitat. Intertidal specimens produced egg cocoons only. Thus, in the Wadden Sea area around Sylt, both holobenthic and pelago-benthic development exist in close proximity to each other. This is the first evidence for the occurrence of one dominant reproductive type associated almost exclusively with either the intertidal or the subtidal population of this polychaete.

Our laboratory experiments were rather characteristic of subtidal conditions. Nevertheless, intertidal females produced egg cocoons. This is at odds with a phenotypic switch in reproductive mode induced by tidal exposure. Moreover, we found no intermediate or individually flexible developmental mode in *S. armiger* to support the existence of poecilogony (Hoagland and Robertson 1988). *S. armiger* individuals either were shedding their eggs into the water column, or, alternatively, were packing them into a jelly mass, the egg cocoon. In contrast, intermediate types of development have been found in *Boccardia proboscidea*, one of the rare, truly poecilogonous polychaetes (Gibson et al. 1999; Schulze et al. 2000). In *B. proboscidea* the developmental mode varies within one single brood of one female. For this species interfertility between different developmental modes and also molecular data provided support for a single species (Gibson et al. 1999; Schulze et al. 2000).

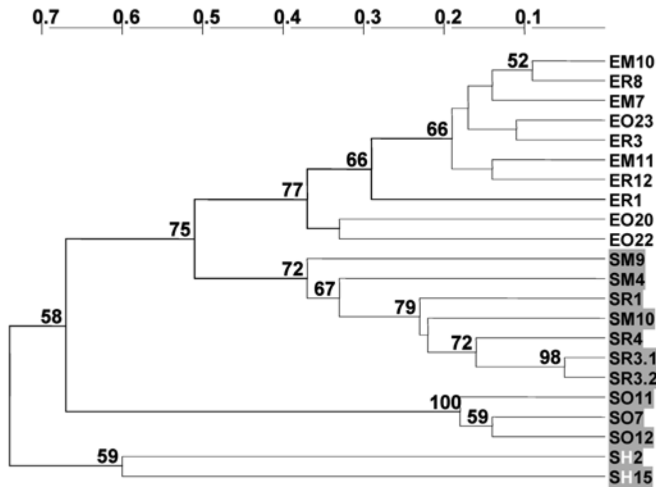

Fig. 4 *Scoloplos armiger*. AFLP genetic distances between subtidal and intertidal *S. armiger* from four different sites. Unrooted tree generated by the cluster analysis of UPGMA using Nei–Li distances. The primer used was Ned-CAT, 10 intertidal and 11 subtidal *S. armiger*. Bootstrap values >50 are indicated at the nodes (*E* intertidal, *S* subtidal$_{habitat}$, *O*, *M*, *R* Sylt locations Odde, Morsum, Rømø, respectively; *H* Helgoland location; *numbers* indicate individuals; *SR3.1*, *SR3.2* are replicates)

Genetic differences between habitats versus differentiation by distance

The existence of two distinct and spatially separated reproductive modes in *S. armiger* can be explained by two different scenarios. (1) The developmental mode is a plastic response to different environmental cues correlated with the division between intertidal and subtidal habitat. Depending on the conditions, individuals change their egg spawning behaviour. Individuals may migrate or drift between these habitats, and together form genetically coherent local or regional populations. (2) The different modes of reproduction are genetically fixed. Individuals perform few or no migrations, and rarely interbreed between habitats. Pelagic larvae of subtidal origin either find their way back to this habitat, or post-settlement mortality in the intertidal is strong. Juveniles hatched from cocoons in the intertidal are rarely entrained in bedload transport towards the subtidal, or their mortality is high once translocated from the intertidal. Due to partial or total reproductive isolation between *S. armiger* from intertidal and subtidal habitats, genetically distinct populations or even species are present.

The two scenarios lead to different predictions on the spatial patterns of genetic variability. While we predict only genetic differentiation through geographic separation (isolation by distance) in the first scenario (Wright 1946), a large genetic divergence between intertidal and subtidal sites irrespective of geographic distance must be present if the second scenario is applied. Clearly, our data support the second scenario for Wadden Sea populations of *S. armiger*. AMOVA analysis of RAPD haplotypes of Sylt specimens attributed a significant proportion of genetic variance to habitat and none to locality, i.e. geographic origin. This pattern is confirmed by our AFLP data. In UPGMA trees, from both AFLP and RAPD data using Nei–Li distances, subtidal and intertidal individuals each were found in the same phylogenetic clade, while individuals from the same locality revealed no phylogenetic proximity. In the phylogenetic analysis bootstrap values are relatively low, but the pattern is clear, and there is high consistency among methods (i.e. AFLP, RAPD).

As the only exception to the overall pattern we found five individuals of the subtidal site Morsum grouping with intertidals. Possibly these were in fact intertidal specimens that had migrated into the subtidal. Since the subtidal site Morsum is surrounded by large areas of intertidal habitat (Fig. 1), it is likely that this site can be most affected by immigration of intertidal *S. armiger*. The laboratory experiment on the origin of larvae also indicated unidirectional migration from the intertidal to the subtidal habitat. We observed two females collected in the subtidal forming egg cocoons. Intertidal *S. armiger* were found to be highly erodible (Armonies 1999), lending support to the notion that the subtidal population occasionally receives migrants from the intertidal.

RAPD and AFLP have been found to be reliable methods in systematics (Harris 1999). Reproducibility of RAPD between laboratories has been doubted by some authors (Jones et al. 1997). In the present study we found high reproducibility. Moreover, the hypothesis was studied in the same laboratory (for each method AFLP or RAPD) during one distinct period of time. We further note that any lack of reproducibility is conservative with respect to the hypothesis being tested. Moreover, AFLP and RAPD methods show essentially the same results. AFLP is considered to be a highly reproducible method (Jones et al. 1997). We conclude that genetic divergence of subtidal and intertidal *S. armiger* supports the hypothesis that *S. armiger* represents two sibling species. However, further experiments, such as cross-breeding trials, are necessary for conclusive validation.

Genetic variability within habitats and dispersal potential

No matter whether populations of poecilogonous species or different species are compared, genetic structure of marine invertebrates may be strongly influenced by the mode of larval development and its dispersal potential (Crisp 1978). The latter may be directly correlated with the effective population size. Populations with larger effective population size tend to have higher heterozygosity (Kijima and Fujio 1984), which corresponds to within-population diversity. It can be expected that planktonically dispersed species exhibit low levels of genetic variation among local populations, but relatively high genotypic diversity within populations. In contrast,

direct or holobenthic developers should undergo highly restricted dispersal and, thus, form sets of closed and relatively inbred local populations, with low diversity and high levels of genetic variation among local populations (Burton and Feldman 1982). We found significantly higher genetic diversity within subtidal than within intertidal sites and also numerous bands, which were unique only to subtidal *S. armiger*, whereas we found none for the intertidals. This is consistent with a higher effective population size in subtidal populations. Retention time in the water column for pelagic larvae of *S. armiger* is estimated to be 2 weeks (Plate and Husemann 1991). In theory, this timespan allows the Sylt subtidal population to perform genetic exchange even with the open North Sea. In contrast, poorly dispersing intertidal *S. armiger* are limited to the near-shore area. In the intertidal habitat the effective population size is supposed to be smaller; thus, many more bands may be lost over time due to drift.

Surprisingly, genetic distances among populations were inconsistent with this model. They were higher between subtidal localities than between intertidal ones, although we would expect the opposite pattern; the reasons for this remain speculative. One possible explanation could be that the subtidal habitat is more heterogenic in selective pressure than the intertidal habitat. Mobility may be another factor diminishing among-population differences. In *Littorina saxatilis*, Wilhelmsen (1999) found remarkably low genetic differentiation within the Wadden Sea around Sylt. She suggests a high colonisation potential, in spite of the ovoviviparous development in this snail. Also *S. armiger* from the intertidal area of the Wadden Sea may be interconnected to a much higher extent by along-shore dispersal than a priori assumed. Erosion and migration via mobile sediments is a common means of transport for marine invertebrates in intertidal soft-sediment communities (Tamaki 1987; Butman and Grassle 1992; Zühlke and Reise 1994; Turner et al. 1997; Grant et al. 1997). Armonies (1999) notes that benthic juveniles of *S. armiger* are exceptionally susceptible to sediment disturbance and displacement within the intertidal.

Population genetic structure of four *Littorina* species does not reflect predictions as to their dispersal potential. Instead, the significance of their historical demography is emphasised (Kyle and Boulding 2000). Very similar to findings for *S. armiger*, in the polychaete *Hediste japonica*, one sibling species exhibits planktonic development, while the other has direct-developing larvae. Genetic marker data revealed that among-population differentiation is consistent with dispersal capacity (higher in the direct-developing form), whereas within-population diversity was unexpectedly lower within the open-water planktonic form (Sato 1999). In *S. armiger* we find a similar partial discordance between population genetic prediction and empirical data, yet the situation is reversed, with concordance based on diversity, while differentiation is at odds with expectations. Obviously, genetic patterns cannot be interpreted based on developmental mode alone; species- and habitat-specific dispersal characteristics need to be considered as well.

Developmental switch and reproductive isolation

Transitions in larval developmental strategies may lead to rapid reproductive isolation, which finally may result in speciation (Hoagland and Robertson 1988). This is supported by numerous discoveries of sibling species, which previously had been regarded as poecilogonous. In particular, in polychaetes, several studies applying genetic markers have been published on this subject for *Capitella* spp. (Grassle and Grassle 1976; Baoling et al. 1988), *Marenzelleria viridis* and *M. wireni* (Bastrop et al. 1998; Jürss et al. 1999), *Streblospio benedicti* and *S. gynobranchiata* (Schulze et al. 2000), and *Hediste* spp. (Sato and Masuda 1997; Sato 1999). *Scoloplos armiger* is a potential candidate to be added to this list in the future. Thus far, the only true and undoubted examples of poecilogonous polychaetes in the literature are *Streblospio benedicti*, *Boccardia proboscidea* and *Pygospio elegans* (Gibson et al. 1999; Morgan et al. 1999; Schulze et al. 2000).

However, reproductive strategies may be derived from both the phyletic history of the group and environmental cues. For *S. armiger* phyletic causation for one reproductive mode appears to be weak, since variability within the family Orbiniidae is high. Four of ten species exhibit direct development and six are free spawning (Giangrande 1997). Instead, physical differences between the intertidal and subtidal environment must have disrupted modes of development. The egg cocoon has been considered to retain the larvae in the intertidal habitat (Gibbs 1968). Thus, the intertidal seems to feature suitable conditions for *S. armiger*, which are absent in the subtidal.

In recent years speciation in the marine habitat has attracted much attention (Palumbi 1992, 1994). According to a strictly allopatric speciation model, the high dispersal and large populations typical for marine macroinvertebrate species suggest a lower speciation rate than is actually indicated by the high marine biodiversity at all spatial and temporal scales. Given numerous marine sister species, which occur in sympatry, it has been argued that reproductive isolation may evolve much faster than expected. Such divergence also applies to large, semi-isolated populations, driven by both well-known evolutionary mechanisms and newly discovered genetic processes. Polychaete species provide an excellent taxonomic group to study the evolution of population divergence in marine organisms, given their high plasticity in developmental modes with probable independent evolution (Giangrande 1997). They also exhibit broad geographic ranges and apparently no strict barriers to gene flow. Many sibling species occur in sympatry or parapatry (Knowlton 1993), while allopatric divergence is seldom apparent, like in *Streblospio* spp. (Schulze et al. 2000). As shown in these species mitochondrial DNA can be used to age a species split

and to interpret intraspecific variation in larval development within a phylogeographic framework. A similar approach may be useful for *Scoloplos armiger*, where in this species the sympatric occurrence of the two developmental modes correlated with genetic divergence, suggesting that incipient or concluded sympatric speciation may be involved.

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