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SYMPOSIUM

Polymorphism in Developmental Mode and Its Effect on Population Genetic Structure of a Spionid Polychaete, *Pygospio elegans*

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Synopsis Population genetic structure of sedentary marine species is expected to be shaped mainly by the dispersal ability of their larvae. Long-lived planktonic larvae can connect populations through migration and gene flow, whereas species with nondispersive benthic or direct-developing larvae are expected to have genetically differentiated populations. Poecilogonous species producing different larval types are ideal when studying the effect of developmental mode on population genetic structure and connectivity. In the spionid polychaete *Pygospio elegans*, different larval types have been observed between, and sometimes also within, populations. We used microsatellite markers to study population structure of European *P. elegans* from the Baltic Sea (BS) and North Sea (NS). We found that populations with planktonic larvae had higher genetic diversity than did populations with benthic larvae. However, this pattern may not be related to developmental mode, since in *P. elegans*, developmental mode may be associated with geography. Benthic larvae were more commonly seen in the brackish BS and planktonic larvae were predominant in the NS, although both larval types also are found from both areas. Significant isolation-by-distance (IBD) was found overall and within regions. Most of the pair-wise F_{ST} comparisons among populations were significant, although some geographically close populations with planktonic larvae were found to be genetically similar. However, these results, together with the pattern of IBD, autocorrelation within populations, as well as high estimated local recruitment, suggest that dispersal is limited in populations with planktonic larvae as well as in those with benthic larvae. The decrease in salinity between the NS and BS causes a barrier to gene flow in many marine species. In *P. elegans*, low, but significant, differentiation was detected between the NS and BS (3.34% in AMOVA), but no clear transition zone was observed, indicating that larvae are not hampered by the change in salinity.

Introduction

Marine invertebrates are known for their diverse life-history characteristics and developmental modes (e.g., Mileikovsky 1971; Wilson 1991; Blake and Arnofsky 1999; Ellingson and Krug 2006; Raff and Byrne 2006). Developmental mode includes the dispersal potential of larvae, whether larvae are planktonic, benthic, or brooded, and the larvae may be the primary dispersal stage for invertebrates that are sedentary or sessile as adults. Differences in the dispersal abilities of larvae can affect effective population size, population stability, gene flow, and genetic structure, as well as rate of speciation (e.g., Hart and Marko 2010). As a result, species with planktonic, dispersive larvae are expected to have effective gene flow over a broader geographic area and large panmictic populations. Direct-developing or benthic larvae are expected to have less ability to disperse, and species with these types of larvae are expected have low gene flow and genetically differentiated populations (Palumbi 1994; Bohonak 1999). Empirical studies are often in agreement with these expectations (Hellberg 1996; Arndt and Smith 1998; Kyle and Boulding 2000; Collin 2001; Dawson et al. 2002; Ellingson and Krug 2006), but there are also an increasing number of studies which reach opposite conclusions (Sotka et al. 2004; Bowen et al. 2006;

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Kenchington et al. 2006; Marko et al. 2007; Miller and Ayre 2008; Shanks 2009; Weersing and Toonen 2009; Zhan et al. 2009; Kelly and Palumbi 2010; and see Hellberg 2009). The unexpected results emphasize that other factors, such as environmental tolerances or larval behavior, can affect population genetic structure and suggest that the ability of larvae to disperse may not be the main determinant of population connectivity and genetic structure in marine species. Nevertheless, the ability of larvae to disperse is still considered the most important factor affecting population stability and connectivity of marine species.

Barriers to dispersal and gene flow in the marine environment may not be immediately obvious. Typically, barriers are inferred after examining patterns of genetic structure among populations. More recently, attempts have been made to explicitly combine models of oceanographic conditions together with genetic analysis to understand influences on gene flow (e.g., Selkoe et al. 2010). Combining physical and genetic data in studies of gene flow provide a powerful way of identifying dispersal barriers and to evaluate whether generalizations of the outcomes of larval dispersal are correct. Since dispersal of marine invertebrate larvae is difficult, or impossible, to track directly (Thorrold et al. 2002; Levin 2006), a generalization of potential capability for dispersal based on developmental mode is still commonplace.

Natural barriers to dispersal may exist in transitional zones where environmental variables, such as salinity, are known to change significantly. For example, the Baltic Sea (BS) is a marginal environment for marine species because of its isolation and low salinity. The BS was formed after the last glacial period (10,000–8000 years ago) and during its young history its salinity has fluctuated, allowing marine species to become established there (Zillen et al. 2008; Pereyra et al. 2009). Nowadays, the BS is an ecologically unique, large brackish sea with limited water exchange with the marine North Sea (NS). As a result, there is a salinity gradient from the NS (>30 psu) through the Kattegat (20 psu), Belt Sea (15–18 psu), and Baltic proper (8–10 psu) to the Northern BS (2–3 psu). In addition to low salinity, the BS has very weak tides, lower water temperature, and lower oxygen levels compared to the NS (Helcom 2003). The drastic environmental change seen between the BS and NS may be a restricting factor for successful migration of larvae between these areas. Species with populations spanning this zone (NS, BS, see Fig. 1) provide a way to assess the

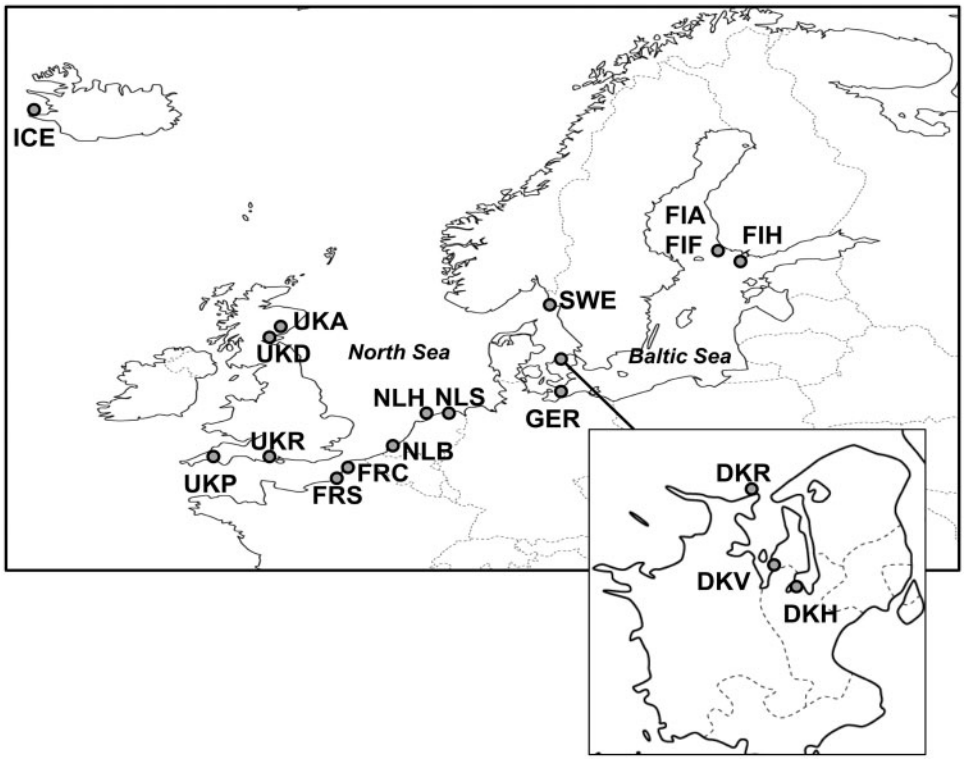

Fig. 1 Map of the sites at which *Pygospio elegans* was sampled (sites are labeled according to their abbreviations in Table 1). Sites FIA and FIF are located in the Finnish archipelago, ~20 km apart.

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relative roles of environmental barriers and potential for larval dispersal to affect gene flow and the genetic structure of populations.

Despite the young geological history of the BS, many Baltic populations have diverged significantly from marine NS populations of the same species. Johannesson and André (2006) conducted a meta-analysis of genetic studies comparing Baltic populations to NS/NE Atlantic populations of the same species. They found that among the 29 species studied, the majority of Baltic populations were statistically less genetically variable and genetically more differentiated from their Atlantic populations. BS populations could be differentiated due to geographic isolation, possible genetic bottlenecks (Nilsson et al. 2001; Härkönen et al. 2005), or adaptation to a different environment (Hemmer-Hansen et al. 2007; Larmuseau et al. 2010; Nissling and Dahlman 2010).

We study the spionid polychaete worm, *Pygospio elegans*, which is found in the NS and BS, exposed to a wide range of salinities. This species is an ideal subject for investigating the relative roles of salinity barriers and developmental mode in determining patterns of population genetics because *P. elegans* is poecilogonous, or polymorphic in developmental mode. *Pygospio elegans* can produce different larval types depending on the number of embryos relative to the number of nurse eggs (a nutritional source) laid by the mother to egg capsules that she broods inside her sand tube (Söderström 1920; Hannerz 1956; Rasmussen 1973; Blake and Arnofsky 1999; J. E. Kesäniemi, personal observation). Planktonic larvae emerge from egg capsules containing a large number of embryos (>20/capsule) and few nurse eggs. At emergence, these larvae are approximately three chaetigers long and have long swimming chaetae. Their pelagic period can last up to 4–5 weeks (Anger et al. 1986) and they actively swim and feed in the plankton (Rasmussen 1973). If only a few embryos (one to two) are laid into the capsules, these will be brooded throughout their development. These benthic larvae feed on the nurse eggs provided by the mother, lack long swimming chaetae and are ready to metamorphose into juveniles soon after emerging from the capsules at the 14–20 chaetiger stage. Intermediate larvae with fewer than 10 larvae per capsule, an intermediate brooding period, and short pelagic phase have also been observed in some populations. Dispersal of larvae is expected to be the primary route for gene flow and to be correlated with developmental mode in *P. elegans*. Adults are motile and will leave their tubes if they are disturbed (J. E. Kesäniemi and K. E. Knott, personal observation), but they quickly build new sand tubes in the sediment (e.g., Mattila 1997) and so are not expected to contribute significantly to dispersal.

Variation in developmental mode in *P. elegans* has been observed both within and among populations, arising from among individual differences rather than variation within the broods of a single individual (Hannerz 1956; Rasmussen 1973; Anger 1984; Gudmundsson 1985; Morgan et al. 1999; J. E. Kesäniemi and E. Geuverink, personal observation). Variation within a population can be seasonal (Hannerz 1956; Rasmussen 1973), but different larval types are also known to occur simultaneously (Rasmussen 1973; Gudmundsson 1985; J. E. Kesäniemi, personal observation). Some populations of *P. elegans* have been reported to be fixed for one developmental mode (either planktonic or benthic) (Anger 1984; Morgan et al. 1999; Bolam 2004), but adults metamorphosing from all larval types are morphologically identical. In addition, studies of allozymes (Morgan et al. 1999) and DNA sequences of the cytochrome c oxidase I gene (Kesäniemi et al. 2012b) provided evidence that *P. elegans* populations with different developmental modes belong to one poecilogonous species.

We examined genetic diversity and population genetic structure in European populations of *P. elegans* with different developmental modes. Our study includes samples over a broad geographic scale, from the Atlantic Ocean (Iceland), through the English Channel and NS to the BS. We wanted to investigate if the transition zone caused by a decrease in salinity between the NS and BS affects population genetic structure in this poecilogonous species. We expected low diversity and low gene flow between populations in the Baltic where benthic larvae are more common. In contrast, we expected high diversity and high effective gene flow among NS populations in which planktonic larvae predominate. We hypothesized that a distinct transition would be visible between these seas, as has been found for many species that span this transition zone (Johannesson and André 2006).

Material and methods

Sampling

We sampled adult *P. elegans* from 18 populations in Europe (Fig. 1). Almost all sampling was conducted during late winter, spring, or early summer of 2010: exceptions included the Swedish sample, which was collected in summer of 2008, and samples from Iceland and Germany, which were collected in 2009. In the nontidal BS, sediment containing *P. elegans* was collected either by scuba diving

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Table 1 Sampling localities, population codes, number of samples genotyped (*n*), observed developmental modes, and estimated density of *Pygospio elegans* (qualitative observations only)

Region	Country	Location	Lat	Long	Population code	*n*	Developmental mode[a]	Estimated density
Baltic Sea	Finland	Ängsö	60.107	21.709	FIA	42	B (A)	Low
		Fårö	59.925	21.772	FIF	39	(A)	Low
		Hanko	59.827	21.772	FIH	40	(A)	Low
	Germany	Germany	54.199	11.840	GER	30		
	Denmark	Vellerup	55.737	11.868	DKV	43	B, I (A)	Medium
		Herslev	55.678	11.987	DKH	42	B, I (A)	Low
		Rorvig	55.965	11.785	DKR	40	I, P (A)	Medium
	Sweden	Gullmar fjord	58.261	11.464	SWE	42		Low
North Sea	Netherlands	Schiermonnikoog	53.472	6.177	NLS	46	B, I, P	Medium/high
		Harlingen	53.167	5.415	NLH	43	P	High
		Breskens	51.386	3.604	NLB	45	P	Medium/high
	France	Canche Bay	50.547	1.598	FRC	43	P	Very high
		Somme Bay	50.227	1.606	FRS	62	P	Very high
	UK	Drum sands	55.994	−3.336	UKD	49	P	High
		Eden estuary	56.365	−2.823	UKA	46		Medium/high
		Plym Bay	50.378	−4.103	UKP	33	P	Low/medium
		Ryde sands	50.734	−1.150	UKR	7		Very low
Atlantic Ocean	Iceland	Iceland	63.978	−22.395	ICE	42	B, I	

[a]Observed larval developmental mode: B = benthic, I = intermediate, P = planktonic, (A) = asexual reproduction.

at 5 m depth (FIA and FIF) or by wading and shoveling the sediment in <1.5 m deep water (FIH and all Danish samples). The German sample was collected from a depth of 18 m using a sediment grab operated from a boat. In the Netherlands, France, and UK, partially exposed sediment was sampled in the intertidal zone during low tides. All of the samples from the Netherlands, UK, and France are called "NS samples," whereas those from Sweden, Denmark, Germany, and Finland are called "BS samples" (Table 1).

Collected sediment was immediately and quickly sieved on location, and sand tubes of *P. elegans* were removed. *Pygospio elegans* emerge from their tubes after disturbance (J. E. Kesäniemi and K. E. Knott, personal observation) and collected tubes were left in seawater in trays without sediment for up to 24 h to allow for such emergence. Afterward, the worms were preserved in ethanol (94–99%) until DNA analysis. During this handling, adults were sexed and examined for the presence of gametes or nurse eggs in the coelom. Tubes were also examined for the presence of egg capsules and brooding females. If capsules were found, the larvae were checked to determine their developmental mode. We did not specifically collect plankton samples, but sea water samples from the collection sites (used for collecting and handling the adults) were examined for the presence of swimming larvae in the laboratory. In addition, we noted whether asexual reproduction was present (indicated by regenerating body segments). According to these observations, we defined a predominant developmental mode for most populations (Table 1). These were consistent with previous reports of observed developmental mode for some populations (Denmark: Rasmussen 1973; Somme Bay: Morgan et al. 1999; Drum Sands: Bolam 2004; Breskens: Rossi et al. 2009), but not others (Ryde Sands: Morgan et al. 1999). Hannerz (1956) reported brooded and intermediate larval types for *P. elegans* in Gullmar Fjord, Sweden, but we could not confirm this since we did not observe reproducing individuals or larvae during our collections at this location.

Molecular methods

DNA was extracted using a Kingfisher magnetic processor (Thermo Fisher Scientific) and Qiagen chemicals or, if the individual was <1 cm in length, Qiagen's DNeasy Blood and Tissue extraction kit with spin columns, following the manufacturer's protocol. All samples were genotyped with eight microsatellite loci described in (Kesäniemi et al. 2012a). Three of these loci (Pe15, Pe17, and Pe18) were amplified using a sequence specific forward primer with additional M13(−21) sequence tail and a fluorescently labeled M13(−21) primer (as described by Schuelke 2000). Amplification was performed in 10 µl reactions with 1 µl of DNA, 1× PCR buffer (Biotools), 200 µM of each dNTP (Fermentas), 8 pmol of reverse primer (TAG Copenhagen), 8 pmol of labeled M13(−21) primer (Applied Biosystems), 2 pmol of the M13(−21) tailed forward

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primer (TAG Copenhagen), 1.5–2 mM of $MgCl_2$ (1.5 mM for Pe15, 2 mM for Pe18 and Pe17) (Biotools), and 0.5 U of *Taq* DNA polymerase (Biotools). Thermocycling conditions were 94°C for 5 min, then 30 cycles of 94°C for 30 s, T_a for 30 s (57°), 72°C for 30 s, followed by eight cycles of 94°C for 30 s, 53°C for 45 s, 72°C for 45 s, ending with a final extension of 72°C for 10 min. The other loci (Pe6, Pe7, Pe12, Pe13, and Pe19) were amplified using two sequence-specific primers, forward primer being labeled. The 10 µl PCR reactions were as above, but 0.5 µM of both primers (one-eighth of the forward primer was labeled, Applied Biosystems) and 1.5–3 mM of $MgCl_2$ (1.5 mM for Pe13, 2 mM for Pe6 and Pe19, 3 mM for Pe7 and Pe12) were used. Thermocycling conditions were: 94°C for 5 min, then 35 cycles of at 94°C for 30 s, T_a for 30 s (54° for Pe6, Pe7, Pe19; 55° for Pe12; 58° for Pe13), 72°C for 30 s, followed by a final extension of 10 min at 72°C. Products were denatured with formamide, separated using an ABI PRISM 3130xl and genotyped using GeneMapper v.3.7 software (all Applied Biosystems).

Analysis

We used Arlequin v. 3.5.1.2 (Excoffier and Lischer 2010) to examine allele frequencies, observed and expected heterozygosity at all loci, and to test conformation to Hardy–Weinberg expectations (HWE). The exact test option was used and *P*-values were adjusted for multiple tests with Bonferroni correction (Rice 1989). Frequencies of null alleles in the loci were estimated with FreeNA (Chapuis and Estoup 2007). Linkage disequilibrium between pairs of loci within populations was tested with Genepop (Raymond and Rousset 1995). Allelic richness and private allelic richness were calculated with HP-Rare (Kalinowski 2005), which uses rarefaction to account for the different sample sizes in the study populations (UKR was excluded from this analysis because of its considerably lower sample size).

To compare genetic diversity between the two regions, the sample locations were divided into two groups (BS and NS; Table 1), excluding the Atlantic population from Iceland. Heterozygosity, gene diversity, and allelic richness in these groups were compared using a Mann–Whitney U-test. Genetic diversity was also compared between the populations that produce planktonic larvae (NLH, NLB, FRC, FRS, UKD, UKP) and the populations that in addition have benthic larvae (FIA, DKV, DKR, DKH, NLS, ICE). Populations for which developmental mode is not known (FIF, FIH, GER, SWE, UKA) were not included in this latter comparison.

Population genetic differentiation was analyzed using population pair-wise F_{ST} calculations using Arlequin (10,000 permutations) and Jost's (2008) pair-wise mean D_{est} calculated in the R package DEMEtics (Gerlach et al. 2010). To determine the proportion of genetic differentiation distributed among areas, a hierarchical analysis of molecular variance (AMOVA) was also conducted with Arlequin (10,000 permutations). In addition, we employed a Bayesian approach to investigate the number of genetic clusters in our sample with the program Structure v.2.3 (Pritchard et al. 2000). In these analyses, we used an admixture model with correlated alleles, and Markov-chain Monte–Carlo (MCMC) sampling with a burn-in of 150,000 followed by 300,000 iterations. Data from six of the microsatellite loci were included: Pe12 and Pe15 were removed due to HWE deviations in most populations. Initially, *K*-values from 3 to 18 were tested, each with two replicates. Afterward, the program was re-run with *K*-values from 4 to 10, each with six replicates. Population information was used as prior information, since it was informative ($r>1$). Furthermore, the number of genetic clusters and their genetic boundaries were also examined with the R package Geneland (Guillot et al. 2005). In addition to using the genetic data, this program incorporates spatial information of the sampling locations (geographic coordinates) while estimating *K*. A spatial model and correlated frequency model were used, and the presence of null alleles was taken into account (Guillot et al. 2008). Ten independent runs with 200,000 MCMC iterations were performed.

Isolation-by-distance (IBD) was measured by Mantel tests and spatial autocorrelation tests using GenAlEx v.6.4 (Peakall and Smouse 2006). Both tests used the default genetic distance as calculated by GenAlEx in which an individual-by-individual squared distance is calculated based on the multilocus genotype (see Smouse and Peakall 1999). Geographic distances were calculated from latitude and longitude of the sampled population (all individuals in a population had identical geographic coordinates). Mantel tests and autocorrelation tests were performed for the whole data set, as well as for smaller spatial scales: NS; BS; Northern BS (Finnish populations only); and Southern BS (Danish populations + GER + SWE). Additionally we tested the correlation between linearized population pair-wise F_{ST} values (calculated in GenAlEx) with population pair-wise geographic distances (linear distances following water-routes between sampling sites measured in Google Earth 6 [http://www.google.com/earth/index.html]).

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To investigate whether there is a genetic break between the BS and the NS we conducted a graphical analysis similar to that performed by Johannesson and André (2006) in their meta-analysis. The analysis is based on genetic differentiation (F_{ST}) between the innermost Baltic population (FIA) and the other populations at increasing geographic distances. For this analysis, following Johannesson and André (2006), the Skagerrak and Kattegat areas were defined to be outside the Baltic area, and we set the German sample to represent the entrance to the BS.

Recent migration rates were examined with two Bayesian methods, BayesAss 3 (Wilson and Rannala 2003) and BIMr v.1.0 (Faubet and Gaggiotti 2008). Since BayesAss assumes relatively low migration rates (a maximum of one-third of the population can be migrants), we also used BIMr (no restrictions on migration) to see if the results of the two methods were similar. For these analyses, the data set was divided into three subsets (to improve performance of the methods). The first data set included the three Finnish populations, the second included Denmark and Sweden, and the third included all of the populations from the Netherlands, UK, and France. For this third data set, some populations were combined, based on the nonsignificant F_{ST} values between them: UKR and UKP ($F_{ST} = 0.0243$); FRC and FRS ($F_{ST} = 0.0041$); and NLB and NLH ($F_{ST} = 0.0048$). NLS was not included in any grouping since it was significantly differentiated from the other populations in this data set. In all BayesAss analyses 1×10^8 iterations of MCMC sampling were run after an initial burnin (1×10^7–5×10^7). Using BIMr, the Finnish data set had 5×10^5 samples and 1×10^6 burnin, and the other data sets were run with 1×10^6 samples and burnin of 1×10^6. In all analyses, at least three runs were conducted for each data set to verify consistent results.

Results

Genetic diversity

A total of 734 *P. elegans* individuals from 18 locations were genotyped. Table 2 shows descriptive statistics for each population and locus. The allelic richness across loci per population ranged from 8.66 to 15.65 (corrected for sample size, UKR excluded) and the number of alleles per locus varied among the populations. Heterozygosity was also high (mean H_E ranging from 0.586 to 0.816 and mean H_O ranging from 0.523 to 0.721). Significant deviations from HWE were observed in six loci in some of the populations and all deviations were caused by heterozygote deficiency. Loci Pe15 and Pe12 proved to deviate from HWE in most populations, so some of the analyses were conducted both with, and without, these loci. Analysis with FreeNA confirmed high null allele frequency for locus Pe12, but moderate or low null allele frequencies for the other loci and populations with HWE deviations. FreeNA implements an ENA correction (Chapuis and Estoup 2007) to samples with null alleles and estimates F_{ST} for both the corrected data and original data. Since F_{ST} estimates were similar either with or without the ENA correction ($F_{ST} = 0.0408$ and $F_{ST(ENA)} = 0.0377$), we assumed that possible null alleles cause no bias to our F_{ST} estimates. Linkage disequilibrium was observed between some pairs of loci (1.8% of all comparisons) in three of the study populations: Sweden (Pe7 × Pe17, Pe15 × Pe17, Pe7 × Pe18, Pe17 × Pe18, Pe7 × Pe13, Pe13 × Pe17), Iceland (Pe7 × Pe13, Pe12 × Pe13), and DKR (Pe15 × P13). Since linkage disequilibrium was not consistent in all populations, we chose not to exclude the loci from the analyses.

When comparing geographic areas, NS populations had significantly higher allelic richness (Md NS = 14.87, BS = 12.35, $N = 16$, $U = 62.00$, $P = 0.002$) and expected heterozygosity (Md NS = 0.801, BS = 0.717, $N = 17$, $U = 62$, $P = 0.012$) than did BS populations. NS populations also had higher observed heterozygosity, gene diversity, and private allelic richness (private alleles are found only from one population), but these results were not statistically significant. Populations with planktonic larvae had higher allelic richness (Md planktonic = 15.19, Md benthic = 12.4, $U = 36$, $N = 12$, $P = 0.004$), expected heterozygosity (Md planktonic = 0.801, Md benthic = 0.717, $N = 12$, $U = 36$, $P = 0.004$) and gene diversity (Md planktonic = 0.740, Md benthic = 0.661, $P = 0.004$) than did populations that also have benthic larvae. Since the populations with different developmental modes are mainly distributed in different geographic areas, the planktonic–benthic groups are similar to those in the NS–BS comparison; however, there are fewer populations in the planktonic–benthic groups, and Iceland and NLS from the Netherlands are included with the populations with benthic larvae.

Population genetic structure

Spatial genetic structure (i.e., significant F_{ST} values among populations) was present among the European *P. elegans* populations, even on a small geographic scale. Overall, pair-wise F_{ST} estimates ranged from 0.001 to 0.170. The geographically

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Table 2 Genetic variation in 18 *Pygospio elegans* populations

	Population																	
Locus	UKP	UKR	FRS	FRC	NLB	NLH	NLS	UKA	UKD	SWE	DKR	DKV	DKH	GER	FIH	FIF	FIA	ICE
Pe6																		
N_a	6	2	8	10	7	10	4	10	7	6	3	4	5	8	4	3	3	4
H_E	0.370	0.143	0.394	0.489	0.248	0.354	0.108	0.446	0.377	0.222	0.267	0.274	0.201	0.332	0.074	0.169	0.301	0.239
H_O	0.424	0.143	0.387	0.581	0.266	0.372	0.111	0.478	0.408	0.238	0.307	0.309	0.190	0.333	0.075	0.179	0.341	0.238
Pe7																		
N_a	21	9	29	23	30	27	29	21	26	20	18	21	25	25	26	26	28	19
H_E	0.944	0.923	0.943	0.924	0.945	0.943	0.953	0.941	0.942	0.927	0.917	0.931	0.946	0.964	0.956	0.954	0.949	0.929
H_O	0.818	0.857	0.887	0.930	0.888	0.860	0.869	0.945	0.878	0.881	0.897	0.884	0.738	0.733	0.900	0.872	0.905	0.881
Pe12																		
N_a	20	3	33	23	27	26	26	24	31	18	18	20	18	19	25	23	28	16
H_E	0.949	0.750	0.961	0.944	0.963	0.960	0.974	0.955	0.957	0.937	0.934	0.953	0.944	0.950	0.957	0.924	0.954	0.900
H_O	0.740	0.500	0.593	0.882	0.694	0.639	0.818	0.594	0.800	0.538	0.700	0.777	0.616	0.600	0.919	0.766	0.838	0.684
Pe13																		
N_a	24	11	33	24	31	29	24	27	31	22	27	26	30	26	24	29	30	17
H_E	0.950	0.967	0.959	0.951	0.960	0.957	0.955	0.954	0.963	0.944	0.955	0.948	0.969	0.966	0.944	0.963	0.961	0.862
H_O	0.848	0.714	0.688	0.791	0.755	0.721	0.891	0.791	0.755	0.809	0.976	0.974	0.881	0.700	0.825	0.816	0.809	0.857
Pe15																		
N_a	24	9	35	30	32	33	24	29	31	24	30	31	28	23	24	23	28	15
H_E	0.957	0.934	0.969	0.967	0.970	0.967	0.907	0.962	0.963	0.944	0.946	0.958	0.953	0.956	0.940	0.945	0.955	0.884
H_O	0.697	0.571	0.737	0.750	0.822	0.721	0.666	0.696	0.829	0.571	0.650	0.881	0.667	0.800	0.692	0.622	0.756	0.619
Pe17																		
N_a	14	6	17	16	15	14	9	17	19	8	9	12	6	12	3	6	7	5
H_E	0.902	0.681	0.851	0.827	0.828	0.881	0.590	0.838	0.885	0.763	0.613	0.667	0.553	0.826	0.143	0.271	0.416	0.708
H_O	0.742	0.571	0.508	0.714	0.666	0.558	0.565	0.435	0.612	0.619	0.447	0.561	0.316	0.643	0.150	0.243	0.500	0.536
Pe18																		
N_a	8	2	11	11	7	13	9	11	9	4	5	5	8	6	3	6	6	3
H_E	0.549	0.264	0.620	0.678	0.577	0.736	0.676	0.737	0.669	0.577	0.606	0.684	0.709	0.694	0.488	0.576	0.663	0.390
H_O	0.290	0.000	0.339	0.447	0.429	0.558	0.659	0.533	0.396	0.293	0.589	0.744	0.658	0.348	0.474	0.553	0.714	0.429
Pe19																		
N_a	8	4	13	9	6	10	8	10	9	5	6	3	5	8	5	6	6	2
H_E	0.583	0.659	0.733	0.664	0.653	0.727	0.593	0.646	0.621	0.447	0.257	0.420	0.466	0.518	0.189	0.437	0.516	0.312
H_O	0.515	0.571	0.613	0.674	0.600	0.767	0.435	0.630	0.638	0.512	0.282	0.381	0.452	0.533	0.150	0.487	0.667	0.333
$N_{A(44)}$	13.76	–	15.42	14.60	15.00	15.65	13.67	14.73	15.38	11.36	11.86	12.16	12.65	14.39	11.65	12.54	13.32	8.66
$N_{PA(44)}$	0.21	–	0.68	0.22	0.37	0.24	0.77	0.36	0.35	0.24	0.21	1.18	0.40	0.42	0.28	0.22	0.20	0.14
GD	0.715	0.653	0.774	0.741	0.715	0.780	0.669	0.768	0.738	0.682	0.622	0.678	0.652	0.730	0.557	0.588	0.680	0.628
Mean H_E	0.776	0.665	0.804	0.806	0.768	0.816	0.720	0.810	0.798	0.720	0.687	0.729	0.718	0.776	0.586	0.655	0.715	0.653
Mean H_O	0.635	0.491	0.594	0.721	0.637	0.650	0.627	0.636	0.667	0.558	0.606	0.689	0.565	0.586	0.523	0.567	0.691	0.572
F_{IS}8loci	0.185	0.280	0.259	0.106	0.172	0.206	0.131	0.217	0.165	0.228	0.120	0.056	0.216	0.240	0.109	0.136	0.033	0.125
F_{IS}6loci	0.156	0.228	0.241	0.089	0.151	0.168	0.090	0.169	0.170	0.138	0.033	0.018	0.160	0.238	0.080	0.066	−0.034	0.049

N_a = number of alleles, H_E = expected heterozygosity, H_O = observed heterozygosity (values with significant departures from HWE are underlined), N_A = allelic richness (based on 44 samples), calculated with HP-Rare. N_{AP} = private allelic richness, based on 44 samples, GD = gene diversity, F_{IS} = inbreeding coefficient, calculated using the whole data set (8loci), and with 6loci (Pe12 and Pe15 removed) (significant values underlined).

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distant Iceland seemed to be most isolated genetically, having the highest pair-wise F_{ST} values (Table 3). On a small scale (within Finland), the pair-wise F_{ST} values were low (0.008–0.028) but significant, indicating genetic structure between the locations. Among the three Danish populations, the F_{ST} values were also low (from 0.006 to 0.010) and the two sample sites within the fjords were not significantly differentiated from each other, but they were significantly differentiated from DKR, the population sampled from the entrance of the fjords (Fig. 1). Interestingly, the German population seemed to be more differentiated from the other Baltic populations than from the NS populations. Some of the geographically close NS populations with planktonic larvae were observed to be genetically similar (nonsignificant F_{ST} values, Table 3). The mean D_{est} estimations were similar to the results of the pair-wise F_{ST} analysis (data not shown). Most of the nonsignificant D_{est} values were between populations within the NS (Table 3). Unlike the F_{ST} results, the UKR population showed more nonsignificant pair-wise D_{est} values, but this might be due to the lower sample size in UKR.

Geneland suggested the presence of eight genetic populations in Europe. Not surprisingly, Iceland formed its own group. In the NS, four clusters with clear genetic boundaries were identified. The two Scottish populations were clustered together (UKD and UKA), and so were the two English Channel populations (UKP and UKR). The two French populations were grouped with the two Dutch populations which had predominantly planktonic larvae (FRS and FRC + NLB and NLH), and there was a clear boundary with NLS (which formed its own cluster), although the latter is geographically close to NLH. Within the BS there were three genetic clusters, but the boundaries among these were more ambiguous. The Finnish populations formed one cluster, which was clearly separated from the others. The German sample formed its own cluster, but alternatively could have been clustered with the Danish samples. The three Danish populations grouped with high probability with the Swedish population, but alternatively the Swedish sample could also have been clustered with NLS. Similar clustering was seen in the Structure analysis, which estimated the presence of nine genetic clusters. As in the Geneland analysis, Structure assigned geographically close populations together, and both analyses distinguished NLS from the other two Netherlands populations and suggested that the German population differs from the other Baltic populations. However, the likelihood values for different K were

Table 3 Population pair-wise F_{ST} values for *Pygospio elegans* in Europe

Code	UKR	UKP	FRS	FRC	NLB	NLH	NLS	UKA	UKD	SWE	DKR	DKV	DKH	GER	FIH	FIF	FIA	ICE
UKR	0																	
UKP	**0.024**	0																
FRS	0.041	0.016	0															
FRC	0.035	0.017	**0.004**	0														
NLB	0.034	0.017	**0.005**	0.009	0													
NLH	0.046	0.014	**0.002**	0.008	**0.005**	0												
NLS	0.026	0.035	0.047	0.039	0.047	0.048	0											
UKA	0.066	0.026	0.024	0.023	0.029	0.021	0.054	0										
UKD	0.058	0.027	0.029	0.029	0.034	0.023	0.050	≥**0.001**	0									
SWE	0.050	0.024	0.037	0.059	0.031	0.036	0.033	0.050	0.050	0								
DKR	0.045	0.035	0.052	0.038	0.054	0.058	0.019	0.058	0.055	0.033	0							
DKV	0.043	0.033	0.042	0.034	0.046	0.046	0.016	0.049	0.046	0.027	0.010	0						
DKH	0.035	0.031	0.039	0.032	0.043	0.046	0.011	0.041	0.040	0.031	0.009	**0.006**	0					
GER	0.058	0.013	0.016	0.022	0.016	0.013	0.044	0.016	0.024	0.021	0.042	0.033	0.031	0				
FIH	0.071	0.098	0.109	0.098	0.107	0.121	0.048	0.113	0.109	0.083	0.034	0.056	0.042	0.105	0			
FIF	0.039	0.061	0.068	0.061	0.067	0.079	0.025	0.076	0.074	0.053	0.023	0.025	0.017	0.066	0.016	0		
FIA	0.029	0.048	0.060	0.046	0.063	0.069	0.018	0.063	0.061	0.047	0.019	0.020	0.009	0.055	0.028	0.008	0	
ICE	0.106	0.055	0.082	0.091	0.074	0.077	0.102	0.096	0.099	0.059	0.104	0.095	0.101	0.064	0.170	0.131	0.124	0

Note that most of the comparisons are significant and only the F_{ST} comparisons with nonsignificant *P*-values are underlined and bold. Comparisons with nonsignificant Jost D_{est} values are on a gray background (D_{est} values not shown).

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very similar, indicating possible problems with the data, likely explained by a pattern of IBD (see below).

According to the AMOVA analysis, most microsatellite variation in European *P. elegans* resides within the populations (93.3%, $P<0.001$). Low, but significant, genetic differentiation was seen between BS and NS groups (3.34%, $P<0.001$), suggesting the presence of variation also on a larger scale. F_{ST} among the NS populations (0.020) was somewhat lower than among the BS populations ($F_{ST}=0.028$), but AMOVA indicated significant within-group structure as well (2.35%, $P<0.001$). A plot of linearized population pair-wise F_{ST} against geographic distance (Fig. 2) indicates that population genetic structure can be explained by a pattern of IBD ($r=0.687$, $P<0.001$). Similarly, Mantel tests of correlations between genetic distance and geographic distance matrices were significant for the whole data set as well within areas: NS, BS, Southern BS (all $P<0.01$); but not within the Northern BS (Finnish populations only). Spatial autocorrelation was positive and significant in only the smallest distance classes estimated for each group (data not shown) also indicating a pattern of IBD.

Using the methods of Johannesson and André (2006), we did not find a strong genetic shift between the BS and NS (Fig. 3). However, we do see increased divergence (F_{ST}) with distance from the northern Baltic, a trajectory resembling a combination of the IBD model and the peripheral-perturbation model in which the Northern Baltic populations are differentiated from all other populations because of their marginal location.

Migration patterns

In Finland, the two methods used to estimate gene flow produced similar results. The benthic FIA population receives the highest amount of migration. The mean migration rates into FIA from FIF were 0.17–0.37 and from FIH 0.10–0.15, but the reverse migration rates from FIA to these populations were lower. BIMr estimated a higher migration rate from FIA to FIF than did BayessAss (0.15 versus 0.03). In Denmark, the results were also fairly consistent between the two methods, showing a high rate of self-recruitment in most populations. Both methods suggested high migration rate from DKR to the inner parts of the fjords (DKV and/or DKH) and migration rate from Sweden to Denmark was low. Among the NS samples, a surprisingly high level of self-recruitment was seen. With BayesAss, the highest outward migration rates were from the French populations, suggesting they are source populations. There were high migration rates from France to the nearby Netherlands sample (NLB and NLH combined) and also to the UK populations across the English Channel and to the northern parts of the UK. However, migration rates in the opposite direction (into France) were very low. On the other hand, BIMr estimated high symmetrical migration rate between France and the planktonic Dutch populations (NLB + NLH), but no migration from France to the other sites. It also estimated zero migration into the UK populations, which is inconsistent with the BayesAss results. The NLS population was estimated to have low migrant proportions and low levels of outward migration to the other populations (using both methods) (Table 4).

Discussion

If a correlation between dispersal ability and larval developmental mode exists, population genetic structure of marine invertebrate species is expected to show a predictable pattern: species with planktonic, dispersive larvae should have larger populations with high genetic diversity and effective gene flow, whereas species with benthic, brooded larvae should have considerable population genetic structure due to limited gene flow among smaller, less diverse populations (e.g., Ellingson and Krug 2006; Lee and Boulding 2009; Binks et al. 2011). We investigated whether the expected correlation between developmental mode, dispersal, and population genetic structure would be predictable for the polychaete *P. elegans*, a poecilogonous species that displays variation in developmental mode primarily among populations, but also within populations. We confirmed our hypothesis that *P. elegans* populations with predominantly planktonic developmental mode had higher genetic diversity than did populations that also had benthic larvae. The same result was found previously using DNA sequence data from the mitochondrial gene cytochrome c oxidase subunit I (Kesäniemi et al. 2012b). However, higher diversity in the populations with planktonic larvae could be explained by factors other than developmental mode.

First, higher genetic diversity in the populations with planktonic larvae could be explained by a larger effective population size (N_e) in these populations. Although we do not have estimates of N_e, all of the populations with predominantly planktonic larvae also appeared to have a high density of worms. Higher N_e could also explain the higher number of private alleles we observed in most of

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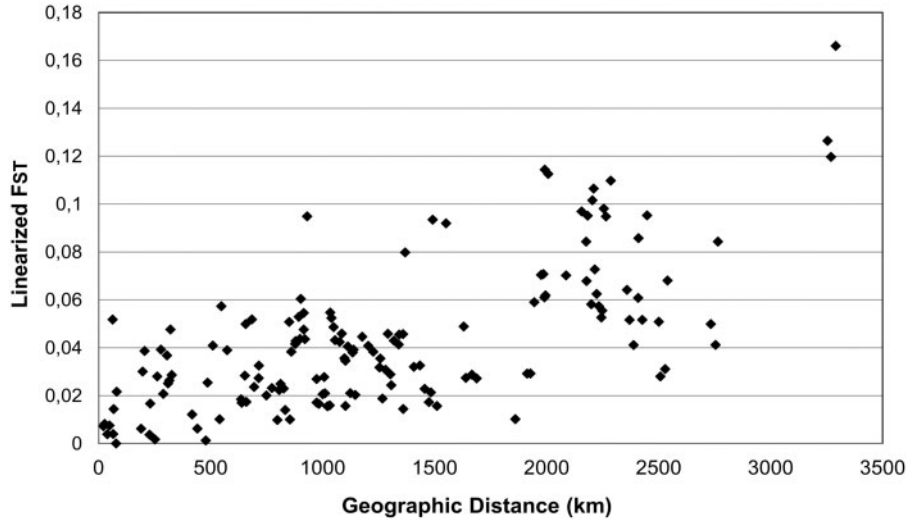

Fig. 2 Scatterplot showing the isolation-by-distance pattern between pair-wise genetic distance (linearized F_{ST}: $F_{ST}/(1-F_{ST})$) and geographic distance (km).

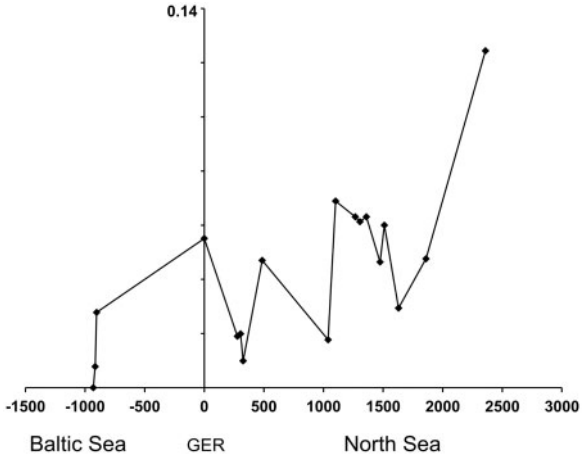

Fig. 3 Genetic differentiation (pair-wise F_{ST} values, *y*-axis) between the innermost Baltic population (FIA) and other populations at increasing distances. The *x*-axis shows distance (km) from the population at the entrance to the Baltic Sea (our GER sample at zero), with negative values going toward the northern Baltic and positive values outside the Baltic through the Kattegat.

the NS populations (although these were not significantly higher). These observations are only qualitative, so a relationship between high density/high N_e and high genetic diversity would need to be addressed with more quantitative methods. However, higher genetic diversity achieved by retention of alleles in large populations has been proposed for other species with planktonic larvae (e.g., Lee and Boulding 2009).

Second, asexual reproduction observed in the Baltic populations of *P. elegans* (Rasmussen 1953; Anger 1984; J. E. Kesäniemi and E. Geuverink, personal observation) could also lower their genetic diversity and complicate interpretations of our findings. Asexual reproduction has not been observed in *P. elegans* in the NS (Morgan et al. 1999; Bolam 2004; K. E. Knott and E. Geuverink, personal observation). The lack of asexual reproduction in the NS could be explained by a higher density of worms in these populations. Wilson (1983) found that in the laboratory, the proportion of asexually reproducing *P. elegans* increased when worms were reared at low densities, and this may also occur in nature. Despite asexual reproduction in some populations, none of the sampled individuals appeared to be clones (multilocus genotypes were not shared); there were, however, significant deviations from HWE and positive F_{IS} values for the loci we studied in most populations, indicating that processes increasing population identity by descent may be common in *P. elegans*. Inbreeding, within population genetic structure (the Wahlund effect) and high local recruitment, in addition to asexual reproduction, could lead to deviations from HWE. These processes may be more common in populations with benthic larvae than in populations with planktonic larvae, but other results (discussed below) indicated that at least high local recruitment was common in most populations. High positive F_{IS} values and deviations from HWE have been noted in other population genetic analyses of marine invertebrates (Addison and Hart 2005; Zhan et al. 2009).

Finally, the difference in genetic diversity could exist because of the association of developmental mode and geographic location. Initial survey of predominant developmental mode indicated that benthic larvae may be more common in the BS (Table 1). However,

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Table 4 Migration rates among *Pygospio elegans* populations within three areas analyzed separately (Northern Baltic Sea, Southern Baltic Sea, and North Sea)

To	From				
Northern Baltic Sea					
	FIA	FIF	FIH		
FIA	*0.68/0.53* (0.01/0.13)	0.17/0.37 (0.07/0.14)	0.15/0.10 (0.07/0.07)		
FIF	0.03/0.15 (0.02/0.12)	*0.90/0.79* (0.06/0.12)	0.07/0.06 (0.06/0.05)		
FIH	0.01/0.06 (0.01/0.08)	0.11/0.19 (0.05/0.13)	*0.88/0.75* (0.05/0.01)		
Southern Baltic Sea					
	DKV	DKH	DKR	SWE	
DKV	*0.68/0.51*(0.01/0.08)	0.01/0.18 (0.01/0.08)	0.29/0.22 (0.02/0.08)	0.02/0.09 (0.02/0.05)	
DKH	0.02/0.00 (0.02/−)	*0.70/1.00* (0.02/−)	0.27/0.00 (0.03/−)	0.01/0.00 (0.01/−)	
DKR	0.02/0.00 (0.01/−)	0.02/0.00 (0.01/−)	*0.86/1.00* (0.03/−)	0.10/0.00 (0.03/−)	
SWE	0.11/0.00 (0.03/−)	0.01/0.00 (0.01/−)	0.02/0.00 (0.02/−)	*0.86/1.00* (0.03/−)	
North Sea					
	FRC + FRS	NLB + NLH	NLS	UKA + UKD	UKP + UKR
FRC + FRS	*0.96/0.75* (0.02/0.06)	0.00/0.15 (−/0.06)	0.01/0.01 (0.01/0.01)	0.03/0.05 (0.02/0.03)	0.00/0.04 (−/0.03)
NLB + NLH	0.30/0.14 (0.01/0.06)	*0.67/0.73* (−/0.06)	0.01/0.02 (0.01/0.01)	0.02/0.03 (0.01/0.02)	0.00/0.01 (−/0.05)
NLS	0.04/0.00 (0.02/−)	0.01/0.00 (0.01/−)	*0.93/1.00* (0.02/−)	0.01/0.00 (0.01/−)	0.01/0.00 (0.01/−)
UKA + UKD	0.11/0.00 (0.03/−)	0.00/0.00 (−/−)	0.01/0.00 (0.01/−)	*0.87/1.00* (0.03/−)	0.00/0.00 (0.00/−)
UKP + UKR	0.22/0.00 (0.05/−)	0.01/0.00 (−/−)	0.06/0.00 (0.03/−)	0.04/0.00 (0.03/−)	*0.67/1.00* (0.01/−)

First values are from BayesAss, the second from BIMr and the respective standard deviations are in brackets (SD values <0.00 are marked with a −). The source populations for migration are given in columns and populations receiving migrants are in rows. Migration values along the diagonal axis are the proportions of individuals with local recruitment (underlined and in italics).

there are exceptions; all developmental modes are observed in NLS, and benthic larvae have been reported for UKR and UKP (Morgan et al. 1999). We found lower genetic diversity in the BS populations compared to those in the NS, but the pattern may not be related to developmental mode. Lower diversity in the Baltic is not unexpected, and is known for marine plants, invertebrates (see Johannesson and André 2006), and vertebrates (Nielsen et al. 2003; Säisä et al. 2005; Hemmer-Hansen et al. 2007). Nevertheless, exceptional populations in the NS that also had benthic larvae, (NLS and possibly UKP and UKR), did have somewhat lower genetic diversity than did other NS populations in which only planktonic larvae were found.

In order to tease out the potential factors influencing diversity in this system, additional analyses contrasting populations which differ only in developmental mode, but not in habitat characteristics, density, or propensity for asexual reproduction would be required. A comparison between populations NLS and NLH perhaps provide one such example, but replicate comparisons would be necessary. Alternatively, concomitant analyses of genetic data and environmental data may provide a way to identify the most probable factors influencing genetic patterns (e.g., Case et al. 2005; Banks et al. 2007; Dionne et al. 2008; Gaggiotti et al. 2009).

Given our hypothesis that developmental mode influences the genetic structure of populations, we expected to find significant genetic differentiation only among the Baltic populations with benthic developmental modes. However, significant genetic structure among populations in both the BS and NS were detected (AMOVA). Pair-wise F_{ST} and D_{est} analyses also indicated significant population differentiation between almost all populations (Table 3). In Finland, all three samples were genetically differentiated, despite short geographic distances between their locations (20–100 km). Here *P. elegans* is strongly associated with seagrasses, and its distribution is likely to be patchy due to the highly fragmented distribution of the sea grass *Zostera marina* in the Finnish archipelago (Boström et al. 2006). In a patchy habitat, brooded, nondispersive larvae should be favored as they would maintain local recruitment (Levin 1984; Pechenik 1999). In the Southern Baltic, where populations show multiple developmental modes, genetic differentiation is also seen, but not among all samples. Interestingly,

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the German population, sampled from deeper water, is more similar to the NS populations than it is to the Baltic populations and it also has higher diversity than do other Baltic populations.

Only a few *P. elegans* populations were not significantly differentiated according to the pair-wise F_{ST} and D_{est} comparisons, and these included primarily the populations with planktonic larvae in the English Channel and adjacent populations in the Wadden Sea (UKP and UKR; FRS, FRC, NLB and NLH). Nevertheless, there was genetic differentiation between the English Channel (UK) populations and those from the French and Dutch sites. Notably, NLS, from which all larval developmental modes have been found, was significantly differentiated from the nearby population NLH, in which planktonic larvae dominate. Clearly, in *P. elegans*, additional factors other than developmental mode must affect the patterns of genetic structure among populations. In analyses of allozyme data, Morgan et al. (1999) found genetic structure in the English Channel to differ between the French coast and the southern coast of UK, probably because of a hydrographic barrier.

Given the many pair-wise population comparisons showing significant genetic differentiation, long-distance dispersal might not be successful in this species. Planktonic larvae of *P. elegans* are expected to have significant dispersal potential since they can live for 4–5 weeks in the plankton (Anger et al. 1986). However, high larval mortality (Pedersen et al. 2008), or higher-than-expected local recruitment could determine realized dispersal in this species. Our estimates of migration rates revealed surprisingly high estimates of self-recruitment in all populations, including those with primarily planktonic larvae (Table 4). As a result, realized dispersal appears not be tightly correlated with developmental mode and the expected association between larval developmental mode and genetic structure of populations is not clear-cut. In principle, oceanic currents can transport different life-stages over large distances, but currents can also promote local differentiation that can help to explain population genetic patterns (Knutsen et al. 2004; Fievet et al. 2006; Kenchington et al. 2006; White et al. 2010). Local habitat characteristics; such as the estuarine environment, or behavioral factors can also affect retention of the larval stage, as well as population genetic structure (Levin 1986; Palumbi 1994; Metaxas 2001; Sponaugle et al. 2002; Swearer et al. 2002). For example, Bolam (2004) suggested that the *P. elegans* larvae in Drum Sands (UKD) might settle locally because of local hydrodynamic conditions. Further study of larval behavior of *P. elegans* would be worthwhile to pursue in order understand limitations on dispersal in this species.

In our study, we found significant IBD across the entire region (Fig. 2) and within both the BS and the NS, regardless of the larval developmental mode. In previous studies, IBD is more often found in species with nondispersive larvae (Duran et al. 2004). For example, in bryozoans, species with planktonic larvae had lower population genetic structure and low or absent IBD, whereas species with nondispersive larvae showed higher genetic differentiation among populations combined with a pattern of IBD (Goldson et al. 2001; Watts and Thorpe 2006). Hellberg (1996) saw a similar pattern in corals from the coast of California. Together, our results of significant IBD on multiple scales, significant genetic autocorrelation at the smallest geographic distance classes (within populations), and high estimated local recruitment support a conclusion of limited dispersal by planktonic larvae of *P. elegans*. In terms of dispersal, the different developmental modes of this species may actually be very similar.

Focusing our geographic study on the natural environmental transition in salinity between the NS and the BS, we also saw evidence of IBD. *Pygospio elegans* did not show strong differentiation over this transition, whereas genetic differentiation is commonly seen in other species with populations in both areas (Bekkevold et al. 2005; Johannesson and André 2006; O'Leary et al. 2007; Wiemann et al. 2010). Additional sampling between Germany and Finland would provide a more rigorous test of the geographic pattern. However, the result implies that the change in salinity does not impose a barrier to gene flow for *P. elegans*. Johannesson and André (2006) noted that the patterns of loss of diversity among regions and the strength of IBD were not different among species with different dispersal potential. However, Hemmer-Hansen et al. (2007) compared population genetic structure of European flounder (*Platichthys flesus* L.) with plasticity in egg type, a phenomenon uncommonly seen in fish. This species spawns benthic eggs in the northern Baltic, but "normal pelagic eggs" in the southern BS and in the NS. A genetic barrier suggesting restricted gene flow was found for the flounder between these areas and was associated with the difference in developmental mode.

The apparent association of developmental mode with habitats of low salinity may indicate that different developmental modes may be advantageous under different environmental conditions.

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Hannerz (1956) and Rasmussen (1973) hypothesized that environmental variation and developmental plasticity would explain larval phenotypic variation in *P. elegans*. However, Anger (1984) did not find plasticity in developmental mode in experiments manipulating temperature and salinity. Environmental variation is known to affect poecilogony in some species, for example, the sea slug *Alderia willowi* (Krug 2007). Also, geographical patterns in poecilogony are not uncommon. In another poecilogonous polychaete, *Boccardia proboscidea*, females at higher latitudes along the western coast of North America invest more in larval nutrition and produce a larger proportion of brooded larvae (Oyarzun et al. 2011).

We examined the role of developmental mode and environment in a broad survey of genetic structure among populations of *P. elegans* extending over a region with extremes of salinity (from the NS to the BS). We found that most genetic variation existed within populations, but that there was significant genetic differentiation at a large spatial scale (across the entire region studied) as well as at moderate scales (within subregions: NS and BS). We observed a significant trend of IBD at both the broadest and regional scales, and the genetic structure of populations did not appear to be affected by the transition zone of changes in salinity, or by the predominant developmental mode of the populations. However, the association of developmental mode and geographic location makes it difficult to separate the effects of these two factors. Our results raise questions about the assumed dispersal potential of the planktonic larvae of *P. elegans*. Local recruitment was estimated to be high regardless of developmental mode or habitat, and could be explained by high mortality rates for planktonic larvae (Pedersen et al. 2008). Alternative explanations of genetic structure in populations with planktonic larvae, such as possible sweepstakes reproductive success (Hedgecock 1994) or temporal Wahlund effects should be investigated.

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References

Addison JA, Hart MW. 2005. Spawning, copulation and inbreeding coefficients in marine invertebrates. Biol Lett 1:450–3.

Anger V. 1984. Reproduction in *Pygospio elegans* (Spionidae) in relation to its geographical origin and to environmental conditions: a preliminary report. Forts Zool 29:45–51.

Anger K, Anger V, Hagmeier E. 1986. Laboratory studies on larval growth of *Polydora ligni*, *Polydora ciliata* and *Pygospio elegans* (Polychaeta, Spionidae). Helgoländer Meeresunters 40:277–395.

Arndt A, Smith MJ. 1998. Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. Mol Ecol 7:1053–64.

Banks SC, Piggott MP, Williamson JE, Bove U, Holbrook NJ, Beheregaray LB. 2007. Oceanic variability and coastal topography shape genetic structure in a long-dispersing sea urchin. Ecology 88:3055–64.

Bekkevold D, Andre C, Dahlgren TG, Clausen LAW, Torstensen E, Mosegaard H, Carvalho GR, Christensen TB, Norlinder E, Ruzzante DE. 2005. Environmental correlates of population differentiation in Atlantic herring. Evolution 59:2656–68.

Binks RM, Evans JP, Prince RJ, Kennington WJ. 2011. Fine-scale patterns of genetic divergence within and between morphologically variable subspecies of the sea urchin *Heliocidaris erythrogramma* (Echinometridae). Biol J Linn Soc 103:578–92.

Blake JA, Arnofsky PL. 1999. Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. Hydrobiologia 402:57–106.

Bohonak AJ. 1999. Dispersal, gene flow, and population structure. Quart Rev Biol 74:21–45.

Bolam SG. 2004. Population structure and reproductive biology of *Pygospio elegans* (Polychaeta:Spionidae) on an intertidal sandflat, Firth of Forth, Scotland. Invertebr Biol 123:260–8.

Boström C, Jackson EL, Simenstad CA. 2006. Seagrass landscapes and their effect on associated fauna: a review. Est Coast Shelf Sci 68:383–403.

Bowen BW, Bass AL, Muss A, Carlin J, Robertson DR. 2006. Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. Mar Biol 149:899–913.

Case RAJ, Hutchinson WF, Hauser L, Van Oosterhput C, Carvalho GR. 2005. Macro- and micro-geographic variation in pantophysin (*Pan*I) allele frequencies in NE Atlantic cod, *Gadus morhua*. Mar Ecol Prog Ser 301:267–78.

Chapuis M-P, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–31.

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Collin R. 2001. Mode of development, phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae) species. Mol Ecol 10:2249–62.

Dawson MN, Louie KD, Barlow M, Jacobs DK, Swift CC. 2002. Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. Mol Ecol 11:1065–75.

Dionne M, Caron F, Dodson JJ, Bernatchez L. 2008. Landscape genetics and hierarchical genetic structure in Atlantic salmon: the interaction of gene flow and local adaptation. Mol Ecol 17:2382–96.

Duran M, Pascual M, Estoup A, Turon X. 2004. Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. Mol Ecol 13:511–22.

Ellingson RA, Krug PJ. 2006. Evolution of poecilogony from planktotrophy: cryptic speciation, phylogeography, and larval development in the gastropod genus *Alderia*. Evolution 601:2293–310.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–7.

Faubet P, Gaggiotti OE. 2008. A new Bayesian method to identify the environmental factors that influence recent migration. Genetics 178:1491–1504.

Fievet V, Touzet P, Arnaud J, Cuguen J. 2006. Spatial analysis of nuclear and cytoplasmic DNA diversity in wild sea beet (*Beta vulgaris ssp. maritima*) populations: do marine currents shape the genetic structure? Mol Ecol 16:1847–64.

Gaggiotti OE, Bekkevold D, Jorgensen HBH, Foll M, Carvalho GR, Andre C, Ruzzante DE. 2009. Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of natural populations: Atlantic herring as a case study. Evolution 63:2939–51.

Gerlach G, Jueterbock A, Kraemer P, Deppermann J, Harmand P. 2010. Calculations of population differentiation based on G(ST) and D: forget G(ST) but not all of statistics!. Mol Ecol 19:3845–52.

Goldson AJ, Hughes RN, Gliddon CJ. 2001. Population genetic consequences of larval dispersal mode and hydrography: a case study with bryozoans. Mar Biol 138:1037–42.

Gudmundsson H. 1985. Life history patterns of polychaete species of the family Spionidae. J Mar Bio Assoc UK 65:93–111.

Guillot G, Mortier F, Estoup A. 2005. Geneland: A program for landscape genetics. Mol Ecol Notes 5:712–5.

Guillot G, Santos F, Estoup A. 2008. Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. Bioinformatics 24:1406–7.

Hannerz L. 1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil and Poecilochaetidae N. fam. in the Gullmar Fjord (Sweden). Zool Bidr Upps 31:1–204.

Härkönen T, Harding KJ, Goodman SJ, Johannesson K. 2005. Colonization history of the Baltic harbor seals: integrating archaeological, behavioral and genetic data. Mar Mammal Sci 21:695–716.

Hart MW, Marko PB. 2010. It's about time: divergence, demography, and the evolution of developmental modes in marine invertebrates. Integ Comp Biol 50:643–61.

Hedgecock D. 1994. Does variance in reproductive success limit effective population size of marine organisms? In: Beaumont AR, editor. Genetics and evolution of aquatic organisms. London: Chapman & Hall. p. 122–34.

Helcom. 2003. Helsinki commission, The Baltic Marine environment 1999–2002. Baltic Sea Environment Proceedings No. 87.

Hellberg ME. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50:1167–75.

Hellberg ME. 2009. Gene flow and isolation among populations of marine animals. Annu Rev Ecol Evol Syst 40:291–310.

Hemmer-Hansen J, Nielsen EE, Grønkjær P, Loeschcke V. 2007. Evolutionary mechanisms shaping the genetic populations structure of marine fishes; lessons from the European flounder (*Platichthys flesus* L.). Mol Ecol 16:3104–18.

Johannesson K, André C. 2006. Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. Mol Ecol 15:2013–29.

Jost L. 2008. GST and its relatives do not measure differentiation. Mol Ecol 17:4015–26.

Kalinowski ST. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–9.

Kelly RP, Palumbi SR. 2010. Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. PLoS ONE 5:e8594.

Kenchington EL, Patwary MU, Zouros E, Bird CJ. 2006. Genetic differentiation in relation to marine landscape in a broadcast-spawning bivalve mollusc (*Placopecten magellanicus*). Mol Ecol 15:1781–96.

Kesäniemi JE, Boström C, Knott KE. 2012a. New genetic markers reveal population genetic structure at different spatial scales in the opportunistic polychaete *Pygospio elegans*. Hydrobiologia. (doi:10.1007/s10750-012-1075-3).

Kesäniemi JE, Rawson PD, Lindsay SM, Knott KE. 2012b. Phylogenetic analysis of cryptic speciation in the polychaete *Pygospio elegans*. Ecol Evol. (doi:10.1002/ece3.226).

Knutsen H, André C, Jorde PE, Skogen MD, Thuróczy E, Stenseth NC. 2004. Transport of North Sea cod larvae into the Skagerrak coastal populations. Proc R Soc Lond B 271:1337–44.

Krug PJ. 2007. Poecilogony and larval ecology in the gastropod genus *Alderia*. Am Malacol Bull 23:99–111.

Kyle CJ, Boulding EG. 2000. Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar Biol 137:835–45.

Larmuseau MHD, Vancampenhout K, Raeymaekers JAM, Van Houdt JKJ, Volckaert FAM. 2010. Differential modes of selection on the rhodopsin gene in coastal Baltic and North Sea populations of the sand goby, *Pomatoschistus minutes*. Mol Ecol 19:2256–68.

Lee HJ, Boulding EG. 2009. Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on

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variation at one mitochondrial and two nuclear DNA markers. Mol Ecol 18:2165–84.
Levin LA. 1984. Life history and dispersal patterns in a dense infaunal polychaete assemblage: community structure and response to disturbance. Ecology 65:1185–200.
Levin LA. 1986. The influence of tides on larval availability in shallow waters overlying a mudflat. B Mar Sci 39:224–33.
Levin LA. 2006. Recent progress in understanding larval dispersal: new directions and digressions. Integ Comp Biol 46:282–97.
Marko PB, Rogers-Bennett L, Dennis AB. 2007. MtDNA population structure and gene flow in lingcod (*Ophiodon elongatus*): limited connectivity despite longlived pelagic larvae. Mar Biol 150:1301–11.
Mattila J. 1997. The importance of shelter, disturbance and prey interactions for predation rates of tube-building polychaetes (*Pygospio elegans* (Claparede)) and free-living tubificid oligochaetes. J Exp Mar Biol Ecol 218:215–28.
Metaxas A. 2001. Behaviour in flow: perspectives on the distribution and dispersion of meroplanktonic larvae in the water column. Can J Fish Aquat Sci 58:86–98.
Mileikovsky SA. 1971. Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Mar Biol 10:193–213.
Miller KJ, Ayre DJ. 2008. Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. J Anim Ecol 77:713–24.
Morgan TS, Rogers AD, Paterson GLJ, Hawkins LE, Sheader M. 1999. Evidence for poecilogony in *Pygospio elegans* (Polychaeta: Spionidae). Mar Ecol Prog Ser 178:121–32.
Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Grønkjær P. 2003. Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea, revealed by individual admixture analysis. Mol Ecol 12:1497–508.
Nilsson J, Gross R, Asplund T, Dove O, Jansson H, Kelloniemi J, Kohlmann K, Löytynoja A, Nielsen EE, Paaver T, et al. 2001. Matrilinear phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonization of the Baltic Sea area. Mol Ecol 10:89–102.
Nissling A, Dahlman G. 2010. Fecundity of flounder, *Pleuronectes flesus*, in the Baltic Sea—Reproductive strategies in two sympatric populations. J Sea Res 64:190–8.
O'Leary DB, Coughlan J, Dillane E, McCarthy TV, Cross TF. 2007. Microsatellite variation in cod *Gadus morhua* throughout its geographic range. J Fish Biol 70(Suppl C):310–35.
Oyarzun FX, Mahon AR, Swalla BJ, Halanych KM. 2011. Phylogeography and reproductive variation of the poecilogonous polychaete *Boccardia proboscidea* (Annelida: Spionidae) along the West Coast of North America. Evol Dev 13:489–503.
Palumbi SR. 1994. Genetic divergence, reproductive isolation, and marine speciation. Annu Rev Ecol Syst 25:547–72.
Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–95.
Pechenik JA. 1999. On the advantages and disadvantages of larval stages in benthic invertebrate life cycles. Mar Ecol Prog Ser 177:269–97.
Pedersen TM, Hansen JLS, Josefson AB, Hansen BW. 2008. Mortality through ontogeny of soft-bottom marine invertebrates with planktonic larvae. J Marine Syst 73:185–207.
Pereyra RT, Bergstrom L, Kautsky L, Johannesson K. 2009. Rapid speciation in a newly opened postglacial marine environment, the Baltic Sea. BMC Evol Biol 9:70.
Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–59.
Raff RA, Byrne M. 2006. The active evolutionary lives of echinoderm larvae. Heredity 9:244–52.
Rasmussen E. 1953. Asexual reproduction in *Pygospio elegans* Claparède (*Polychaeta sedentaria*). Nature 171:1161–2.
Rasmussen E. 1973. Systematics and ecology of the Isefjord marine fauna (Denmark). Ophelia 11:1–495.
Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–9.
Rice WR. 1989. Analyzing tables of statistical tests. Evolution 43:223–5.
Rossi F, Vos M, Middelburg JJ. 2009. Species identity, diversity and microbial carbon flow in reassembling macrobenthic communities. Oikos 118:503–12.
Säisä M, Koljonen M-L, Gross R, Nilsson J, Tähtinen J, Koskiniemi J, Vasemägi A. 2005. Population genetic structure and postglacial colonization of Atlantic salmon (*Salmo salar*) in the Baltic Sea area based on microsatellite DNA variation. Can J Fish Aquat Sci 62:1887–904.
Schuelke M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–4.
Selkoe KA, Watson JR, White C, Horin TB, Iacchei M, Mitara S, Siegel DA, Gaines SD, Toonen RJ. 2010. Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. Mol Ecol 19:3708–26.
Shanks AL. 2009. Pelagic larval duration and dispersal distance revisited. Biol Bull 216:373–85.
Smouse PE, Peakall R. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity 82:561–73.
Söderström A. 1920. Studien über die Polychaeten familie Spionidae [dissertation]. Sweden: University of Uppsala.
Sotka EE, Wares JP, Barth JA, Grosberg R, Palumbi SR. 2004. Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. Mol Ecol 13:2143–56.
Sponaugle S, Cowen RK, Shanks A, Morgan SG, Leis JM, Pineda J, Boehlert GW, Kingsford MJ, Lindeman KC, Grimes C, et al. 2002. Predicting self-recruitment in marine populations: Biophysical correlates and mechanisms. B Mar Sci 70:341–75.
Swearer SE, Shima JS, Hellberg ME, Thorrold SR, Jones GP, Robertson DR, Morgan SG, Selkoe KA, Ruiz GM, Warner RR. 2002. Evidence of self-recruitment in demersal marine populations. B Mar Sci 70:251–71.
Thorrold SR, Jones GP, Hellberg ME, Burton RS, Swearer SE, Neigel JE, Morgan SG, Warner RR. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. B Mar Sci 70:S291–308.

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Watts PC, Thorpe JP. 2006. Influence of contrasting larval developmental types upon the population-genetic structure of cheilostome bryozoans. Mar Biol 149:1093–101.

Weersing K, Toonen RJ. 2009. Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393:1–12.

White C, Selkoe KA, Watson J, Siegel DA, Zacherl DC, Toonen RJ. 2010. Ocean currents help explain population genetic structure. Proc R Soc B 277:1685–94.

Wiemann A, Andersen LW, Berggren P, Siebert U, Benke H, Teilmann J, Lockyer C, Pawliczka I, Skóra K, Roos A, et al. 2010. Mitochodrial control region and microsatellite analyses on harbor porpoise (*Phocoena phocoena*) unravel population differentiation in the Baltic Sea and adjacent waters. Conserv Genet 11:195–211.

Wilson GA, Rannala B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–91.

Wilson WH. 1983. The role of density dependence in a marine infaunal community. Ecology 64:295–306.

Wilson WH. 1991. Sexual reproductive modes in polychaetes: classification and diversity. B Mar Sci 48:500–16.

Zhan A, Hu J, Hu X, Zhou Z, Hui M, Wang S, Peng W, Wang M, Bao Z. 2009. Fine-scale population genetic structure of Zhikong Scallop (*Chlamys farreri*): do local marine currents drive geographical differentiation? Mar Biotechnol 11:223–35.

Zillen L, Conley DJ, Andren T, Andren E, Björck S. 2008. Past occurrence of hypoxia in the Baltic Sea and the role of climate variability, environmental change and human impact. Earth Sci Rev 91:77–92.

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