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Are there two species of the polychaete genus *Marenzelleria* in Europe?

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Abstract North Sea and Baltic Sea populations of the introduced polychaete *Marenzelleria viridis* (Verrill, 1873) reproduce at different times (spring and autumn, respectively). Enzyme separation by starch gel electrophoresis revealed major differences between specimens from the Baltic Sea and those from the North Sea (collected in 1992 and 1993) but a high degree of homogeneity among populations from the same sea. Three enzyme loci, glucose-6-phosphate isomerase (*GPI-A*, *GPI-B*) and malate dehydrogenase (*MDH*), were fixed to 100% by different alleles in the North and Baltic Sea populations, respectively. Different alleles are dominant for mitochondrial aspartate aminotransferase (*mAAT*) with allele frequencies of ca. 0.97 in all sampled populations from the North Sea and Baltic Sea, respectively, but heterozygotes were found in all populations. These genetic differences could be due to environmentally induced selection or genetically different origins of the populations, suggesting that populations of the genus *Marenzelleria* in the North and Baltic Seas may be two different species.

Introduction

Marenzelleria viridis (Verrill, 1873; syn. *Scolecolepides viridis*, Verrill, 1873), a spionid polychaete formerly considered indigenous to the North American Atlantic coast, has been colonizing brackish water ecosystems in the North and Baltic Seas for more than 10 yr. It is proliferating rapidly in some areas of these seas and has become an important component of the macrozoobenthos in many biotopes.

Marenzelleria viridis was discovered in European waters in the Forth estuary (west North Sea) in 1982 (McLusky et al. 1993), and in 1983 specimens were found in the Ems estuary (Netherlands, southeast North Sea; Essink and Kleef 1988). According to Essink and Kleef (1993), the species had spread into the Baltic Sea by 1985 (Bick and Burckhardt 1989; Fig. 1 present study). If the species continues to spread at its present rate, it can be expected to colonized most suitable European biotopes within the near future.

Essink and Kleef (1993) suspect that *Marenzelleria viridis* was transported across the Atlantic to Europe as larvae or juveniles in the ballast water of tankers, and this could also account for its appearance in the Baltic Sea. Moreover, after studying the time sequence for records of *M. viridis* in Europe, they formulated a hypothesis regarding the possible mode by which the spionid polychaete was able to spread within the North and Baltic Seas. According to their hypothesis, colonization of the southeast (Ems estuary) and northwest North Sea (Tay Estuary in Scotland) resulted from two distinct immigration events. Thereafter, the spionid spread with the anti-clockwise water circulation system (Lozan et al. 1990; Otto et al. 1990), possibly entering the Baltic Sea in the ballast water of ships or with the water flowing from the North Sea into the Baltic.

Since the planktic larvae of *Marenzelleria viridis* have a life-span of several weeks and often occur in abundances of several million individuals m^{-3}, and since juveniles can also be present in considerable numbers in the plankton (Bochert et al. 1994), the modes of immigration to Europe and propagation in the North Sea hypothesized by Essink and Kleef (1993) appear probable (Carlton and Geller 1993).

There are two reasons for questioning Essink and Kleef's (1993) assumption that colonization of the Baltic Sea started with polychaetes from the North Sea: (1) The North American (George 1966; Dauer et al. 1982) and North Sea (Atkins et al. 1987; Essink and Kleef 1993) populations of *Marenzelleria viridis* reproduce in spring, their pelagic larvae being found from February to May. The Baltic population (Bochert et al. 1994) reproduces in autumn, and pelagic larvae in this area are present from September

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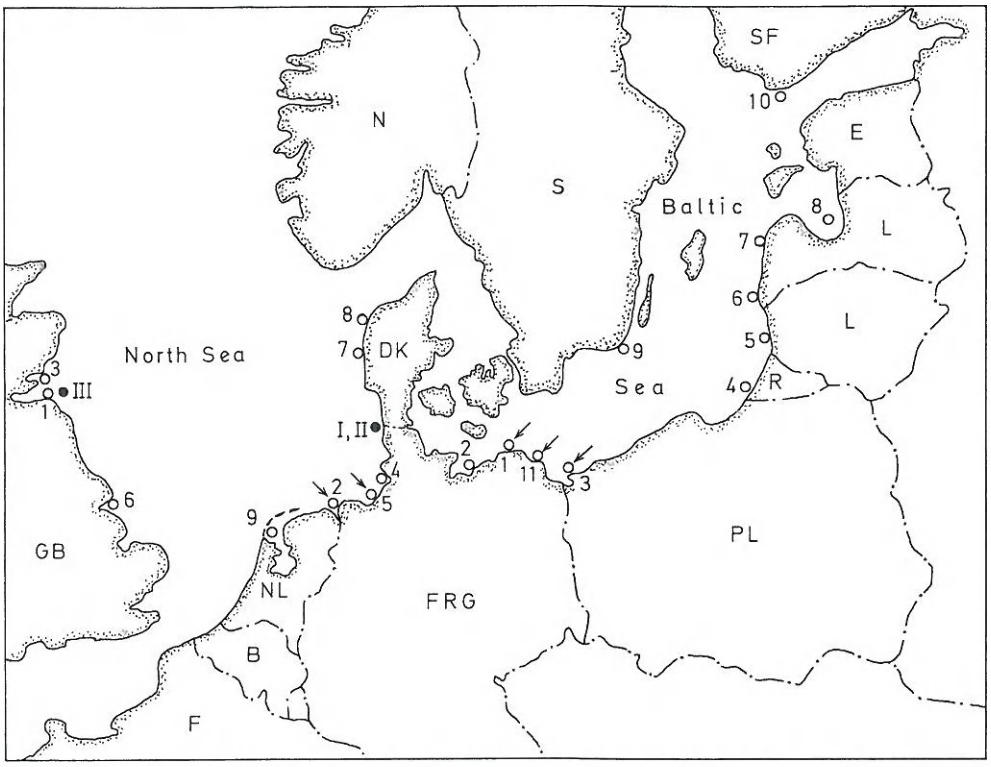

Fig. 1 *Marenzelleria* spp. Distribution in the North Sea and Baltic Sea. Arabic numbers (open circles) represent collections of *M. viridis*, Latin numbers (filled circles) collections of *M. wireni*. Arrows indicate sampling sites for present study. North Sea: *1* McLusky et al. (1993); *2* (Ems 1, Ems 2) Essink and Kleef (1988); *3* Atkins et al. (1987); *4* Leling (1986); *5* (Weser estuary); *6, 8* Essink and Kleef (1993); *7* Kirkegaard (1990); *9* Dekker (1991). *I* Wohlenberg (1937); *II* Otte (1979); *III* Atkins et al. (1987). Baltic Sea: *1* (Bodden chain south of Darss-Zingst); *2* Bick and Burckhardt (1989); *3* (Oderbucht) Gruszka (1991); *4, 5* Chubareva and Olenin (1992); Zmudzinski et al. (1993); *6, 7, 8* Andersin et al. (personal communication); *9* Persson (1991); *10* Andersin et al. (personal communication), Norkko et al. (1993); *11* Greifswalder Bodden) Gruszka (1991)

to December. (2) Finds of *M. viridis* have so far been rare in the Baltic Sea west of the Darss-Zingst Peninsula (Mecklenburg Bay, Kiel Bay, Wismar Bay), where only isolated specimens have been reported. If the species entered the Baltic Sea in ballast water or in the water flowing from the North Sea (ca. 1200 $km^2 yr^{-1}$; Lozan et al. 1990), one would expect abundances to be far higher in these areas, particularly since the species was able to spread to other parts of the North and Baltic Seas so quickly and successfully.

We employed starch gel electrophoresis in order to answer the following questions: Do genetic differences exist between the North Sea and Baltic populations of *Marenzelleria viridis* in addition to the above mentioned difference in reproduction period, and, if such differences exist, what caused them?

As far as we know, no biochemical genetic studies have been undertaken for either the genus *Marenzelleria* or the species *M. viridis*. Therefore, besides studying the composition of the North Sea and Baltic populations, we were also seeking suitable methods for demonstrating the corresponding zymograms and detectable polymorphic and genetically interpretable enzyme loci.

Materials and methods

Polychaetes

All worms used for the studies described here were adult *Marenzelleria viridis* specimens that were stored at –70 °C until prepared for analysis or used immediately after sampling. For sampling sites and dates see Table 1.

Table 1 *Marenzelleria viridis*. Populations studied and sampling sites. Ems 1 and Ems 2 are two samples collected at different times in the same area (Dollard, Ems estuary)

Sampling site	Sample collection
North Sea	
Dollard, Ems estuary, Holland	Sep 1992 (Ems 1)
	Apr 1993 (Ems 2)
Weser estuary/Blexter Plate	Apr 1993
Baltic Sea	
Bodden chain south of Darss-Zingst:	
Bliesenrade, Bodstedter Bodden	Sep 1992
Körkwitz, Saaler Bodden	Mar 1992
Born, Bodstedter Bodden	Mar 1992
Dabitz, Grabow Bodden	Sep 1992
Greifswalder Bodden	Mar 1993
Oderbucht	Apr 1993

The salinities fluctuated at the sampling sites in both the North Sea (8 to 21‰) and the Baltic Sea (Darss-Zingst Bodden chain: Körkwitz 4 to 10‰ Dabitz; Oderbucht: ca. 7‰; Greifswalder Bodden: ca. 7.8‰).

Worms were collected from the waddens of the North Sea, from shallow regions up to 1 m deep in the Darss-Zingst bodden chain of the Baltic Sea, and down to 10 m in the Greifswalder Bodden and Oderbucht. *Marenzelleria viridis* individuals were found in muddy and sandy sediments at all sampling sites, but abundances were higher in muddy sediments (Essink and Kleef 1993). Further information concerning the habitats and abiotic factors can be found in the publications of Essink and Kleef (1993) and Bick and Burckhardt (1989).

All chemicals used were from SIGMA, Boehringer, Roth, Aldrich or Merck.

Horizontal starch gel electrophoresis (SGE) was used to determine allele frequencies at the given loci. The following buffers were used: (I) Amine-citrate (Morpholine), pH 6.1 (Murphy et al. 1990), modified. Electrode buffer: 0.04 *M* citric acid, 0.01 *M* EDTA, 0.01 *M* $MgCl_2$, adjusted to pH 6.1 by adding *N*-(3-aminopropyl)-morpholine. Gel buffer: 1:19 dilution of electrode buffer. (II) See (I), but pH adjusted to 6.5. (III) Tris-maleic anhydride-EDTA, pH 6.9 (Pasteur et al. 1988).

Starch gel electrophoresis

The 11.5% gels were made from a mixture of starch hydrolysates (time of hydrolysis: 0, 50, 60, 70, 80 min; Dr. Marek, Brno, personal communication).

Electrophoresis

SGE was carried out in Multiphor II horizontal electrophoresis chambers with cooling plates from Pharmacia-LKB. The cooling plates were cooled to below 8 °C by an external cooling circulation. The power pack was an E 443 from Consort.

Sample preparation

Whole individuals (10 to 100 mg) were homogenized in bidistilled water or gel buffer (weight: volume ca. 1:1.5) by ultrasonic disintegration (homogenizer: HD 60, Bandelin). Both liquid phases were found to be equally suitable. After centrifugation (K 23 centrifuge, Janetzky) at 7000 *g* and 4 °C for 30 min, the supernatant was placed on the gel on stripes of filter paper (Filtrak FN 8; 2 to 3×10 mm) 8 cm from the cathode end of the gel. Electrophoresis was terminated after the samples (containing bromphenolblue) had migrated ca. 10 cm towards the anode. The gel was cut into layers ca. 2 mm thick, which were placed in solutions containing stains specific to the various enzymes (Pasteur et al. 1988; Murphy et al. 1990; May 1992) and incubated at 37 °C.

Staining was terminated by decanting the solution with the stain and washing the gel with water. The gel was fixed in a mixture of water and glycerol (1:1) to prevent fading of the gels stained with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide or thiazolyl blue) and stored in a refrigerator for photography.

The results of separation for enzymes were the same for frozen whole individuals and for fresh samples.

Results

The enzyme nomenclature is as proposed by Shaklee et al. (1990). The first survey involved 13 enzymes. The activities of alcohol dehydrogenase, fumarate hydratase, glucose dehydrogenase, glutamate dehydrogenase, L-iditol dehydrogenase, L-lactate dehydrogenase, mannose-6-phosphate isomerase, octanol dehydrogenase, superoxide dismutase and phosphogluconate dehydrogenase were very low or not detectable.

Aspartate aminotransferase (AAT; E.C. 2.6.1.1; dimer; buffers I, II, III)

In *Marenzelleria viridis* AAT has two loci named according to their migration properties (Harris and Hopkinson 1976). They are supernatant AAT (*sAAT**) and mitochondrial AAT (*mAAT**), which migrate towards the anode and cathode, respectively.

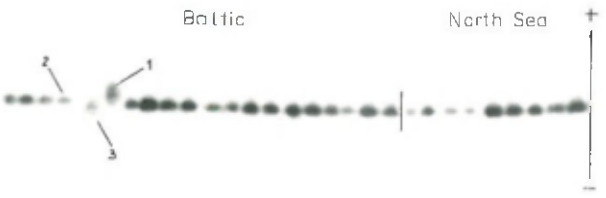

Fig. 2 *Marenzelleria viridis*. Supernatant aspartate aminotransferase (*sAAT*) in populations from the North Sea and Baltic Sea. The most common allele *sAAT*2* in all populations denoted by 2. The rare alleles **1* and **3* also shown

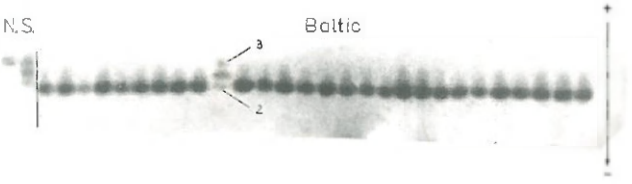

Fig. 3 *Marenzelleria viridis*. Mitochondrial aspartate aminotransferase in North Sea and Baltic Sea specimens. The most common allele *mAAT*3* in North Sea (*N.S.*) *M. viridis* denoted by 3 and the most common allele *mAAT*2* in Baltic polychaetes by 2. Figure also shows heterozygotes mAAT 2/3 in both populations

In the case of sAAT, the four alleles we found are denoted *sAAT*1*, **2*, **3* and **4*, respectively, starting with the one that migrates farthest towards the anode. The three alleles of mAAT are denoted *mAAT*1*, **2* and **3*, respectively, starting with the allele that migrated farthest towards the cathode.

sAAT (locus A)

Table 2 lists the individuals in our samples according to sampling site and allele frequency. Allele *sAAT*2* predominates in the specimens from the North Sea ($n = 411$) and Baltic Sea ($n = 534$; Fig. 2 present study).

Heterozygous individuals of the types 1/2, 2/3 and 1/4 were rare, and no homozygous individuals of the types 1/1, 3/3 or 4/4 were found. This also applies to heterozygous combinations of these alleles.

mAAT

The distribution of the alleles we found was not identical in the North Sea and Baltic Sea populations. The predominant allele was *mAAT*3* in the North Sea populations ($n = 355$) and *mAAT*2* in the Baltic populations (Table 2, Fig. 3). Heterozygous individuals of type 2/3 were found in both the North Sea and Baltic populations. The characteristic zymogram for heterozygous with 25% 2/2, 50% 2/3 and 25% 3/3 was obtained by mixing homogenates of homozygous worms of types 2/2 and 3/3, freezing the mix-

Table 2 *Marenzelleria viridis*. Allele frequencies for the enzymes sAAT, mAAT, (MDH/ME), GPI-A, and GPI-B in the investigated population. Most common frequencies shown in boldface

Enzyme / Allele	Sampling sites								
	North Sea			Baltic Sea					
	Ems 1	Ems 2	Blexter Plate	Bliesenrade	Körkwitz	Born	Dabitz	Greifswalder Bodden	Oderbucht
sAAT	*n*= 80	197	134	73	90	118	61	105	87
Allele 1	0	0	0	0	0.006	0.008	0	0	0.006
2	**1**	**0.997**	**0.996**	1	**0.994**	**0.988**	**1**	**0.995**	**0.988**
3	0	0.003	0.004	0	0	0	0	0.005	0.006
4	0	0	0	0	0	0.004	0	0	0
mAAT	*n*= 67	190	144	52	119	102	23	158	70
Allele 1	0	0	0	0	0	0.005	0	0	0.007
2	0.030	0.029	0.017	**0.981**	**0.975**	**0.990**	**0.935**	**0.981**	**0.971**
3	**0.970**	**0.971**	**0.983**	0.019	0.025	0.005	0.065	0.019	0.022
MDH/ME	*n*= 252	200	149	237	90	118	98	161	76
Band A	1	1	1	1	1	1	1	1	1
B	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**
B′	**1**	**1**	**1**	0	0	0	0	0	0
C	0	0	0	**1**	**1**	**1**	**1**	**1**	**1**
C′	**1**	**1**	**1**	0	0	0	0	0	0
GPI-A	*n*= 232	–	–	–	–	–	–	–	–
Allele 1	0.259	–	–	–	–	–	–	–	–
2	0.469	–	–	–	–	–	–	–	–
3	0.272	–	–	–	–	–	–	–	–
GPI-B	*n*= 232	172	139	–	–	–	–	–	–
Allele 1	0.244	0.189	0.180	–	–	–	–	–	–
2	0.472	0.532	0.615	–	–	–	–	–	–
3	0.284	0.279	0.205	–	–	–	–	–	–

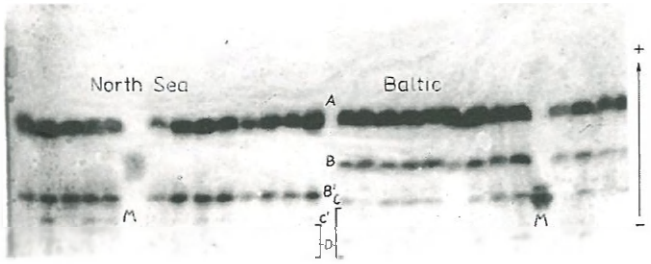

Fig. 4 *Marenzelleria viridis*. Malate dehydrogenase/malic enzyme in individuals from the Baltic Sea and North Sea. Bands in the zymogram classified as A, B ... according to their electrophoretic mobilities towards the anode. Differences between specimens from both populations visible in bands B/B′ and C/C′. M are samples from *Nereis diversicolor*. Activity in bands D is very low

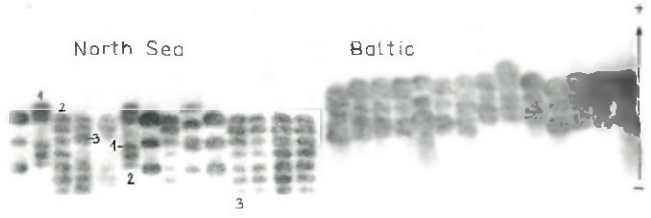

Fig. 5 *Marenzelleria viridis*. Glucose-6-phosphate isomerase in North Sea and Baltic Sea specimens. Figure shows marked differences at these two loci between populations. The observed alleles of *GPI-A* (nearest the anode) and *GPI-B* denoted by 1, 2 and 3, respectively. Running time: 4 to 5 h

ture and subsequently thawing it again. Homozygous individuals with the allele **3*, which predominates among the North Sea populations, were also found in Baltic populations. Allele **1* is very rare.

Malate dehydrogenase (MDH; E.C. 1.1.1.37)/ malic enzyme (ME; E.C. 1.1.1.40; buffers I, II)

No polymorphous loci were found in the North Sea or in Baltic populations for these enzymes. We were unable to shed light onto the genetic basis for the electropherograms we obtained for the NAD^+-specific dimer MDH or the $NADP^+$-specific tetramer ME since no alleles were found for the same pattern in either case. However, the MDH/ME zymograms obtained for all polychaetes permit 100% accurate differentiation between worms from the North Sea and those from the Baltic (Fig. 4).

The electropherograms of individuals from the Baltic and North Seas exhibit several bands. Band A, which is nearest the anode, shows the highest activity for all individuals when stained specifically for MDH (+NAD) and ME (+NADP).

Band B is actually the marker distinguishing all Baltic individuals studied so far (n=780; Table 2) from those collected from the North Sea (n=601), owing to the striking differences in electrophoretic mobility.

Band C contains considerably less activity, but here too the electrophoretic mobility for North Sea individuals is

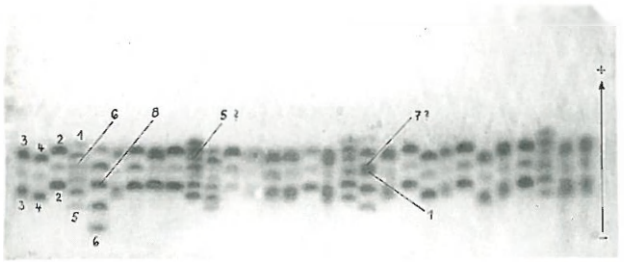

Fig. 6 *Marenzelleria viridis*. Glucose-6-phosphate isomerase in Baltic specimens. Increase in running time (7 to 8 h) of electrophoresis enhances resolution at the two GPI loci. Observed alleles for *GPI-A* (nearest the anode) and *GPI-B* denoted by 1, 2, 3, 4, 5?, 6, 7?, 8 and 1, 2, 3, 4, 5, 6, respectively. Question marks for alleles **5* and **7* of *GPI-A* indicate that alleles can not definitely be identified

appreciably different to that for individuals from the Baltic. Several additional bands, D, with very low enzyme activities were also found.

Glucose-6-phosphate isomerase (GPI; E.C. 5.3.1.9; dimer; buffer I)

Our experiments show that GPI is encoded at two loci in each of the populations we investigated (Fig. 5). The same figure indicates that the alleles located at the two highly polymorphous loci are completely different in individuals from the North Sea ($n=683$) and Baltic ($n=892$) populations. The resolution of the alleles of individuals from Baltic populations can be enhanced by increasing the running time (Fig. 6).

North Sea populations

The locus that migrated farthest towards the anode is denoted *GPI-A**, and its alleles are denoted **1*, **2* and **3*, respectively, starting with the one nearest the anode. The alleles of *GPI-B** are likewise denoted **1*, **2* and **3*, also starting with the one nearest the anode. Three alleles were found with the allele frequencies stated in Table 2 at each of these loci.

Baltic populations

Neither of the two GPI loci in any of the individuals we studied from the Baltic Sea expressed the alleles that were typical of the North Sea populations, nor were alleles typical of Baltic specimens expressed at either GPI locus from North Sea individuals.

At least six alleles were found at each locus (Fig. 6). Unfortunately, we have not yet been able identify the number of alleles or their frequencies with absolute certainty.

Like MDH/ME, the two GPI loci are diagnostic (Avise 1974) and allow *Marenzelleria viridis* individuals from the North Sea to be differentiated from Baltic individuals.

Discussion and conclusions

Our investigations into the genetic structure of the polychaete *Marenzelleria viridis* have revealed major differences between the North Sea and Baltic populations, although the structure within each of these populations is largely homogeneous. Considerable genetic differences between isolated populations of marine invertebrates have been described several times, for instance in the case of the common mussel *Mytilus edulis* (e.g. Johannesson et al. 1990).

The results presented here are particularly interesting because it has been only a little more than 10 yr since *Marenzelleria viridis* was first reported in European coastal waters (Atkins et al. 1987; Essink and Kleef 1988; Bick and Burkhardt 1989).

Like the genetic difference we have discovered, the different reproduction periods (North Sea and North American populations in spring, Baltic populations in autumn) (George 1966; Atkins et al. 1987; Essink and Kleef 1993; Bochert et al. 1994) suggest that the North and Baltic Seas populations may differ fundamentally. Only speculative reasons can be given for the different reproduction periods (Bochert et al. 1994) since no comparative experimental studies have been performed. Both reproduction period and the genetic results vary only slightly within each of the two distribution areas (Essink and Kleef 1993; McLusky et al. 1993; Bochert et al. 1994; our results).

At least two hypotheses can be formulated to explain the differences: (1) The genetic differences have arisen due to the selective pressure of environmental factors. (2) The polychaetes in the North and Baltic Seas are of different genetic origins.

If, like Essink and Kleef (1993), we assume that *Marenzelleria viridis* was imported to Europe from a single North American population during an immigration event (possibly two discrete immigration waves into the North Sea according Essink and Kleef 1993), the effects of environmental selection pressures (hypothesis 1 above) would provide an acceptable explanation of the differences observed so far. The gametes of numerous spawning *M. viridis* individuals are released into the water simultaneously during the reproduction phase. The larval stage following fertilization leads a planktic life for several weeks (George 1966). Bochert et al. (1994) reported abundances of 21.8×10^6 larvae m^{-3} of water and initial colonization densities of up to 270 000 ind m^{-2} of sediment by juvenile benthic forms in the bodden chain south of Darss-Zingst (Baltic Sea). These figures show vividly the enormous potential of the species for colonizing new habitats and also, if they possess polymorphous enzymes, for possible environmentally induced short-term selection. However, we found absolutely no common allele for three enzyme loci (*GPI-A*, *GPI-B* and *MDH/ME*), and other alleles are almost completely fixed for each of the mAAT that migrate towards the cathode. Although the adaptive benefits of allelic isozyme polymorphism is a controversial subject, many studies suggest that it does confer adaptational ability, in-

cluding Nevo's (1990) field studies and laboratory experiments showing that pollution can act as a selection factor. He concluded that a species with a higher level of isozyme diversity would be more resistant to pollutants than one with a low isozyme diversity. Pollution could conceivably act as a selection factor in North Sea and Baltic estuaries. In view of Smith's (1964) investigations with *Nereis diversicolor*, the influence of salinity on the larval development of *Marenzelleria viridis* must naturally also be regarded as a possible selection factor causing the genetic differences we found between the *M. viridis* populations in the North Sea and Baltic Sea, respectively. However, since the selection hypothesis has not been supported by appropriate studies it remains pure speculation.

Atkins et al. (1987) considered another, perhaps rather improbable, explanation for the origin of the European *Marenzelleria viridis* populations besides Essink and Kleef's (1993) colonization model: "If, however, the two forms [*M. viridis* and *M. wireni*] are synonymous, then an origin in the European Arctic is possible, and natural processes may be sufficient to account for the presence of the Tay population..."

The morphological similarity of *Marenzelleria viridis* to *M. wireni* forms the basis for this possible explanation. Maciolek (1984) revised the description of the genus *Marenzelleria* comprising the species *M. wireni*, *M. viridis* and *M. jonesi* and assigned the common North American estuarine species known previously as *Scolecolepides viridis* to this genus. Discussing the species, she mentioned that "*Marenzelleria viridis* is very similar to *M. wireni* (Augener). The two species differ only in subtle respects..." However, *M. wireni* is reportedly restricted largely to the Arctic Circle, where it usually occurs in low abundances (Holmquist 1967, 1973; Maciolek 1984). It has also been found in waddens of the island Sylt in the southern North Sea (*Microspio wireni*, Wohlenberg 1937; Otte 1979) and possibly in the Forth estuary (Atkins et al. 1987) in the northwest North Sea. Its presence in the Sylt waddens in 1937 and 1979 suggest that small populations of *M. wireni* exist in the North Sea and could provide starting points for the possible colonization of estuaries.

Besides the isolated finds of *Marenzelleria wireni* in the North Sea (Wohlenberg 1937; Otte 1979), some evidence also shows that *M. wireni* is able to migrate into estuaries where it can survive and reproduce at low salinities (below 1‰) (Holmquist 1967, 1973). Although little is known concerning the biology of *M. wireni*, these reports suggest that both *M. viridis* and *M. wireni* can successfully colonize the same biotope (estuaries, brackish waters).

Unfortunately, the planned studies into the differentiation of *Marenzelleria viridis* and/or *M. wireni* that Atkins et al. (1987) mentioned have never been published to our knowledge. Therefore, at present it is impossible to preclude definitely the simultaneous presence of both *M. viridis* (very probably imported as larvae or juveniles in the ballast water of ships) and *M. wireni* (from European Arctic waters) in the North Sea and/or the Baltic.

The available literature concerning the population genetics of, and differentiation between, polychaete species seems to indicate that populations of the genus *Marenzelleria* in the North and Baltic Seas are not of identical origin. Grassle (1984) and Grassle and Grassle (1976, 1978) analyzed a group of sibling species of cosmopolitan polychaete worms from the genus *Capitella* which were originally thought to be of the same species, *Capitella capitata*. The six sibling species they identified differ only slightly in morphology, and exact assignment is also impossible on the basis of life history alone. However, the sibling species can be identified with certainty if an analysis of eight genetic loci is taken into account in addition to the above traits. Grassle (1984) and Grassle and Grassle's (1976, 1978) conclusions were confirmed by Wu et al. (1988), who identified at least three sibling species in the *Capitella* group by means of a similar approach.

Manchenko and Radashevsky (1993) were able to distinguish two sibling species of the *Polydora ciliata* complex as a result of allozyme variation revealed by starch gel electrophoresis of 18 enzymes (27 loci) and ecological differences.

Studying six enzymes from two morphological variations of *Arenicola marina* (the blow lug and black lug) in South Wales, Cadman and Nelson-Smith (1990) found a high value for Nei's genetic distance and a low one for genetic identity. They therefore concluded that the two morphs constitute separate species.

All species of the genus *Nephtys* (*N. longosetosa*, *N. caeca*, *N. cirrosa* and *N. hombergii*) can be identified by analyzing six isoenzymes and the unspecific protein pattern (Schmidt and Westheide 1992). Using the same traits, these two authors also found out that *N. longosetosa* is a complex consisting of two morphologically identical species.

Population genetic studies (Hateley et al. 1992; Fong and Garthwaite 1994) have revealed considerable genetic differences between *Nereis diversicolor* populations in geographically close regions. In contrast, our results revealed much smaller genetic differences between *Marenzelleria viridis* populations living at much greater distances from each other in the North and Baltic Seas. This is almost certainly linked to the long planctic phase of *M. viridis* larvae (Bochert et al. 1994).

Summarizing, we see that biochemical studies have led to the description and differentiation of polychaete species and in several cases to their separation into sibling species and even populations. This approach, therefore, appears suitable for settling various questions relating to the polychaetes, including the problems we have addressed.

We found that the North Sea and Baltic Sea populations of *Marenzelleria viridis* had no common alleles for two gene loci (*GPI-A**, *GPI-B**) and MDH/ME although hundreds of worms collected in some cases on different occasions from each population were studied. In other words, so far, each of these three enzymes is sufficient to identify the origin of an individual with absolute certainty [diagnostic loci in Avise's (1974) sense]. The alleles for the mAAT are also fixed to a great degree, although heterozygotes also occur in this case.

Our results so far permit us to conclude provisionally that, if the selection hypothesis (hypothesis 1) proves to be wrong, the North Sea and Baltic Sea populations of the genus *Marenzelleria* are of different genetic origin (hypothesis 2). The genetic differences we have found might reflect the genetic statuses of *M. viridis* and *M. wireni*, respectively, or genetically distinct populations of *M. viridis* imported from different parts of the North American east coast.

It is possible that samples from hitherto unknown, but quite conceivable, hybrid zones colonized by the North Sea and Baltic populations (species?) (Hewitt 1988), crossbreeding of worms from these two populations, examination of autochthonous North American *Marenzelleria viridis* and Arctic *M. wireni* populations and studies into the effects of environmental conditions as possible selection factors will help to identify the causes of the differences.

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