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Short communication

Mytilus galloprovincialis-type foot-protein-1 alleles occur at low frequency among mussels in the Dutch Wadden Sea

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Abstract

The presence of *M. galloprovincialis*-type genes among the population of mussels in the Dutch Wadden Sea, historically described as *M. edulis*, was assessed. We applied the molecular technique in which a fragment of the gene coding for an adhesive protein of the byssus of mussels is amplified by PCR and assayed for length using electrophoresis. Among 321 individual mussels collected in August–October 2001 at 14 sites (5 intertidal, 9 subtidal) widely dispersed over the Dutch Wadden Sea, 6 specimens (collected at 5 sites) were found that showed a heterozygote genotype with both the *M. edulis*- and the *M. galloprovincialis*-type alleles being amplified; all others were identified as homozygotes for the *M. edulis*-type allele. Differentiation in frequencies of heterozygotes among sites was not detected. The ofact that the *M. galloprovincialis*-type allele was present at low frequency (0.0093) may be attributed to one of three possible, and not mutually exclusive, causes: incomplete diagnosticity of this marker, an historically stable introgression zone in the Wadden Sea, or a recent invasion.

Keywords: *Mytilus edulis*; Hybridisation; Introgression; Population genetics; Geographic structure

1. Introduction

One of the best studied cases of hybridisation and introgression in the marine environment is the *Mytilus edulis* species complex of mussels. The complex is described as encompassing three species: *M. edulis*, *M. galloprovincialis*, and *M. trossulus* (McDonald et al., 1991). Wherever the distributions of any pair of these species overlap, they hybridise. The hybrid zone may be narrow, as e.g. in the Danish waters, where *M. trossulus* from the Baltic Sea meets *M. edulis* from the North Sea (Väinölä and Hvilsom, 1991), or broad and complex, as is the area of contact between *M. edulis* from the northeastern Atlantic and *M. galloprovincialis* from the Mediterranean. Hybridisation and introgression is in some cases probably an example of an historical equilibrium contact zone (Väinölä and Hvilsom, 1991; Rawson et al., 1996), while in other cases there is evidence for recent invasion by species of the *M. edulis* complex (Geller, 1999; Inoue et al., 1997).

The eastern Atlantic *M. edulis* / *M. galloprovincialis* hybrid zone is especially well studied around the British Isles (e.g. Skibinski et al., 1978, 1983; Willis and Skibinski, 1992; Wilhelm and Hilbish, 1998; Hilbish et al., 2002). Here one can find true hybrid populations as well as introgression zones, but the extent of the broader introgression zone has not been

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mapped in detail. The main reason for this is that the traditionally employed allozyme markers for the *M. edulis* species complex are not completely differentiated between species, so that species identity can be rather reliably assigned to populations but not to individuals (Rawson et al., 1996). The advent of PCR-based techniques has opened up new possibilities for finding diagnostic markers (e.g. Daguin and Borsa, 1999; Geller, 1999; Hilbish et al., 2000). One set of recently described DNA markers, amplified by PCR from the foot-protein-1 gene (Inoue et al., 1995a; Rawson et al., 1996), is very promising in that its alleles appear to be the most differentiated markers for the *M. edulis* species complex to date (Inoue et al., 1995b, 1997; Hilbish et al., 2002).

The Dutch Wadden Sea mussels are considered to be *M. edulis* (Dankers et al., 1983; Gosling, 1992) and a nearby population from the Oosterschelde in the south of The Netherlands was identified as *M. edulis* with the use of allozymes (Hummel et al., 2001). It is interesting to know the frequency of foot-protein-1 alleles in the Dutch Wadden Sea for the purpose of studying the broader extent of the British historical hybrid and introgression zone with the use of this possibly more powerful marker, and for future research on potential contemporary invasion events. In this note we report on the application of this marker for estimating the frequencies of *M. edulis* and/or *M. galloprovincialis*-type alleles in the Dutch Wadden Sea.

2. Study area, sampling strategy and methods

From 29 July to 20 October 2001, at 14 different sites as widely dispersed as practically possible over the entire Dutch Wadden Sea, samples of mussels were collected (Fig. 1; for a detailed description of the Wadden Sea environment see Wolff, 1983). Five of the sites were in the subtidal, and apart from the Breezand-site (site 5, Fig. 1), they all contained large, one-year or older mussels as well as spat-size mussels. The nine intertidal sites only yielded spat, i.e. mussels up to a length of 30 mm that had settled during the preceding summer. Only the sample from the intertidal flats south of Ameland (site 11, Fig. 1) contained a one-year old mussel of 32 mm. For comparison we

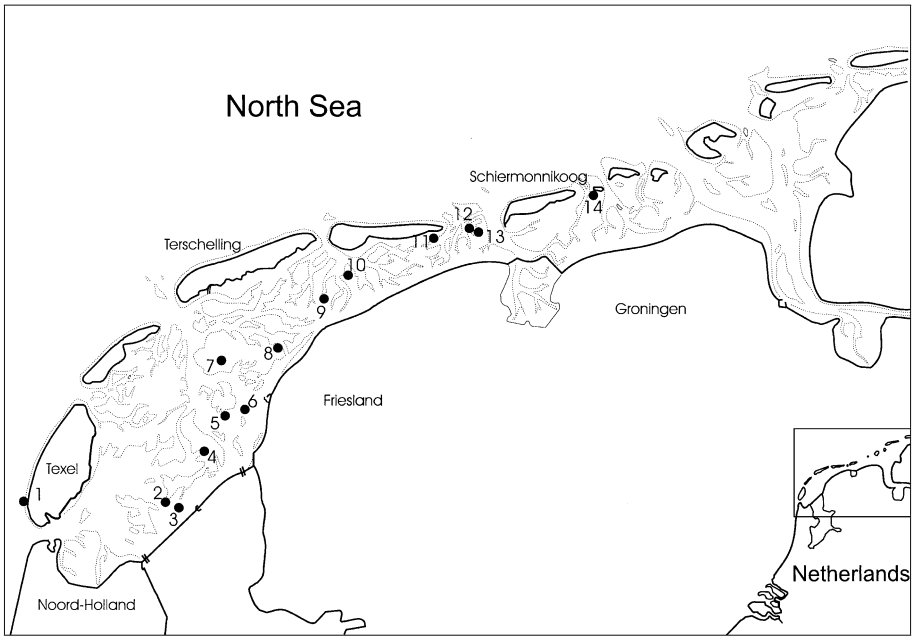

Fig. 1. The Wadden Sea, with the intertidal and subtidal sampling sites where mussels were collected in August–October 2001. The hatched lines indicate mean low water.

also obtained samples of mussel spat from two subtidal sites in the Oosterschelde (Middelplaat and Vondeling) in the south–west Netherlands. Samples from the Wadden Sea were frozen at −20 °C, and samples from the Oosterschelde were kept in 95% ethanol, until further processing.

Of each of the samples about 20 (or 40) mussels were selected randomly and their length measured with electronic callipers to the nearest mm. Total DNA was extracted from approximately 1 mm^3 of tissue using a DNA extraction kit (GenElute, Sigma, USA) and the manufacturer's protocol. Of the dissolved DNA, 1 mm^3 was used in a 25 mm^3 PCR reaction containing 1 μM of both primers Me15 and Me16 (Inoue et al., 1995a), 1 μM of each dNTP, and 1 unit Taq DNA polymerase (Sigma, USA). Temperature cycling consisted of an initial denaturation step of 94 °C for 10 min, 30 cycles of 30 s at 94 °C, 30 s at 56 °C and 90 s at 70 °C, and a final extension step of 72 °C for 7 min. PCR products were visualised on 2% agarose (FMC Bioproducts, USA) gels (Fig. 2), running a positive control for both *M. edulis* (from the Dutch Wadden Sea) and *M. galloprovincialis* (from Banyuls-sur-Mer on the French Mediterranean coast).

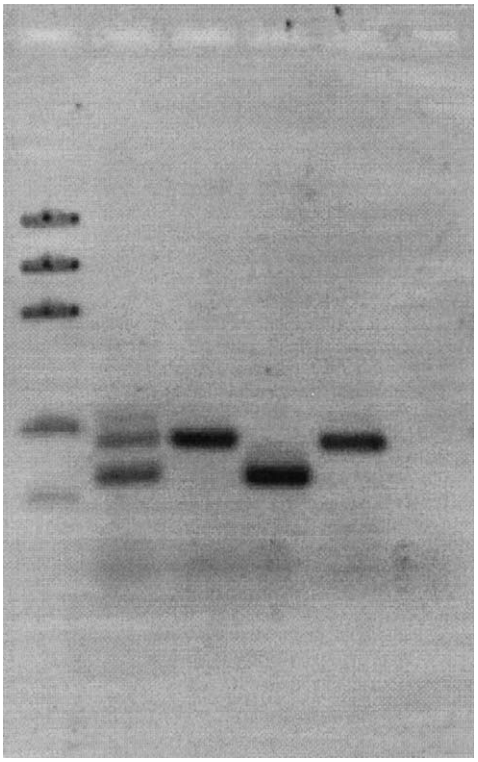

Fig. 2. Agarose gel of the PCR product of the adhesive protein gene using primers Me15 and Me16 of four individual mussels with from left to right in lane (1) molecular marker with band sizes 1000, 700, 500, 200 and 100 base pairs, (2) individual from which both *M. edulis*- and *M. galloprovincialis*-type alleles were amplified, (3) homozygote control for *M. edulis*-type allele, (4) homozygote control for *M. galloprovincialis*-type allele, (5) individual with only the *M. edulis*-type allele amplified, and (6) negative control.

3. Results

Of the 321 mussels from the Dutch Wadden Sea examined, six (1.9%) carried both the *M. edulis*- and the *M. galloprovincialis*-type alleles and were thus heterozygotes (Table 1). The remainder of the population were homozygotes for the *M. edulis*-type allele. Two of 141 subtidally collected mussels (1.4%) were heterozygotes and so were four of the 180 intertidally collected ones (2.2%). Of the heterozygotes, only the one, collected in the subtidal Scheurrak channel (site 3, Fig. 1), was a large specimen (53 mm long) over 1 year old. The remaining five heterozygote specimens measured 7 to 25 mm, with a mean of 12 mm; all belonged to the mussel spat of 2001 and were found widely distributed between the western subtidal (site 1, Molenrak) and the eastern intertidal (site 7, Engelsmanplaat). The Wadden Sea homozygote specimens measured 5–29 mm (mean 13 mm, $n = 263$) for spat, and 32–71 mm (mean 48 mm, $n = 52$) for one-year-old mussels. The 30 mussels from Oosterschelde all showed *M. edulis*-type alleles. For the Wadden Sea data, the frequency of the *M. edulis*-type allele is estimated as 0.9907, that of the *M. galloprovincialis* allele as 0.0093. The corresponding genotype frequencies conform to Hardy-Weinberg equilibrium (Fisher's exact test, n.s.).

4. Discussion

In the present study, 321 individuals were genotyped for the foot-protein-1 alleles that are amplified by the primers Me15 and Me16. Although we demonstrate the presence of *M. galloprovincialis*-type alleles among the mussels found in the Dutch Wadden Sea,

Table 1
Breakdown of the occurrence of homozygote mussels for the *M. edulis*-type adhesive protein allele and heterozygotes carrying alleles for *Mytilus edulis*/*M. galloprovincialis* in the Dutch Wadden Sea in August 2001. The site numbers correspond with those in Fig. 1. LLWS = low low-water spring

Site no.	Name of (nearby) location	Tidal level	Height relative to LLWS (m)	Number of homozygotes	Number of heterozygotes
1	basalt piers in North Sea, Texel	intertidal	+0.3	20	0
2	Javaruggen	subtidal	−3.5	40	0
3	Breezand	subtidal	−3.0	20	0
4	Scheurrak	subtidal	−0.7	39	1
5	Molenrak	subtidal	−0.7	20	0
6	Molenrak	subtidal	−1.5	20	1
7	Griend	intertidal	+1.7	19	1
8	Ballastplaat	intertidal	+1.3	18	2
9	Kromme Balg	intertidal	+1.5	20	0
10	Kromme Balg	intertidal	+0.4	20	0
11	Ameland	intertidal	+1.4	20	0
12	Engelsmanplaat	intertidal	+1.5	19	1
13	Engelsmanplaat	intertidal	+1.0	20	0
14	Simonszand	intertidal	+2.2	20	0

no homozygotes for the *M. galloprovincialis*-type allele were found. Because the *M. galloprovincialis*-type allele frequency was 0.0093, with random mating and in the absence of selection, homozygotes are expected to be exceedingly rare: 0.0093^2, or 0.01% of the population. Hilbish et al. (2002) mapped the distribution of the two alleles in the well-known hybrid zone in southwest England. There, around Cornwall, the authors found a region of heterozygotes flanked on one side by a region from which samples contained only the *M. edulis*-type allele and on the other side by an area with very low frequencies of the *M. galloprovincialis*-type allele. In Japan, 174 homozygotes for the *M. galloprovincialis*-type allele and 56 heterozygotes for that allele and the *M. trossulus*-type allele were observed (Inoue et al., 1997). Inoue et al. (1995b) report on the identification of 4 homozygotes for the *M. edulis*-type allele from northern Norway as well as from Brittany, France, and four homozygotes for the *M. galloprovincialis*-type allele from the French Mediterranean coast. With the use of different primers, but also amplifying from the foot-protein-1 gene, Rawson et al. (1996) identified only homozygotes from three North American allopatric sites, one for each species (total number: 191), as well as 17 homozygotes for the *M. galloprovincialis*-type allele that originated from cultures at the French Mediterranean coast.

Our study thus confirms the notion that the foot-protein-1 alleles are highly differentiated between the *M. edulis* and *M. galloprovincialis* ecotypes (Inoue et al., 1995b, 1997; Hilbish et al., 2002). The fact that we observed the *M. galloprovincialis*-type allele in low frequency can be an indication of any of three possible situations. First, the alleles may not be completely diagnostic between *M. edulis* and *M. galloprovincialis*. Second, the Dutch Wadden Sea may be part of the (historically stable) introgression region that forms the outskirts of the hybrid zone between *M. edulis* and *M. galloprovincialis* around the British Isles. Such a low level of introgression would not be detected when using the less strongly differentiated allozyme markers. This possibility, however, seems less likely because populations purely containing the *M. edulis*-type foot-protein-1 allele were observed on the southern coast of Cornwall in England (Hilbish et al., 2002). And third, we may be seeing the result of a recent invasion of *M. galloprovincialis* or some of its genes into the study area. Future research should focus on the question whether the occurrence of *M. galloprovincialis*-type foot-protein-1 alleles in the Dutch Wadden Sea is due to the fact that the marker is not diagnostic, because the area belongs to an historically stable introgression zone, or because of recent invasion.

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