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Distribution of *Mytilus* taxa in European coastal areas as inferred from molecular markers

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ABSTRACT

The genetic constitution of mussels (*Mytilus* spp.) was studied by means of three nuclear (Me 15/16, EF-bis, ITS) and one mtDNA (ND2-COIII) marker on a large European scale. In addition to a sharp cline between Atlantic and Mediterranean *M. galloprovincialis*, we observed a clear genetic distinction between the Black Sea and Mediterranean populations and a higher incidence of *M. trossulus* than reported so far in northern European populations. The frequency of *M. galloprovincialis* nuclear alleles was high along the Iberian Peninsula and decreased abruptly along the French coasts with a high frequency of *M. edulis* alleles in the Bay of Biscay, The Netherlands, Germany, Iceland, Barents and White Seas, and with little evidence of introgression between the two taxa. *M. trossulus* alleles were observed in the Baltic Sea and Danish Straits as expected. In addition, occurrence of *M. trossulus* alleles in cold waters of Iceland, Barents Sea and White Sea is reported for the first time.

1. Introduction

Mussels *Mytilus* are distributed in northern and southern hemispheres (Hilbish et al., 2000; Borsa et al., 2007; Gerard et al., 2008). In Europe, the presence of three *Mytilus* taxa has been traditionally reported (Gosling, 1992). *M. trossulus* is present in the Baltic Sea as a hybrid with *M. edulis* (Riginos and Cunningham, 2005; Kijewski et al., 2006) and in Scotland (Dias et al., 2008). *M. trossulus* has been diverging in the North Pacific for about 3.5 million years (Rawson and Hilbish, 1995) and has colonised the Atlantic coasts of North America and Europe most probably following the last glacial maximum 18 thousand years ago (Riginos and Cunningham, 2005; Rawson and Harper, 2009), while *M. edulis* evolved in the North Atlantic and colonised eastern coasts of North America and Europe during the Pleistocene (Riginos and Henzler, 2008; Riginos et al., 2004). In Europe *M. edulis* has been reported from the Atlantic coastal waters of France, The Netherlands, Germany, Great Britain, Ireland and Norway (Hummel et al., 2001; Luttikhuizen et al., 2002; Śmietanka et al., 2004; Riginos and Henzler, 2008). *M. galloprovincialis* diverged in the Mediterranean about 2 million years ago (Rawson and Hilbish, 1995; Daguin and Borsa, 2000). As a pure taxon, it occurs in the Black and Mediterranean Seas (Daguin et al., 2001; Śmietanka et al., 2004). Outside of these regions it has been reported from the Atlantic coastal areas of Spain, France, Great Britain and Ireland (Gosling et al., 2008; Quesada et al., 1998) hybridising with *M. edulis*. Secondary introgression in *M. galloprovincialis* has been reported (Quesada et al., 1995a; Diz and Presa, 2008). However, the knowledge about the present distribution of *Mytilus* taxa in Europe requires updating due to changes in their geographic range. The recent discovery of *M. trossulus* on the coasts of western Scotland (Dias et al., 2008; Zbawicka et al., 2010) and *M. edulis* on Spitsbergen (Berge et al., 2005) are good examples.

Mussels have been cultured in Europe for hundreds of years (Smaal, 2002). Mussel culture depends on natural resources in terms of phytoplankton, and recruitment and exploitation capacities of ecosystems. Mussel seed is collected from wild beds or collector ropes and transferred to other areas for growing. The geographic distribution of *Mytilus* in Europe may therefore result not only from natural processes, but also from introductions and translocations (Kijewski et al., 2009).

Mytilus taxa in Europe are difficult to identify using only shell characteristics because of adaptations of the shell to different environmental conditions, and also because of hybridisation between pairs of taxa (Bierne et al., 2002; Hilbish et al., 2003; Riginos and Cunningham, 2005; Gardner and Thompson, 2009). Large coastal areas in Europe are occupied by hybrid *M. edulis*/*M. galloprovincialis* and/or *M. edulis*/*M. trossulus* populations (Bierne et al., 2003; Kijewski et al., 2006; Gosling et al., 2008). In order to overcome difficulties with *Mytilus* taxa

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designation based solely on shell morphology in North Atlantic and Pacific, diagnostic nuclear DNA markers have been constructed (e.g. Heath et al., 1995; Inoue et al., 1995). Several DNA nuclear markers have been used in an attempt to characterise European *Mytilus* populations: EF-bis (Bierne et al., 2003; Kijewski et al., 2009), Me 15/16 (Hilbish et al., 2002; Śmietanka et al., 2004; Gosling et al., 2008) and M7 Lysin (Riginos and McDonald, 2003; Kijewski et al., 2009). Mitochondrial DNA (mtDNA) has also been used as a marker in European *Mytilus* population genetic studies (Śmietanka et al., 2004, 2009) and several studies have indicated that Baltic *M. trossulus* is introgressed by *M. edulis* mtDNA (Wenne and Skibinski, 1995; Quesada et al., 1998; Zbawicka et al., 2003; Kijewski et al., 2006). However, knowledge about European populations of *Mytilus* is still fragmentary. Most research on molecular population genetics of *Mytilus* has been limited to regional or local coastal areas in Europe (e.g. Hilbish et al., 2002; Kijewski et al., 2006, 2009; Gosling et al., 2008). In most other studies, only a limited number (1–2) of molecular markers have been used (e.g. Quesada et al., 1998; Ladoukakis et al., 2002; Śmietanka et al., 2004; Gosling et al., 2008).

In this study, the genetic composition of wild mussel populations was studied over a large geographic range on the European coastline, from Iceland and Barents Sea to Gibraltar and the Black Sea using 22 samples, with three nuclear DNA diagnostic markers, Me 15/16, EF-bis and ITS, and portions of the mtDNA coding region for the first time. Information on the distribution of *Mytilus* genotypes and alleles in Europe as contrary to traditionally named taxa is provided in this paper.

2. Materials and methods

Samples of approximately 50 *Mytilus* individuals were collected from 22 sites on European coasts in 2003–2004, except for the AZO sample collected in 1997 (Table 1 and Fig. 1). Prior to DNA extraction, whole mussels were frozen at −70 °C or preserved in 96% ethanol.

2.1. Molecular methods

For DNA extraction pieces of mantle tissue were removed, homogenised and extracted using the CTAB method of Hoarau et al. (2002). DNA was suspended in deionized water. Three nuclear DNA markers, Me 15/16, EF-bis and ITS, that diagnostically differentiate the *Mytilus* taxa in their North Atlantic and Pacific range were analysed. A pair of primers Me15 and Me16 was used to amplify a segment of gene coding an adhesive protein of byssus (Me 15/16), with diagnostic PCR products length differences among the three studied taxa: *M. edulis*, *M. trossulus* and *M. galloprovincialis* (Inoue et al., 1995). Diagnostic differences between *M. trossulus* and the other two taxa in the internal transcribed spacer (ITS) regions between the 18S and 28S rDNA genes were detected with the restriction enzyme *HhaI* digestion (Heath et al., 1995; Kijewski et al., 2006). The EF-bis marker is an intron in the elongation factor 1 α (Bierne et al., 2003) with double digestion RFLP variation diagnostic between *M. trossulus*, *M. edulis* and *M. galloprovincialis* (Kijewski et al., 2006, 2009). Restriction fragments were separated by electrophoresis in 2% high-resolution agarose gels

Table 1
A — genotype frequencies of two nuclear markers Me 15/16 and EF-bis in the 22 samples. n — number of specimens genotyped; E–*M. edulis*, T–*M. trossulus*, G–*M. galloprovincialis* allele. He — heterozygosity expected. No *M. galloprovincialis*/*M. trossulus* heterozygotes were observed for Me 15/16. B — phenotype frequencies of the ITS marker in the eight samples where *M. trossulus* alleles were recognised.

A

		Glu-5′ (ME 15–16)							EF-bis							
Sample site		n	EE	EG	ET	GG	TT	He	n	EE	EG	ET	GG	GT	TT	He
Asko	ASK	56	0.27		0.41		0.32	0.61**	56					0.02	0.98	0.02
Gdansk Bay	GDA	54	0.46	0.02	0.41		0.11	0.64**	54					0.04	0.96	0.04
Mecklenburg Bight	MEB	63	0.76		0.24			0.39**	63			0.11			0.89	0.11
Tjarno	TJA	35	0.94		0.06			0.33**	32	0.09		0.22			0.69	0.33
White Sea	ONE	39	1.00					0	39	0.03		0.10			0.87	0.14
Barents Sea	BAR	20	0.65	0.10	0.10		0.15	0.47**	20	0.10		0.10			0.80	0.26**
Iceland	ICE	46	1.00					0	48	0.02		0.29			0.69	0.28
Balgzand	BAL	44	0.95	0.05				0.04	44	0.82	0.09		0.09			0.24**
Westerschelde	WES	56	1.00					0	55	0.87	0.04	0.05	0.04			0.15**
Somme	SOM	52	1.00					0	51	0.94	0.06					0.06
Seine	SEI	48	1.00					0	46	0.98	0.02					0.02
Loire	LOI	46	0.98	0.02				0.02	45	0.31	0.53		0.16			0.49
Il′Re	IDR	49	0.96	0.04				0.04	49	0.31	0.51		0.18			0.50
Bidasoa	BID	51	0.02	0.08		0.90		0.11*	50	0.02	0.06		0.92			0.10*
Mundaka	MUN	52	0.02	0.08		0.90		0.11*	51	0.04	0.27		0.69			0.29
Vigo	VIG	47		0.04		0.96		0.04	44		0.23		0.77			0.20
Punta Camarinal	CAM	48		0.04		0.96		0.04	46		0.17		0.83			0.16
Gerona	GER	42		0.05		0.95		0.05	32	0.06	0.22		0.72			0.29
Banyuls	BAN	44				1.00		0	38	0.05	0.21		0.74			0.27
Oristano	ORI	51		0.02		0.98		0.02	49	0.02	0.16		0.82			0.19
Odessa	ODE	46				1.00		0	46				1.00			0
Azov Sea	AZO	52				1.00		0	52				1.00			0

B

Sample site		n	Not *M.trossulus* homozygote	Heterozygote	*M.trossulus* homozygote
Asko	ASK	60	0.32	0.68	0.00
Gdansk Bay	GDA	55	0.29	0.71	0.00
Mecklenburg Bight	MEB	63	0.70	0.30	0.00
Tjarno	TJA	22	1.00	0.00	0.00
White Sea	ONE	28	1.00	0.00	0.00
Barents Sea	BAR	11	0.73	0.00	0.27
Iceland	ICE	42	0.98	0.00	0.02
Westerschelde	WES	56	1.00	0.00	0.00

Significance of deviation from HWE $p <= 0.05$ indicated with * for χ^2 and with ** for both χ^2 and G^2 tests.