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Regional genetic differentiation in the blue mussel from the Baltic Sea area

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ABSTRACT

Connectivity plays an important role in shaping the genetic structure and in evolution of local adaptation. In the marine environment barriers to gene flow are in most cases caused by gradients in environmental factors, ocean circulation and/or larval behavior. Despite the long pelagic larval stages, with high potential for dispersal many marine organisms have been shown to have a fine scale genetic structuring. In this study, by using a combination of high-resolution genetic markers, species hybridization data and biophysical modeling we can present a comprehensive picture of the evolutionary landscape for a keystone species in the Baltic Sea, the blue mussel. We identified distinct genetic differentiation between the West Coast, Baltic Proper and Bothnian Sea regions, with lower gene diversity in the Bothnian Sea. Oceanographic connectivity together with salinity and to some extent species identity provides explanations for the genetic differentiation between the West Coast and the Baltic Sea (Baltic Proper and Bothnian Sea). The genetic differentiation between the Baltic Proper and Bothnian Sea cannot be directly explained by oceanographic connectivity, species identity or salinity, while the lower connectivity to the Bothnian Sea may explain the lower gene diversity.

1. Introduction

Connectivity plays an important role in shaping the genetic structure and in evolution of local adaptation (Palumbi, 1994). In marine environments barriers to gene flow are in most cases caused by gradients in environmental factors (temperature, salinity regimes etc.), ocean circulation and/or larval behavior (Kelly and Palumbi, 2010). Most marine organisms produce free-swimming larvae that spend hours to weeks in the water column (Paris et al., 2007) and this pelagic development and passive dispersal has traditionally been assumed to result in high connectivity and low genetic differentiation over long distances (Bohonak, 1999). Several studies have however revealed genetic structuring also on small geographical scales, even in marine organisms with a long pelagic larval stage (Barber et al., 2002; Gilg and Hilbish, 2003; Knutsen et al., 2003; Johannesson and Andre, 2006; Kelly and Palumbi, 2010). Identification of spatial population structure and connectivity patterns is achieved through a range of methods including trace elements, stable isotopes and the use of genetic markers (Crooks and Sanjayan, 2006). Neutral genetic markers can provide insights of reduced gene flow and breaks in population connectivity. Methods based on genome wide genetic markers, for example amplified fragment length polymorphism (AFLP) (Vos et al., 1995), cover a larger scope of both selective and neutral markers that can provide a more comprehensive description of gene flow and population structures (Stinchcombe and Hoekstra, 2008; Nosil et al., 2009). Supplemented with biophysical models genome wide markers may provide a better understanding on how demographic factors, connectivity barriers and selection shape the marine evolutionary landscape (Cowen and Sponaugle, 2009; Jacobi et al., 2012).

The Baltic Sea ecosystem is an example of an evolutionarily young marine ecosystem (Johannesson and Andre, 2006) connected to the North Sea only by narrow and shallow sills in Öresund

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and the Danish Belts. The salinity gradient present in the Baltic Sea area spans from 17 to 25 along the Swedish West Coast, from 5.5 to 7.3 in the Baltic proper, approximately 5 in the Bothnian Sea and 2 to 4 in the Bothnian Bay (Elmgren, 2001). The adaptation of species to the brackish conditions in the Baltic Sea has caused both morphological and genetic differences compared to their salt/fresh water ancestors. Many species in the Baltic Sea constitute unique evolutionary lineages with a lower genetic diversity compared to the North Sea, and a strong genetic differentiation between populations in North Sea and the Baltic Sea (Johannesson and Andre, 2006; Johannesson et al., 2011).

A keystone species in the Baltic Sea ecosystem is the blue mussel (*Mytilus edulis trossulus* complex). The blue mussel has several ecologically important features including filtering water and providing a link between the benthos and the pelagic by cycling nutrients and organic matter (Kautsky and Evans, 1987). It also creates complex structures acting as substrate for many organisms and it is an important food source for many fish and bird species (Koivisto, 2011), hence sustainability of the blue mussel populations is a key issue for the Baltic Sea ecosystem. The blue mussel has a long planktonic larval phase, 3–4 weeks (Seed, 1969) in general and as long as 5–6 weeks in the Baltic Sea (Kautsky, 1982), which gives a potential for long distance dispersal. Despite this high potential for dispersal strong genetic and morphological differentiation between the Baltic Sea and North Sea populations have been observed (Bulnheim and Gosling, 1988; Väinölä and Hvilsom, 1991; Johannesson et al., 1990). The Baltic Sea blue mussels are smaller in size, have a lower growth rate and thinner shells (Remane and Schlieper, 1972; Kautsky et al., 1990) compared to the mussels from the North Sea. These differences are partly explained by the lower salinity, but may also partly be due to the occurrence of lineages from two sister species of *Mytilus* within Baltic Sea blue mussels. Along the Swedish West Coast populations are dominated by *Mytilus edulis* with limited hybridization with *Mytilus trossulus* while the Baltic Sea is considered a hybrid complex with populations dominated by the *M. edulis trossulus* individuals (with an extensive mixing of the *M. edulis* and *M. trossulus* genomes) (Väinölä and Strelkov, 2011). Although the hybridization pattern and the gross genetic differentiation between the Baltic Sea and the West Coast are well studied (Bierne et al., 2003; Riginos and Cunningham, 2005; Kijewski et al., 2006, 2011; Stuckas et al., 2009; Väinölä and Strelkov, 2011; Zbawicka et al., 2012), population genetic structure and connectivity within the Baltic Sea are less well studied. Early allozyme studies assessing the genetic structure of the Blue mussels in the Baltic Sea have used few markers and only one or a few sites, all in the Baltic Proper, (Bulnheim and Gosling, 1988; Väinölä and Hvilsom, 1991; Johannesson et al., 1990), giving a low resolution of the genetic structure in the Baltic Sea. More recent studies based on neutral microsatellite data (Gardeström et al., 2008), and neutral single nucleotide polymorphisms (SNP) (Wennerström et al., 2013) suggests a genetic differentiation between the different Baltic Sea regions; Baltic Proper (BP), Bothnian Sea (BS) and Gulf of Finland (GF) (Gardeström et al., 2008; Wennerström et al., 2013).

The aim of this study was to further explore the causes of the genetic structure and barriers to gene flow in the blue mussel between the West Coast and the Baltic Sea and within the Baltic Sea by using a combination of genome wide, high-resolution genetic markers, biophysical connectivity modeling and species hybridization data. The first objective was to analyze the genetic structure using AFLP, a method that yields a large number of genetic markers randomly distributed across the genome including both neutral and non-neutral regions to achieve a higher resolution than previous studies. The second objective was to apply a comprehensive method for connectivity measurements in marine environments i.e. a biophysical model based on ocean circulation, depth and the duration of the pelagic larval phase. The third objective was to evaluate the relative effects of connectivity, geographical distance and salinity on the genetic structure. The fourth objective was to evaluate the influence of species identity (based on a diagnostic molecular marker for the different species *M. edulis*, *M. trossulus* and *Mytilus galloprovincialis*) on the genetic structure but also to remove gross effects of species identity before analyzing other structuring variables.

2. Material and methods

2.1. The blue mussel

The blue mussel has a life span of around 12 years and a generation time of 1–2 years. Spawning takes place during spring, but depending on temperature and food abundance a second period of spawning can occur during summer or autumn (Seed, 1969; Kautsky, 1982). In the Baltic Sea the first spawning takes place during the second year of age (Kautsky, 1982).

2.2. Sampling sites

Blue mussels were sampled at 12 sites; along the Swedish West Coast (WC), in the Baltic Proper (BP) and in the Bothnian Sea (BS) during June, July and August 2012, 2013 (Fig. 1, Table 1). The West Coast sites were; *Tjärnö* (TJA), *Kristineberg* (KRI). *Kullen* (KUL), *Bjärred* (BJA). Baltic Proper sites: Sweden, *Karlskrona* (KAR), Poland, *Gdansk*, (GDA), Sweden, *Askö* (ASK), Finland, *Tvärminne* (TVA). Bothnian Sea sites: Sweden, *Simpnäs* (SIM), *Grisslehamn* (GRI), *Singö* (SIN), *Höga Kusten* (HKU). The sampled sites were all situated in the outer part of the archipelago and therefore minimizing the between site differences in terms of exposure to water currents and pollution. Temperature regimes and salinity differ between the different regions (WC, BP, BS) but are similar among the sampled sites in each of the regions (Leppäranta and Myrberg, 2009).

At each site 30–60 individuals (14–20 individuals used in the subsequent AFLP analysis) were sampled using either a benthic sledge, a triangular bottom scraper or by scuba/by hand from a depth between 1 and 10 m, depending on distribution and population densities.

2.3. Generation of genetic markers

2.3.1. DNA isolation

Each individual was measured and age was estimated by counting growth rings (Haskin, 1954) all individuals included in this study had an estimated age between 3 and 5 years. The adductor muscle was dissected, snap frozen and immediately stored in −80 °C. To avoid batch/site biases all samples were randomly ordered prior to DNA isolation. Total genomic DNA was isolated from a small piece of the muscle tissue ~2 × 2 mm, of each specimen using the E. Z.N.A. Mollusc DNA Kit (OMEGA Bio-Tek, Norcross, GA, U.S.A.) with minor modifications of the manufacturers' protocol. In short, tissue was incubated overnight at 56 °C in 250 μl ML1 lysis buffer and 25 μl proteinase K. Followed by a chloroform:isoamyl (24:1, 300 μl) step with 3 min centrifugation. DNA suspended in an aqueous phase was then carefully separated from the interphase (200–300 μl) and treated with 300 μl MBL buffer and 10 μl RNAse, and incubated for 10 min in 70 °C. The DNA was precipitated from the solution with 99.7% ethanol and transferred to a DNA HiBind spin columns and centrifuged to remove any trace of alcohol. Purified DNA was eluted with 50 μl (preheated to 70 °C) 10 mM Tris-Hcl (pH 8.5) buffer. To estimate yield and quality of obtained DNA an agarose gel 1.5% electrophoresis (100 V,