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**Phylogeography and hybrid swarms:
history of brackish water bivalve diversity
in North European marginal seas**

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- II. Nikula, R., Strelkov, P. & Väinölä, R. 2007: Diversity and trans-Arctic invasion history of mitochondrial lineages in the North Atlantic *Macoma balthica* complex (Bivalvia: Tellinidae). – *Evolution* 61: 928-941.
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This study addressed the large-scale molecular zoogeography in two brackish water bivalve molluscs, *Macoma balthica* and *Cerastoderma glaucum*, and genetic signatures of the postglacial colonization of Northern Europe by them. The traditional view poses that *M. balthica* in the Baltic, White and Barents seas (i.e. marginal seas) represent direct postglacial descendants of the adjacent Northeast Atlantic populations, but this has recently been challenged by observations of close genetic affinities between these marginal populations and those of the Northeast Pacific. The primary aim of the thesis was to verify, quantify and characterize the Pacific genetic contribution across North European populations of *M. balthica* and to resolve the phylogeographic histories of the two bivalve taxa in range-wide studies using information from mitochondrial DNA (mtDNA) and nuclear allozyme polymorphisms.

The presence of recent Pacific genetic influence in *M. balthica* of the Baltic, White and Barents seas, along with an Atlantic element, was confirmed by mtDNA sequence data. On a broader temporal and geographical scale, altogether four independent trans-Arctic invasions of *Macoma* from the Pacific since the Miocene seem to have been involved in generating the current North Atlantic lineage diversity. The latest trans-Arctic invasion that affected the current Baltic, White and Barents Sea populations probably took place in the early post-glacial. The nuclear genetic compositions of these marginal sea populations are intermediate between those of pure Pacific and Atlantic subspecies.

In the marginal sea populations of mixed ancestry (Barents, White and Northern Baltic seas), the Pacific and Atlantic components are now randomly associated in the genomes of individual clams, which indicates both pervasive historical interbreeding between the previously long-isolated lineages (subspecies), and current isolation of these populations from the adjacent pure Atlantic populations. These mixed populations can be characterized as self-supporting hybrid swarms, and they arguably represent the most extensive marine animal hybrid swarms so far documented. Each of the three swarms still has a distinct genetic composition, and the relative Pacific contributions vary from 32 to 90 % in local populations. This diversity highlights the potential of introgressive hybridization to rapidly give rise to new evolutionarily and ecologically significant units in the marine realm.

In the south of the Danish straits and in the Southern Baltic Sea, a broad genetic transition zone links the pure North Sea subspecies *M. balthica rubra* to the inner Baltic hybrid swarm, which has about 60 % of Pacific contribution in its genome. This transition zone has no regular smooth clinal structure, but its populations show strong genotypic disequilibria typical of a hybrid zone maintained by the interplay of selection and gene flow by dispersing pelagic larvae. The structure of the genetic transition is partly in line with features of Baltic water circulation and salinity stratification, with greater penetration of Atlantic genes on the Baltic south coast and in deeper water populations.

In all, the scenarios of historical isolation and secondary contact that arise from the phylogeographic studies of both *Macoma* and *Cerastoderma* shed light to the more general but enigmatic patterns seen in marine phylogeography, where deep genetic breaks are often seen in species with high dispersal potential.

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CONTENTS

1. INTRODUCTION	5
1.1. Biogeography of the North European marginal seas	5
1.2. Intraspecific genetic structure of European marine biota	7
1.2.1. Atlanto-Mediterranean patterns of genetic structuring	8
1.2.2. Trans-Arctic and trans-Atlantic affinities of European marine biota	10
2. AIMS OF THE THESIS	11
3. MATERIAL AND METHODS	12
3.1. Samples	12
3.2. Molecular data	12
3.3. Data analyses	13
3.3.1. Phylogeny reconstruction	13
3.3.2. Estimation of lineage and population divergence times	14
3.3.3. Geographical patterns of genetic variation and admixture	15
3.3.4. Assessment of genotypic structure at the contact of Pacific and Atlantic <i>Macoma</i> genomes	15
3.3.5. Geography of the genetic transitions in the Baltic <i>Macoma</i>	15
4. RESULTS AND DISCUSSION	16
4.1. Phylogeography of <i>Cerastoderma glaucum</i> and the NE Atlantic <i>Macoma balthica</i> (<i>M. b. rubra</i>) (I, II)	16
4.2. Trans-Arctic invasion history of the <i>Macoma balthica</i> complex (II)	18
4.3. Hybridization and introgression of <i>M. balthica</i> subspecies in the North European marginal seas: hybrid swarms and a broad transition zone (III, IV)	21
5. CONCLUSIONS	25
6. ACKNOWLEDGEMENTS	28
7. REFERENCES	29

1. INTRODUCTION

1.1. Biogeography of the North European marginal seas

During the last glacial maximum the Baltic and White Sea basins and most of the Barents Sea in Northern Europe were covered by the Scandinavian ice sheet (Fig. 1). Since the final melting of the ice, hydrographical and climatological settings have regulated reconstitution of biotic communities in these sea areas, collectively designated as the North European marginal seas in the present work.

Reconstructions of postglacial sea surface temperatures in the North Atlantic show that the Holocene warming was often interrupted by variably long cooling phases (Mayewski et al. 2004; Andresen et al. 2007). The variability of the Holocene salinity conditions has been most extensive in the Baltic, where the postglacial hydrogeological evolution of the basin repeatedly opened and closed the connection to the NE Atlantic and brought about alternating freshwater and saline phases (review in Gustafsson & Westman 2002). It also caused marked variation in the salinity during the Littorina stage, when the salinity first rapidly rose to a level ca 5 ppt higher than at present across the basin, and then started declining since about 6000 years ago (Björck 1995). Because of their differing geomorphology, the more northerly Barents and White seas did not experience drastic salinity changes but were still affected by the varying Holocene temperatures (Berger et al. 2001).

As a consequence of the variable history, the present marginal sea biotas are collections of invaders of different origin that were able to colonize the areas during the various postglacial phases and could cope

with any change ever since their arrival. The complexity of the colonization process of the North European marginal seas was recognized and discussed early on (Ekman 1953, Segerstråle 1957, Zenkevitch 1963). In the classical works on the Baltic Sea zoogeography, truly marine species have been classified according to their supposed geographical origin either as Atlantic-boreal euryhaline species or arctic marine relict species (Ekman 1953, Segerstråle 1957). The Atlantic-boreal element includes taxa that extended their range postglacially to the Baltic Sea from the adjacent fully marine areas and still survive in both. The Arctic marine relicts of the Baltic Sea, on the other hand, are such marine species that invaded the Baltic during phases that were cooler than the present and whose main marine distribution today is at higher latitudes in the NE Atlantic, disjunct from the Baltic range (Ekman 1953).

The classification conventionally applied to the White Sea biota is somewhat different: species are considered either boreal, arctic-boreal or arctic (e.g. Berger et al. 2001); no relict status is emphasized for the arctic species because of the present day close proximity of the arctic biogeographical zone. Zenkevitch (1963), however, considered the White Sea to harbour both cold-water relicts and warm-water relicts, as some of the arctic or boreal taxa occur in the White Sea isolated from their main area of distribution. He also assigned White Sea fauna into “forms common to the Baltic Sea” and “forms common to the Far Eastern Seas”; the first group would include species that were arctic marine relicts in the Baltic Sea, and the latter involved species that otherwise are found in the Pacific Ocean but have their westernmost oupost in the White Sea owing to dispersal during the Holocene climatic optimum.

Prime examples of euryhaline marine organisms that have been successful in colonizing the marginal seas are bivalves: yet in the Baltic there are only five marine bivalve species whose distributions extend all the way to the oligohaline northern Baltic (Segerstråle 1957, Petersen & Russell 1971). They are, however, mostly key species in the ecosystem, with high abundance and biomass. Traditional concepts about the zoogeographical origin and arrival of marine bivalves in marginal seas were based on their global distribution and, when available, on fossil record. By default then, naturally dispersed bivalves, as any marine species, were assumed to be closely related to and derived from geographically closest populations found in the fully marine areas. In a way this view is axiomatic – a species cannot colonize a new area unless it is within immediate dispersal range from it – but does not necessarily imply that marginal sea populations of marine species are closely related to their nearest *present-day* marine conspecifics.

Development of molecular markers that can be used to study genetic affinities at intraspecific level revolutionized biogeography by allowing direct inference about evolutionary ancestry of and gene flow between geographical populations. A common feature of most studied species of marine origin in the marginal seas is – in compliance with the traditional biogeographical view – that their populations are closely related to but genetically depauperate compared to conspecific populations in the adjacent North-East Atlantic (reviewed by Johannesson & André 2006). Bivalves make an exception however, which necessitated formulating a novel biogeographical hypothesis regarding origins of marine North European marginal sea biota.

Studies of the two most common Baltic bivalves, *Macoma balthica* and *Mytilus trossulus*, revealed that there are populations in the North European marginal seas that have their genetically most similar present-day relatives not in the adjacent North-East Atlantic but in the North East Pacific or the North-West Atlantic (Varvio et al. 1988, McDonald & Koehn 1988, Väinölä & Varvio 1989, Väinölä & Hvilsum 1991, Väinölä 2003). The first results on the unconventional biogeographical affinities were based on nuclear allozyme characters, and they were then corroborated by phylogenetically more direct information on DNA characters. (Borsa et al. 1999, Luttikhuisen et al. 2003). Since anthropogenic introductions of marine species into the Baltic Sea are frequent (Leppäkoski et al. 2002), the possibility could not be ruled out that the exotic genetic elements in the bivalves are due to recent human activity (Väinölä 2003) – a scenario that at the outset appears more plausible than natural dispersal over such long distances.

This thesis explores molecular genetic signatures of postglacial colonization of North European marginal seas in two marine bivalve taxa. The lagoon cockle *Cerastoderma glaucum* belongs to the well-established group of Atlantic-boreal marine species, while the marginal sea populations of the Baltic clam *Macoma balthica* have been suggested to include a Pacific genetic contribution, the status and arrival history of which is still largely unrecognized and unexplored in the North-East Atlantic zoogeography. The genealogical relationships of both *C. glaucum* and *M. balthica* populations are studied across their distributional ranges to test the conceptions of the ancestry of the species' marginal populations. The primary focus of the work is on verify-

ing, quantifying and characterizing the contribution of the Pacific genetic components across North European populations of *M. balthica*.

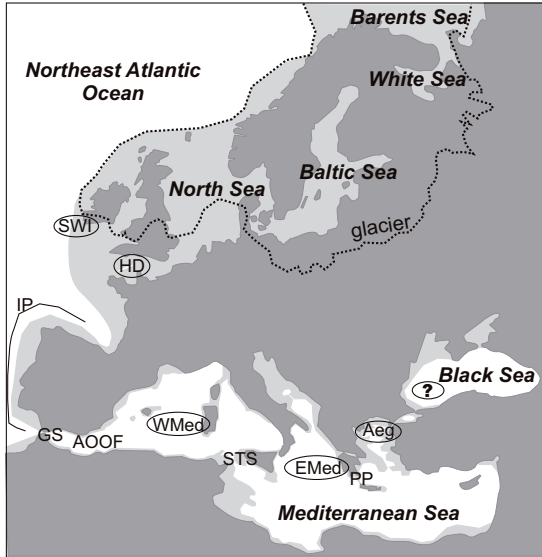


Figure 1. European coasts during the last glacial maximum. Paleoshorelines (light grey) of the Mediterranean and Black seas were drawn after Lambeck & Purcell (2005) and the shoreline in the NE Atlantic after Frenzel et al. (1991). The limits of the Scandinavian ice sheet (dotted line) were drawn after Svendsen et al. (2004). Regions suggested as places of phylogenetic sundering in multiple marine species are marked on the map: GS – Gibraltar Strait; AOO – Almeria-Oran oceanographic front; STS – Siculo-Tunisian Strait; PP – Peloponnese peninsula. Areas appearing to have been important Pleistocene refugia (encircled, but not to indicate the exact locations which are largely unknown) are: HD – Hurd Deep in the English Channel; SWI – Southwest Irish coast; IP – Iberian peninsula; WM – Western Mediterranean; EM – Eastern Mediterranean. Persistence of marine species in the Black Sea during the last glacial maximum is controversial.

1.2. Intraspecific genetic structure of European marine biota

With the aim to give the findings of this thesis a broader context, I will in the following review genogeographic (phylogeographic and more generally population genetic) patterns of European marine taxa that share their distribution with *C. glaucum* and *M. balthica*. The ranges of the two bivalves overlap on the NE Atlantic coasts from France to Norway and in the Baltic Sea (I, III). *C. glaucum* is confined to the NE Atlantic and the adjacent Mediterranean, Black and Caspian seas. *M. balthica* is more widespread, with a trans-Arctic distribution in the boreal-subarctic zone, on both coasts of the Atlantic and the Pacific oceans.

Much of the shallow fringes of these marine regions repeatedly turned into dry land in course of the Pleistocene (Fig. 1), and those habitats that remained submerged experienced a changing climate. Which species persisted glacial environmental changes and where? How did marine species reclaim the regions deserted in course of the glaciations? How did climatic fluctuations affect genetic diversity of marine species? Comparisons of the genogeographic structures of codistributed species with similar life history traits (e.g. Arbogast & Kenagy 2001) enable generalizations about importance of certain glacial refugial regions and of (re)colonization pathways for the present genetic structure of species. I will first take a look at within-European patterns, and then consider the trans-Atlantic and trans-Arctic affinities of European marine species that share the passive planktonic dispersal mode of *Macoma* and *Cerastoderma*.

1.2.1. Atlanto-Mediterranean patterns of genetic structuring

Occurrences of geographically localized evolutionary lineages or differentiated gene pools within European marine species are common and have been commonly interpreted to reflect persistence of differentiation that was induced by Pleistocene glaciations (e.g. Quesada, Beynon & Skibinski 1995, Quesada, Zapata & Alvarez 1995, Pannacciulli et al. 1997, Luttkhuizen et al. 2003), in concordance with terrestrial phylogeographic inference (e.g. Hewitt 2004). Within the Mediterranean basin genetic breaks in many species seem to be attracted to regions “of shallow depth” or straits that likely impeded migration repeatedly during the phases of low glacial sea level (Fig. 1): i.e. the Strait of Gibraltar (Saavedra et al. 1995, Sanjuan et al. 1997, Quesada et al. 1998, Pérez-Losada et al. 2002), the Siculo-Tunisian Strait (Saavedra et al. 1995, Procaccini et al. 2001, Peijnenburg et al. 2004; Arnaud-Haond et al. 2007, Iannotta et al. 2007,), and the Peloponnese Peninsula (I, Pérez-Losada et al. 2007). Also the hydrographical Almeria-Oran oceanographic front near the Gibraltar has been suggested to promote genetic discontinuity in some taxa (Quesada, Beynon & Skibinski 1995, Quesada, Zapata & Alvarez 1995, Pannacciulli et al. 1997, Saavedra & Peña 2005).

Ages of mtDNA lineage splits have been estimated in many taxa, and notably often they are assumed to date several glacial phases back (e.g. I, Provan et al. 2005, Papadopoulos et al. 2005, Pérez-Losada et al. 2007). This implies existence of multiple refugia during glacial phases and persistence of phylogeographic structure through the intervening interglacials. However, in some species only subtle geographic dif-

ferentiation is found that points to contemporary dispersal barriers or isolation by geographic distance as the most immediate cause for population structure (e.g. Launey et al. 2002, Duran et al. 2004a, b; Dieckmann et al. 2005). Also, there are cases of genetic homogeneity over vast distances in taxa whose dispersal efficiency does not seem to differ from those of sundered species. Such a pattern may indicate glacial survival in a single small population from where recolonization took place quickly, or anthropogenic introduction and spread (e.g. Iannotta et al. 2007).

In between the extremes of deep genetic sundering and large-scale homogeneity stand taxa where evidence of admixture (secondary contact) between distinct evolutionary lineages has been found, but where the lineages still remain largely allopatric across most of their geographic ranges (Quesada, Beynon & Skibinski 1995, I, Iannotta et al. 2007). Also, some postglacially established marine coastal populations seem to harbour several deep mtDNA lineages whose geographic origins are not resolved, and therefore it cannot be discerned whether the pattern is due to dispersal from multiple refugia or transfer of the diversity from a single large refugial population to postglacially established populations (e.g. Olsen et al. 2004, Peijnenburg et al. 2006).

Based on high levels of local intraspecific genetic diversity, Pleistocene marine refugial areas have been inferred to have existed in the NE Atlantic around the Iberian Peninsula, in the English Channel and off the southwestern coast of Ireland (Coyner et al. 2003, Provan et al. 2005, Jolly et al. 2005, Hoarau et al. 2007, see Fig.1). The Mediterranean Sea as a whole is a plausible Pleistocene refugium for NE Atlantic marine species. The phylogeographic splits into western and eastern Mediterranean

lineages found in some taxa (e.g. I, Papadopoulos et al. 2005, Iannotta et al. 2007, Arnaud-Haond 2007) further suggest that allopatric refugia may have existed within the Mediterranean basin. Surprisingly, the suggested refugial areas around the British Isles were in the immediate vicinity or even covered by the Scandinavian ice sheet during the last glacial maximum according to glacial reconstructions. Phylogeography of the English Channel populations exemplifies how genetic patterns repeated in multiple co-distributed taxa potentially refine our understanding of the past environmental settings; more commonly, phylogeographic patterns are construed under the available paleoclimatological knowledge that is taken as granted.

In conclusion, the large-scale genetic structure of some Atlanto-Mediterranean species appears to be governed by past events even today, while in others Pleistocene extinctions seem to have reset the genetic memory and even the large scale patterns are product of Holocene environmental settings. These aspects combine in species exhibiting local intergradation of differentiated evolutionary lineages, or secondary contact zones. A contact of distinct gene pools that involves interbreeding and backcrossing (i.e. hybridization) may result in introgressive hybridization or “permanent incorporation of genes from one set of differentiated populations into another genetic material from one distinct gene pool to another”, as defined by Rieseberg & Wendel (1993). Given low dispersal/cross-fertilization rates and/or reduced fitness of hybrids, introgressive hybridization may be fairly limited and lead to formation of a geographically narrow genetic transition or a hybrid zone flanked by pure parental populations (Barton & Hewitt 1985). If interbreeding between contacting popula-

tions is relatively free from intrinsic and extrinsic constraints, introgressive hybridization may eventually lead to formation of a hybrid swarm or a population where the genetic characters that earlier segregated between isolated parental populations are completely disassociated at level of individual genotypes (e.g. Taylor et al. 2006). The possible spatial and genomic outcomes of introgressive hybridization in natural environments are as diverse as are the environmental and genetic settings of the meeting populations; the above examples serve to provide definitions of some key terms used in this thesis.

Patterns of genetic structure in secondary contact zones have been widely studied especially in the terrestrial environment, and they have been viewed as “windows on evolutionary processes” of adaptation, natural selection and rise of species boundaries (Harrison 1990). Less is known of existence of hybrid zones and their genetic dynamics in marine environments (Gardner 1997), although it could be argued that marine hybrid zones in particular could shed light on genetic mechanisms of speciation and reproductive isolation as marine species are perhaps less than terrestrial species inflicted by physical constraints to gene flow due to their high dispersal capacity and fecundity. This would facilitate recognition of intrinsic mechanisms driving population differentiation and speciation.

However, among the most studied instances of marine hybridization are the European contact zones between the members of the *Mytilus* blue mussel complex (Skibinski & Beardmore 1979, Bierne et al. 2003). Divergence of *M. edulis* and *M. galloprovincialis* is suggested to have been promoted by the same history as intraspecific breaks in many European marine species, namely Pleistocene lowering of sea level

and isolation of the Mediterranean from the Atlantic (Barsotti & Meluzzi 1968). The secondary contacts were likely initiated by natural postglacial dispersal, but transplantsations by humans likely contribute to them too (e.g. Bierne et al. 2003). In regions colonized postglacially, from SW France to British Isles, interdigitated pure populations of *M. edulis* and *M. galloprovincialis* are found alongside sympatric populations and populations that consist mainly of hybrid genotypes (Skibinski et al. 1983, Bierne et al. 2003). Ecological specialization and habitat heterogeneity is suggested to underlie the mosaic-like occurrence of the pure and hybrid mussels along the NE Atlantic coast (e.g. Bierne et al. 2003, but see Coghlan & Gosling 2007).

1.2.2. Trans-Arctic and trans-Atlantic affinities of European marine biota

The northern connection between the Atlantic and Pacific basins via the Bering Strait opened for the first time perhaps as early as in the Late Miocene (Marincovich & Gladenkov 1999). However, movement of Pacific biota into the Atlantic through the Bering Strait started effectively only after formation of the Panama land bridge which converted Pacific water to flow across the Strait into the Arctic and the Atlantic about 3.5 million years ago (Marincovich 2000). Owing to that event, some of the North Atlantic marine taxa have their origin in the Pacific Ocean (Durham & McNeil 1967, Vermeij 1991). Lowering of the global sea level in the Pleistocene hindered trans-Arctic exchange of Pacific and Atlantic biotas until the end of the Pleistocene, when the Strait became again more permanently inundated about 12 000 years ago (Keigwin et al. 2006).

A realization that cryptic trans-Arctic gene flow may have fairly recently influenced the North Atlantic marine biota has arisen from molecular studies that show little or no trans-Arctic differentiation in such taxa that according to the fossil record were pre-Pleistocene invaders in the North Atlantic (McDonald & Koehn 1988, Palumbi & Kessing 1991, Orti et al. 1994, Väinölä 2003, Olsen et al. 2004, Addison & Hart 2005). They suggest that Pacific invasions to the Atlantic and Europe are more of a recurring (Pleistocene interglacial and Holocene) phenomenon than a one time event diagnosable from the first fossil appearance of a species. Patterns of genetic variation indicate that in some cases the original Pliocene – Early Pleistocene invaders have been totally replaced by much more recent invasion waves (Orti et al. 1994, Addison & Hart 2005).

Phylogeographic patterns of amphiatlantic marine species suggest that intraspecific genetic connections over the Atlantic after the latest glaciation have been asymmetrical: European ancestry of North American coastal populations is more common than the converse (Wares & Cunningham 2001). This is likely due to commonness of glacial extinctions of especially rocky shore species in the North-West Atlantic, where suitable refugia were not much available during the glacial periods, and persistence of populations in Europe, from where recolonization could take place (Wares & Cunningham 2001, cf. Addison & Hart 2005).

More generally, it has been proposed that successful establishment of any invasion wave is facilitated by emergence of new unoccupied habitat and/or extinctions of earlier dwellers of the habitat (Vermeij 1989, Cunningham & Collins 1998). From this point of view, the postglacially reformed North European marginal seas

appear as primary areas of investigation when assessing trans-Arctic or trans-Atlantic connectivity and genetic patterns. Still, trans-Atlantic phylogeographic studies often ignore the Baltic and White Sea populations (e.g. Wares 2001, Jørstad 2004, Hickerson & Cunningham 2006, Coulson et al. 2006, Ellingson & Krug 2006).

The Baltic Sea has been rather viewed as a natural laboratory for testing hypotheses of genetic adaptation to environmental gradients through natural selection (e.g. Gabrielsen et al. 2002, Bekkevold et al. 2005, Jørgensen et al. 2005, Riginos & Cunningham 2005). Genetic clines found in Baltic species are readily interpreted as resulting from clinal selection along the strong salinity or temperature gradient (e.g. Christiansen & Frydenberg 1974, Theisen 1978, Johannesson et al. 1990, Gabrielsen et al. 2002). The use of the Baltic Sea as a laboratory may however be challenging in species whose North Sea and Baltic Sea gene pools are distinctive due to their long separate evolutionary histories prior to their contact in Baltic waters, as the effects of selection versus mixing intensity of North Sea and Baltic waters for formation of clines may be hard to tease apart. Yet, new genetic combinations brought about by contact and recombination between distinct evolutionary lineages may indeed provide the variation that is prerequisite of natural selection and adaptation to novel environments to occur (Anderson & Stebbins 1954, Hilbish 1996). From this point of view, species with secondary contact of deep evolutionary lineages in the geologically young, physiologically demanding North European marginal seas could indeed prove fruitful for demonstrating natural selection and creative power of reticulate evolution (Arnold 1992, Rhymer & Simberloff 1996).

The Baltic *Mytilus* population was the first recognized case of contact of deep evolutionary lineages of a marine taxon in the North European marginal seas (Varvio et al. 1988, Väinölä & Hvilsum 1991). It has remained unclear, however, whether its history involves direct trans-Arctic or indirect trans-Atlantic -routed migration of the Pacific *M. trossulus* type mussels to meet the native *M. edulis* in the NE Atlantic. The trans-oceanic ancestry of Baltic *Mytilus* cannot be traced with help of mitochondrial phylogeography, since only *M. edulis* type mitochondria have been found to survive in the Baltic Sea (Rawson & Hilbish 1998). On the other hand, the Baltic *Macoma* population which represents a similar contact of deeply diverged stocks or subspecies (Väinölä & Varvio 1989, Väinölä 2003), has been shown to possess mtDNA distinct from the main European lineage (Luttikhuisen et al. 2003). *Mytilus* and *Macoma* have a largely common distribution and tolerance of the physical environment in the boreal-subarctic NW Atlantic and Pacific coasts, and a similar dispersal ability via planktonic larvae. Therefore, a phylogeographic study of *Macoma*, such as conducted in this thesis, has the potential to illuminate in general the origin of the remote genetic affinities of biota of the postglacially reformed North European marginal seas.

2. AIMS OF THE THESIS

In this thesis, I explore molecular zoogeography of two common brackish-water bivalve species that colonized the North European marginal seas after the latest glaciation. A primary objective of the work is to test the biogeographic hypothesis of cryptic trans-Arctic invasions into Northern

Europe, and to characterize impacts of these invasions through introgressive hybridization on the population genetic composition of *Macoma* across the marginal seas. A common goal in the studies of both species is to place the genealogy and genetic diversity of the evolutionarily young North European populations in the context of the phylogeographic and population genetic diversity of the species on a larger geographical scale. Paper I addresses the phylogeographic structure of the lagoon cockle, *Cerastoderma glaucum*, throughout its range across Europe using mitochondrial DNA and nuclear allozyme markers, and assesses hypotheses presented on its systematic subdivisions. In paper II, we study mitochondrial phylogeography of the Baltic clam, *Macoma balthica*, on a circum-polar scale to test and elaborate a hypothesis of repeated trans-Arctic invasions from the Pacific Ocean into the North East Atlantic. In papers III-IV, we study population- and genome level consequences of the secondary contacts between two *M. balthica* subspecies in three North European marginal sea areas.

3. MATERIAL AND METHODS

3.1. Samples

Variation of *Cerastoderma glaucum* was studied from 15 localities across the distributional range of the species, from the Baltic, North, Mediterranean, Black and Caspian seas (I). Clams of the *Macoma balthica* complex were studied from 114 European sampling sites in the Baltic, White, Barents, Pechora and North seas and the Bay of Biscay; from two localities in the North West Atlantic; and from two trans-Arctic localities, in the Chukchi Sea

and the North East Pacific (II, III, IV). For the mtDNA analysis (II), samples of *M. petalum* from three North American localities were also included. Samples were collected between 1985-2006, and mostly stored deep-frozen (-80° C) until analysis.

3.2. Molecular data

Two types of genetic polymorphisms were employed to study the structure and evolutionary relationships of bivalve populations: sequence variation in mitochondrial DNA (mtDNA) and nuclear enzyme polymorphisms (or allozymes). In most organisms mtDNA is inherited clonally from mother to offspring and does not undergo recombination, thus allowing reconstruction of maternal phylogenetic relationship based on mutational differences accumulated between matrilineages in the mtDNA sequence (Avise et al. 1987). Further advantages of mtDNA are its high (neutral) mutation accumulation rate and low effective population size due to haploidy and maternal transmission: in animal taxa mtDNA variation patterns typically may diagnose/reveal subdivision events of tens of thousands of years or older (Avise et al. 1987). Electrophoretically detectable allozyme variation takes a longer time to accumulate between isolated populations as rates of detectable mutations are slower and as genetic drift at diploid biparentally inherited nuclear loci is slower/weaker than in mtDNA. The multilocus nature of allozyme data however gives a more general view to the history of the population genome, and the segregation of diploid genotypes provides information of the breeding structure of populations. Due to the difference in inheritance modes, the mtDNA and allozyme markers are complementary

when both phylogenetic origins and degree of hybridization and genomic admixture in a population or individuals need to be resolved. MtDNA traces roots of maternal genealogy extending millions of years back owing to its clonal inheritance, whereas allozyme diversity and genotypic structure that are subject to recombination can reveal dynamic population level processes that have taken place within generations.

An exception to the common maternal transmission pathway of mtDNA is the mode of doubly uniparental inheritance (DUI) that has evolved polyphyletically in bivalves (see review by Breton et al. 2007). A presence of DUI would potentially confound straightforward phylogenetic inference, as in species with DUI the males carry different mtDNA types in their somatic and gonad tissues. The mitochondria present in somatic tissue are clonally inherited from the mother and those present in the gonad tissue from the father. However, no signs of DUI (frequent and consistent double peaks in sequencing chromatograms or heteroplasmic patterns in RFLP phenotypes) were found in *Macoma* or *Cerastoderma* mtDNA raw data in the present work.

MtDNA variation was studied by directly sequencing parts of the genes coding for cytochrome oxidase subunit I (COI, in *C. glaucum*) and subunit III (COIII, in *M. balthica*). The COIII haplogroup identities of *Macoma* individuals were also determined by a restriction enzyme assay of the PCR product. The assay was designed to separate the observed main lineages by their diagnostic restriction fragment profiles in agarose gel electrophoresis (II-IV). Allelic and genotypic variation at allozyme loci was studied in both taxa with starch gel electrophoresis (I, III and IV).

The nuclear genetic structure of *C. glaucum* was studied from 16 allozyme loci

from seven localities across the European range, and the mtDNA variation was studied by sequencing altogether 129 individuals from 15 localities for the COI gene. Allozyme variation of *Macoma* at up to 10 loci was studied from 78 localities in the North European marginal seas, and in eight sites from NE Atlantic and NE Pacific reference areas. MtDNA variation of *Macoma* was studied by sequencing from 180 individuals from 52 localities in the NEMS and reference areas for a part of the COIII gene. In *Macoma*, cytonuclear character combinations were recorded as the mtDNA and allozyme data were prepared from same individuals, but in *Cerastoderma*, different individuals were used for the allozyme and mtDNA analyses. The molecular assay conditions are explained or referred to in the original papers. The sequence data acquired for the thesis were deposited in the GenBank database with accession numbers AY226908-AY226940 and EF044063-EF044136.

3.3. Data analyses

3.3.1. Phylogeny reconstruction

Genealogical relationships of mtDNA haplotypes were reconstructed under the maximum parsimony (MP) criterion using the program PAUP* (Swofford 2002). Heuristic searches were employed to find optimal trees, as the number of different haplotypes in the data sets were too high for exhaustive searches. Hypotheses of genealogical relationships of the trans-Arctic mtDNA lineage of *Macoma* and the Ponto-Caspian phylogroup of *Cerastoderma* are also illustrated by statistical parsimony networks that allow presentation of alternative branching schemes and multifurca-

tions unlike conventional dichotomous MP trees (Clement et al. 2000). Main lineages of the *M. balthica* complex are summarized by a tree reconstructed with the neighbour-joining method in MEGA program (Kumar et al. 2001).

3.3.2. Estimation of lineage and population divergence times

The rate of accumulation of detectable nucleotide differences between mitochondrial lineages is not linear through extended periods of time, as mutations may repeatedly occur at the same nucleotide site. Therefore, nucleotide substitution models that correct for this saturation effect were used in estimating the actual amount of divergence between mtDNA lineages. Uneven substitution rates across nucleotide sites in the studied mtDNA segments (product of their triplet codon structure) were taken into account by using the synonymous Li-Wu-Luo distance (Li et al. 1985) for estimating age of *Cerastoderma* lineage splits, and the general time reversible mutation model with a 0.70 proportion of invariant sites for estimating age of lineage splits in *Macoma* (Posada & Crandall 1998).

Estimates of absolute substitution rates in mtDNA were obtained by linking certain lineage splits in the genealogies with major paleohydrographical events that plausibly induced them. For *Cerastoderma*, such an event is the Messinian stage of the Mediterranean Sea about 6 million years ago, to which the divergence of the *C. glaucum* and its closest, currently solely NE Atlantic relative *C. edule* has been attributed (e.g. Brock 1991). During the Messinian stage, non-marine (brackish) environments

existed in the Mediterranean basin which was isolated from the Atlantic for some hundreds of thousands of years (Krijgsman et al. 1999). The rate calibration for *Macoma* relied on the assumption that the divergence of the extant Pacific and NE Atlantic mtDNA lineages began somewhere between the start of the great trans-Arctic interchange (Vermeij 1991) and start of the Pleistocene, where also the earliest known *M. balthica* fossils in Europe date back to (Simonarson et al. 1998), i.e. 3.5-2 million years ago – an assumption also commonly used in other currently vicariant Pacific-Atlantic taxa inferred to have participated in the interchange (e.g. Wares & Cunningham 2001, Hickerson & Cunningham 2006, Govindarajan et al. 2005, Hyde & Vetter 2007).

A prerequisite for estimation of absolute time from the amount of nucleotide substitutions is an equal rate of their accumulation across diverged evolutionary lineages; this molecular clock hypothesis could not be rejected by a likelihood ratio test (Posada 2003) for the mitochondrial lineages of *Macoma* (II). To account for the over-estimation of population divergence times caused by the ancestral polymorphism in the splitting population, ancestral polymorphism was approximated by the extant within-lineage polymorphism (Nei & Li 1979), and subtracted from the between-lineage distances in *Macoma*. Additionally, a coalescence simulation method of population isolation time estimation which does not require reciprocal monophyly of mtDNAs between the assessed populations (Nielsen & Wakeley 2001, Hey 2005) was applied in II for *Macoma* to assess the age of the most recent trans-Arctic isolation (or invasion) event.

3.3.3. Geographical patterns of genetic variation and admixture

As the geographic variation in *Macoma* was judged to have arisen either through mechanical mixing of two taxa or introgressive hybridization between them (admixture of gene pools), the main tool used for investigating multilocus patterns of allele frequency (co)variation between samples was the principal component analysis (PCA). Ideally, the first PC directly reflects relative contributions of the gene pools to the mixed population, and the PC1 scores of samples can be interpreted as a mixing index. Furthermore, the remaining principal components summarize patterns of (co)variation that are independent of the main component, and thus reflect genetic diversity that has evolved by processes other than the principal admixture (cf. Kontula & Väinölä 2001, Väinölä & Väinölä 2003).

For this summary, a joint PCA of allele frequencies at eight allozyme loci from all *Macoma* samples across the North European marginal seas was conducted. In **III** and **IV**, admixture proportions of Atlantic and Pacific type genomes in *Macoma* samples were estimated also separately in each sample with the maximum likelihood method of Wang (2003). The first principal component of the joint PCA essentially represents the same mixing gradient as the ML-admixture estimates; the latter are however derived from somewhat broader data, as all scored loci per local sample were utilized. Importantly, a joint PCA enables demonstrating the degree of uniqueness of the Baltic, Barents and White Sea *Macoma* populations. Allozyme differentiation between the main evolutionary units of *Cerastoderma* was quantified with Nei's standard genetic distance measure (Nei 1972).

3.3.4. Assessment of genotypic structure at the contact of Pacific and Atlantic *Macoma* genomes

The degree of interbreeding between the Pacific and Atlantic genetic types of *Macoma* in the marginal seas was examined by looking at genotypic associations of alleles in the samples. To simplify this examination, data were made diallelic by classifying all alleles either as Atlantic or Pacific, according to their direction of correlation with the first principal component that, as expected, reflected a transition from pure Atlantic type genetic composition to a highly Pacific like (reference) composition. Allelic associations between nuclear loci were then estimated according to Weir's (1996) formula of composite genotypic disequilibrium and cytonuclear allelic associations according to Asmussen & Basten (1994) (see **IV**). The sample-wise estimates of disequilibria over all loci were proportioned to their maximum values under two population contact scenarios: mechanical mixing (no interbreeding) and one generation of random mating between the pure reference populations. The average mixing index values derived from PC1 scores of the samples were utilized as estimates of sample-wise mixing proportions when calculating the expected maximum values of disequilibria.

3.3.5. Geography of the genetic transitions in the Baltic *Macoma*

The geographical extent of the genetic transitions and location of the areas of steepest transitions in the Baltic *Macoma* were looked for by plotting the average mixing index (standardized PC1 score from allozyme data) or mtDNA haplotype

frequency of a sample against its geographical distance from the Baltic entrance (the Darss and Drogden sills), and by fitting a logistic function $X = 1/(1 + \exp(-4(d-m)/w))$ to the data. Here m is the cline midpoint and w “cline width”, or inverse of the curve slope at the cline midpoint (see Cahan et al. 1998). Fitting was made separately for subsets of data representing opposing Baltic shores, different depth ranges and different genomes (mitochondrial and nuclear).

4. RESULTS AND DISCUSSION

The two most important outcomes of this thesis are a confirmation of the presence of a recent Pacific genetic contribution in bivalve populations of the North European marginal seas, and a geographically comprehensive basic description of the integration and organization of the Pacific and Atlantic genomic components of *Macoma balthica* in the marginal seas. In addition, the thesis includes a description of the phylogeographic structure of another prominent marginal sea bivalve species, *C. glaucum*, a work that contributes more generally to conceptions about Pleistocene history of European marine biota. With reference to the Baltic Sea and the postglacial reassembly of its biota, the thesis offers a view into the genetic structure of two species with different zoogeographical origins and arrival history. A rare population genetic and biogeographic phenomenon in the marine environment – a hybrid swarm, or complete secondary amalgamation of diverged lineages – is highlighted by the descriptions of several hybrid swarms that have formed postglacially in parallel after secondary contacts of Pacific and Atlantic subspecies of the *M. balthica* complex.

4.1. Phylogeography of *Cerastoderma glaucum* and the NE Atlantic *Macoma balthica* (*M. b. rubra*) (I, II)

The working hypotheses in the phylogeographical study of *C. glaucum* were the conflicting views previously presented on its systematic divisions. It has been argued to be divided into an Atlantic and a Mediterranean subspecies that had separated several million years ago; these subspecies would have, respectively, invaded the Baltic Sea and the Ponto-Caspian basins in postglacial times (Brock 1987, 1991, Brock & Christiansen 1989). Alternatively, *C. glaucum* was perceived as a complex of four largely codistributed species occurring in different habitats (Scarlato and Starobogatov 1972). A recent study of allozyme variation of *C. glaucum* in the Mediterranean, Atlantic and Baltic seas however failed to reveal any deep genetic subdivisions that would deserve systematic consideration (Mariani et al. 2002).

Our survey of mtDNA and allozyme variation across the species range could not support any of these previous hypotheses on systematics and population structure of *C. glaucum*. Nevertheless, notable genetic breaks and phylogeographic structuring were disclosed. The deepest split separates the Ponto-Caspian and eastern Mediterranean populations from the western Mediterranean, Atlantic and Baltic populations (Fig. 2a). The locations and depths of the breaks are by and large concordant in the mitochondrial and nuclear markers. The 6.2 % observed mtDNA sequence difference between these two major phylogroups would correspond to roughly 1 million years of divergence time under our tentative substitution rate calibration, which is in line with the allozyme estimate of Nei's genetic distance $D = 0.15$ between the two

major clusters. In two Greek samples, however, the cytoplasmic and nuclear markers discord, suggesting secondary contact and subsequent differential introgression of the markers to have taken place.

Apart from the major break, considerable phylogeographic structuring exists within the Atlantic-Mediterranean phylogroup of *C. glaucum* also: six well differentiated haplotype groups with geographic affinities were revealed. In contrast, the Ponto-Caspian phylogroup exhibits a shallow pattern of variation that is indicative of a fairly recent population bottleneck/growth and recent genetic connections between the eastern Mediterranean, Black Sea and Caspian Sea (Fig. 2a). The existence of the two major phylogroups and geographically localized subgroups within one of them indicates that *C. glaucum* survived the Pleistocene climatic fluctuations in multiple refugial populations. The most important refugial area would seem to have been the eastern Mediterranean (Aegean and Ionian seas), which would have retained, through multiple glacial cycles, both the two main lineages (assuming that the Ponto-Caspian lineage could not have a refuge in the Black Sea), and in addition a great amount of intra-lineage diversity within the Atlantic-Mediterranean lineage – indeed a higher diversity of haplotypes than found in the remaining western Mediterranean, Atlantic and the Baltic samples in total. The hydrographical events that could have enabled the early post-glacial dispersion of *C. glaucum* from the Black Sea to the Caspian Sea (a lake with no natural outlet) – as documented in the subfossil record and conforming to our data – remain poorly understood. Transportation of spat by migrating waterfowl is considered an important means of dispersal for *C. glaucum* around British Isles by Boyden & Russell (1972), and it may account even for

the dispersal from Black Sea to the Caspian Sea, as major migration routes of waterfowl pass between the basins.

All sampled Baltic individuals carried the same haplotype, which was found also right outside the Baltic entrance where it was accompanied by other haplotypes of the same clade (Fig. 2a). Our sampling density is too low to judge if this haplotype group is unique to the Baltic and the adjacent Danish Straits. In any case, it is too divergent from the other Atlantic-restricted haplotype group to share a postglacial common ancestor with it, i.e. to have evolved within the Baltic basin. Rather, the divergence of the two Atlantic haplotype groups appears no younger than previous Eemian interglacial (I), and suggests multiple Atlantic Pleistocene refugial populations to have been maintained for *C. glaucum*.

Similarly, the phylogeographic structure of indigenous Atlantic populations of *Macoma balthica* (i.e. subspecies *M. b. rubra*) was found to comprise three relatively deep mtDNA lineages that have geographically clearly distinct core areas while their distributions still largely overlap (Fig. 2b). Their estimated divergence times also far exceed the postglacial period (II). Notably, the geographical distributions of these old lineages are presently segregated in such marine areas that were colonized only post-glacially. In all, it seems that three marine Pleistocene refugial areas in the Atlantic should be postulated to account for the lineage diversity. The most simple explanation of the current differential post-glacial distributions is that these areas should have been located in a way that facilitated parallel dispersal into the areas emerging from the ice. Therefore, it is not likely that all the refugia were located in areas south of the glaciers, as has previously been assumed (Fig. 1). Instead, parallel colonizations

would have been more likely achieved by longitudinal dispersal, from putative marine refugia west of the Scandinavian glaciers. Such longitudinal range shifts during cool climatic phases would require that the

glacial environmental conditions were mild enough in the areas presently offshore. Unfortunately, little is known of Pleistocene marine habitats and their benthic biota west of the Scandinavian ice sheet. Reconstructions of the ice sheet however suggest that the sheet did not quite reach the edge of the continental shelf in all areas north of the British Isles (Svendsen et al. 2004), leaving some room for speculations concerning refugia of coastal marine benthic taxa there. Radiocarbon datings of thermophilous terrestrial species along the western margin of the Scandinavian ice sheet have been interpreted to indicate glacial persistence of populations close to the ice sheet (Stewart & Lister 2001), and similar suggestions are emerging from marine phylogeographic studies in areas adjacent to the British Isles (see Introduction). In the NE Pacific, *M. balthica* has been considered a species that has a high likelihood of surviving in northern refugia near glacial margins (Hetherington & Reid 2003). A major difficulty in searching phylogeographic evidence for offshore refugial areas is that the species hypothetically inhabiting them during the periods of low sea levels do not occur there any more.

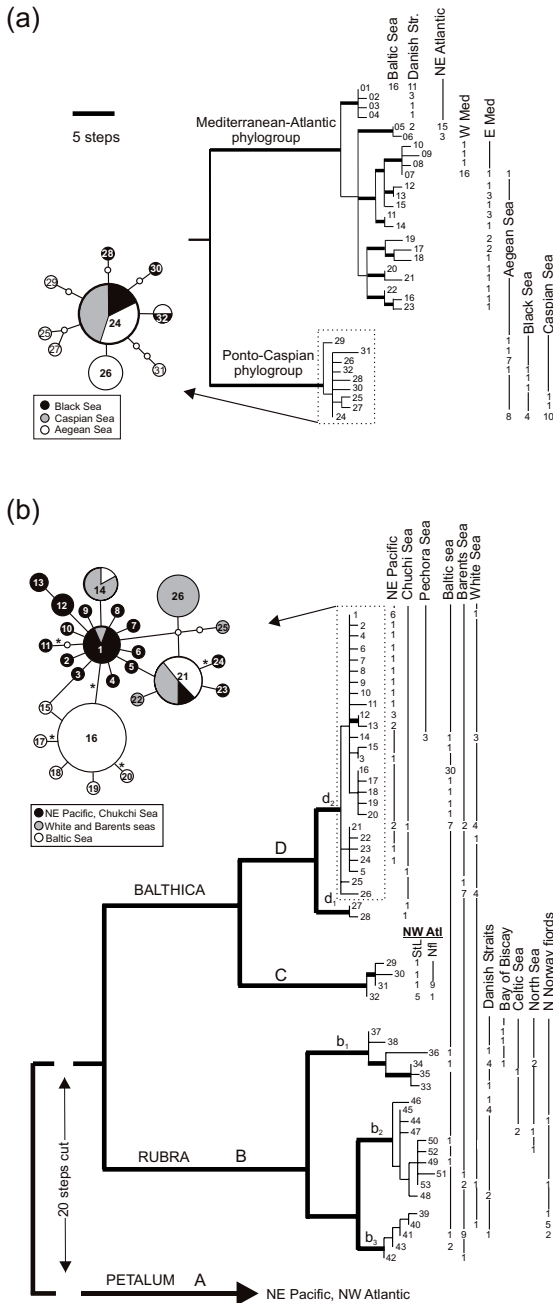


Figure 2 a-b. Reconstructions of genealogical relationships of mitochondrial haplotypes found in (a) *Cerastoderma glaucum* and (b) *Macoma balthica*. Robust clades that are present in all equally parsimonious topologies found are drawn with thick lines. The geographic distributions and frequencies of the haplotypes are indicated to the right. Unrooted MP networks of subsets of the haplotype trees which better depict recent derivation of many rare haplotypes from the common and widespread ones are presented to the left.

4.2. Trans-Arctic invasion history of the *Macoma balthica* complex (II)

The circumpolar study of mtDNA diversity of *Macoma balthica* disclosed four deep genealogical lineages (Figs. 2a and 3), whose geographical distribution suggests

(i) several independent trans-Arctic passages to have taken place in *Macoma* since the Miocene, (ii) an absence of Late Quaternary trans-Atlantic exchange between NE Atlantic (European) and NW Atlantic populations and (iii) a mixed NE Pacific and NE Atlantic ancestry of clams

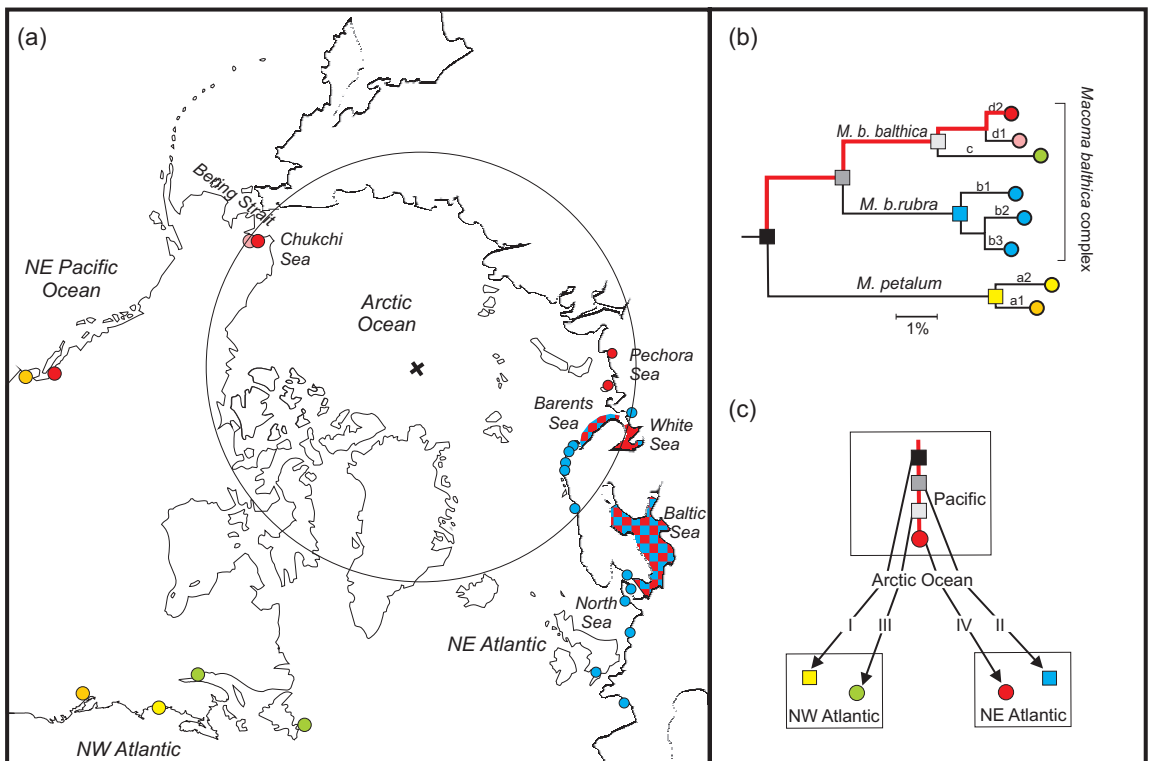


Figure 3 a-c. Phylogeography of *Macoma* supports a scenario of repeated trans-Arctic invasions from the Pacific into the Atlantic (II). – (a) Circum-polar distribution of the main haplotype lineages found in the *Macoma balthica* complex and *M. petalum*. – (b) The main mtDNA lineages in the *M. balthica* complex and *M. petalum*, depicted by a NJ tree based on (uncorrected) p -distances. Circles encompass groups of individual haplotypes that were truncated for simplicity (cf. Fig. 2b). The series of branches drawn with red represents the assumed Pacific stem lineage of *Macoma*. Squares denote common ancestors of branching lineages. – (c) A scenario of four unidirectional trans-Arctic invasions (I-IV) of the *M. balthica* complex and its common ancestor with *M. petalum* from the Pacific to the Atlantic to explain the phylogeographic structure. Assuming that invasion II took place 2-3.5 million years ago (Ma) during the major trans-Arctic exchange, the other invasions were tentatively dated to have occurred 5.2-9.3 Ma (I), 0.8-1.4 Ma (III) and the Early Holocene (IV). Coalescence analysis of the haplotype diversity and distribution in the trans-Arctic lineage d2 (red; see MP network in Fig. 2b for its phylogeography) was utilized in dating the latest invasion IV, which refutes anthropogenic introduction of the *M. b. balthica* of NE Pacific origin in the North European marginal seas.

in the North European marginal seas (Fig. 3). Also, the mtDNA genealogy clarified the taxon limits of the *Macoma balthica* complex by showing the old separation of North American boreal-temperate zone *M. petalum* – still often treated as *M. balthica* – from the more northern *M. balthica* complex (Fig. 3 legend).

The mixed evolutionary ancestry of NEMS *Macoma* is verified by the co-occurrence of two deep mtDNA lineages there, one of which has a trans-Arctic, Pacific-Atlantic distribution (branch d2); the other lineage (branch B) is confined to the NE Atlantic (Fig. 3). The mtDNA data thus confirms the hypothesis of a secondary trans-Arctic invasion as an explanation of the intermediate genetic composition of NEMS populations between the North American and NE Atlantic populations, initially put forward on the basis of allozyme data (Väinölä 2003). The mtDNA data also corroborates the trans-Arctic reinvasion hypothesis over the alternative trans-Atlantic one. The amount of differentiation detected in the trans-Arctic mitochondrial lineage between the Pacific and NEMS samples and the endemic (post-invasion) Baltic diversity are high enough to refute a hypothesis of very recent anthropogenic introduction, but too low to be attributed to divergence since the Eemian interglacial (II). Considering the amount of endemic mutations in the Baltic Sea and paleoclimatological limitations, an invasion during the Early Holocene may be regarded as the most plausible historical scenario for arrival of the Pacific type *M. balthica* in the NE Atlantic (II).

Application of long-term substitution rates (e.g. obtained with a calibration point older than 2 million years) to short-term events – like the suggested latest trans-Arctic invasion of *Macoma* – has been criticized

by Ho et al. (2005) based on the conclusion that mutation rates are time-dependent due to purifying selection that acts only on longer evolutionary time scales than age of intraspecific populations. Similar views have arisen from some population data of invertebrate mtDNA (Audzijonytė & Väinölä 2006). Therefore, an application of a long-term clock to a recent divergence event would lead to overestimation of the age of the isolation. However, even if we assumed that our maximum likelihood estimates of trans-Arctic isolation time in lineage d₂ (II) were ten-fold overestimates, the hypothesis of recent anthropogenic introduction would still not get support. Should the level of rate overestimation be similar in *Macoma* as in humans according to Ho et al. (2005), the youngest ML-estimate would fall to the warm Allerød interstadial about 14 000 years ago.

Conceptually, the definitions of Baltic “arctic marine relict species” by Ekman (1953) and the White Sea “forms common to the Far Eastern seas” by Zenkevitch (1963) fit with the scenario that we suggest to explain the presence of Pacific *Macoma* lineage in the North European marginal seas (II). According to Ekman (1953), a relict status of a species in a given area requires that the species occurs there in isolation from its main present area of distribution, and that the gap in the distribution was caused by regional extinction of the species following an environmental change. However, calling *M. balthica* an arctic marine relict in the Baltic Sea would be misleading, as its distribution extends only to the sub-arctic climatic zone (III), and the arrival of the Pacific type in NEMS via the Arctic was likely facilitated by regional warming of the arctic zone (II), not by its extension southward as in case of the species traditionally called arctic marine relicts. A com-

mon term applicable to the Pacific genomic component of *Macoma* in all NEMS areas could be “trans-Arctic relict”; it could be applied also to other species with a similar biogeographical history. So far, candidates for the group are the Baltic *Mytilus*, the Barents and White Sea *Clupea* (Berger et al. 2001, Jørstad 2004) and the Barents Sea *Theragra finmarchicus (chalcogramma)* (Ursvik et al. 2007).

An explanation for the geographical and temporal association of the Pacific type *Macoma* in the Baltic Sea with conventional arctic marine relicts, such as *Halicryptus spinulosus*, *Mysis mixta* and *Pontoporeia femorata* (Väinölä 2003), could be found in the spatial heterogeneity of the circumpolar Holocene warming: the North Sea was still experiencing a cold climate when the thermal maximum occurred in Northern America and Siberia (Kaufman et al. 2004; Caseldine et al. 2006). Earliest fossils of *M. balthica* in the Skagerrak region are indeed of similar age with the latest occurrences of purely arctic species there, like *Portlandia (= Yoldia) arctica* (Petersen 2004). The way to the cool deep brackish waters of the Baltic Sea opened perhaps at the last minute for the arctic marine species that were about to be completely replaced by Boreo-Lusitanian biota in the North Sea and for *M. b. balthica* that was approached by *M. b. rubra* from the south.

4.3. Hybridization and introgression of *Macoma* subspecies in the North European marginal seas: hybrid swarms and a transition zone (III, IV)

The genetic composition of the North European marginal sea *Macoma* populations is shown to be intermediate between samples from the Pacific and Atlantic

reference populations (or pure subspecies *M. b. balthica* and *M. b. rubra*) by principal component analyses of multilocus allozyme variation (III, IV, Fig. 3). The ordination of samples based on the first and second PC from the joint analysis of data from III and IV also illustrates a clear distinction between the genetic identities (allele frequencies) of the Baltic, Barents and White Sea populations.

The genetic composition of marginal sea populations resembles the pure Pacific subspecies *M. b. balthica* most closely in the central White Sea: ML admixture analysis estimates show 66-90 % Pacific contribution there, while the average estimates for Barents Sea samples are 32-50 %. The White and Barents Sea clusters are each fairly homogeneous regarding the contributions of the Pacific and Atlantic genomes, and a similarly homogeneous cluster is found in the Northern Baltic Sea. The ML admixture analyses suggest that the nuclear gene pool of the Northern Baltic *Macoma* is about 60 % of the Pacific type. In these three groups of populations, no significant deviations from interlocus equilibria between the Pacific and Atlantic type allozyme alleles are found (Fig. 4a-b). Thus, the nuclear *Macoma* genomes of Pacific and Atlantic origin are thoroughly amalgamated there at level of individuals and the admixed genetic composition is self-supported, i.e. a considerable current recruitment from pure parental populations is not taking place. Apart from the absence of genetic disequilibria, the independence of the White and Barents Sea swarms from pure Atlantic type *Macoma* is supported by the observation that the contribution of the *M. b. rubra* genome in these areas seems not correlated with the proximity of pure *M. b. rubra* populations (III, Fig. 5). The kind of outcomes of secondary contact and

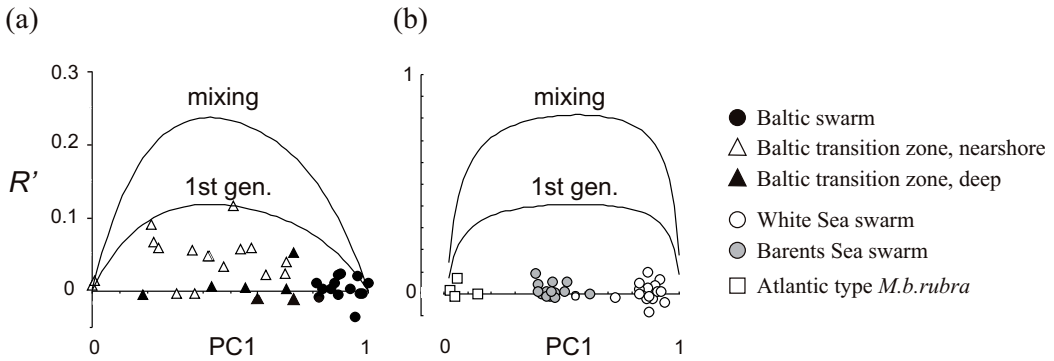


Figure 4 a-b. Estimates of nuclear inter-locus disequilibria between Pacific alleles in North European marginal sea populations of *Macoma balthica*, plotted against scaled PC1 scores of the samples from PCAs presented in **III** and **IV**. – (a) The Baltic Sea estimates are from 8 loci. – (b) The Barents and White Sea estimates are from those 5 loci only that are most diagnostic between the pure Pacific and Atlantic *Macoma* types. The curves show the expected values under scenarios of mechanical mixing of pure parental subspecies and after one generation of random mating of such a mixture. In (a), the PC value of 1 corresponds to the most Pacific-like Baltic sample, in (b) to the pure Pacific sample. Grouping of populations corresponds to Fig. 3. The nearshore and deep bottom samples from the Baltic transition zone are marked with different symbols.

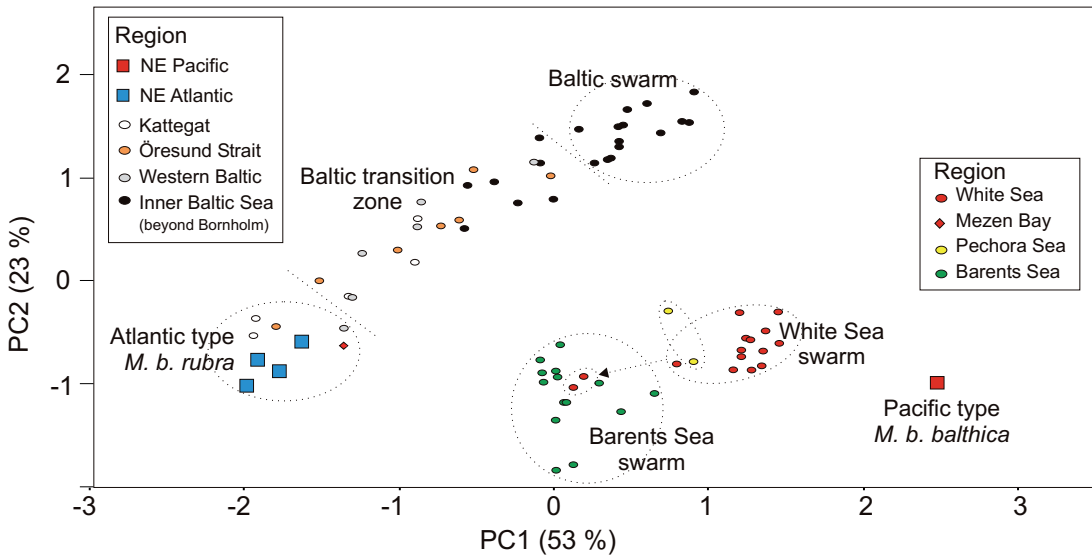


Figure 5. Ordination of *Macoma balthica* samples from the North European marginal seas by principal component analysis of allele frequencies at 8 allozyme loci. “Swarm” refers to a geographical group of samples where Pacific and Atlantic type alleles are randomly associated at the level of individuals, i.e. the average values of pairwise disequilibrium between nuclear loci are close to zero (see Fig. 4a-b). To make the data presented in **III** and **IV** suited for a simultaneous analysis, the allele pooling used in **IV** for the *Gpi* alleles was applied to the data of **III**, and the pooling used in **III** for *Ap* alleles was applied to the data of **IV**.

introgressive hybridization that we here describe fit the concept of hybrid swarm.

The Pacific and Atlantic mitochondrial genomes (that do not recombine in sexual reproduction) are both found in all marginal sea swarms, yet in differing proportions: The White Sea and the Northern Baltic swarms are almost fixed for the Pacific mtDNA yet for different haplotypes of the lineage, while both the Pacific and Atlantic lineages are strongly present in the Barents Sea swarm. In conclusion, three *Macoma* hybrid swarms, each with a unique nuclear and mitochondrial genomic composition, are here described from the North European marginal seas. Hybrid swarms are a rare – or at least a rarely documented – phenomenon in marine taxa (III, Roques et al. 2001). The *Macoma* hybrid swarms of the North European marginal seas seem to represent the best examples so far, in terms of geographical extent, thoroughness of the mixing of genomes of different origin (III) and perhaps also regarding the importance of the hybrid swarm populations for the ecosystem they are part of.

In contrast to the compact clusters formed by the northern Baltic, White and Barents Sea hybrid swarm populations, the wide dispersion of the more southern Baltic samples along the first axis in the PCA ordination (Fig. 5) indicates a close and active contact of the southerly Baltic *Macoma* with the Atlantic subspecies *M. b. rubra* in the North Sea. The dynamic contact is also evident from the strong inter-locus associations of the Atlantic (or of the Baltic = Pacific, c.f. III) alleles within the samples from the Baltic entrance (Öresund Strait) and in the Western Baltic (IV). In the Öresund, allelic associations between allozyme loci are on average 28 % of the maxima expected in the case of mere mechanical admixture, and cytonuclear associations are

of the same magnitude, on average 21 % of the respective maxima (IV). The *Baltic transition zone* between the fairly homogeneous northern Baltic hybrid swarm and the pure Atlantic *M. b. rubra* is here defined as the geographical area (i) which encompasses the majority of allele frequency change, and corresponds to the cline width as estimated by fitting a smooth geographical cline model to the average mixing index, and (ii) where notable inter-locus allele associations (disequilibria) are recorded within samples, disproving an equilibrium swarm type genomic population structure and indicating a degree of mechanical admixture of different genetic origins.

Plottings of the mixing index and fitting of the geographical cline model indicate that the transition zone extends far beyond the steepest ecotone of the Öresund Strait: about 300 km into the Baltic from the Darss and Drogden sills (defined as the Baltic entrance) along the northern Baltic coast and as far as 600 km along the S/E Baltic shore (Fig. 6a, IV). The midpoint of the nuclear transition (steepest point of the fitted cline) along the northern Baltic coast is 23-92 km from the entrance, within the Baltic. The similarly fitted transition in mtDNA is as wide as the nuclear one, but approx. 100 km further into the Baltic; this is mainly due to the high share of Atlantic mtDNA around the Baltic entrance (Fig. 6a). Yet, the most distinctive features of the Baltic transition zone are its geographical irregularity regarding the local contribution of the Atlantic genome, i.e. poor fit to the regressions that are used to estimate the cline shape and location (Figs. 5 and 6a-c), and the variability in the degree of local genotypic associations even between geographically close samples (see Table 3 in IV).

Despite the general irregularity, three clear geographical trends are recognized

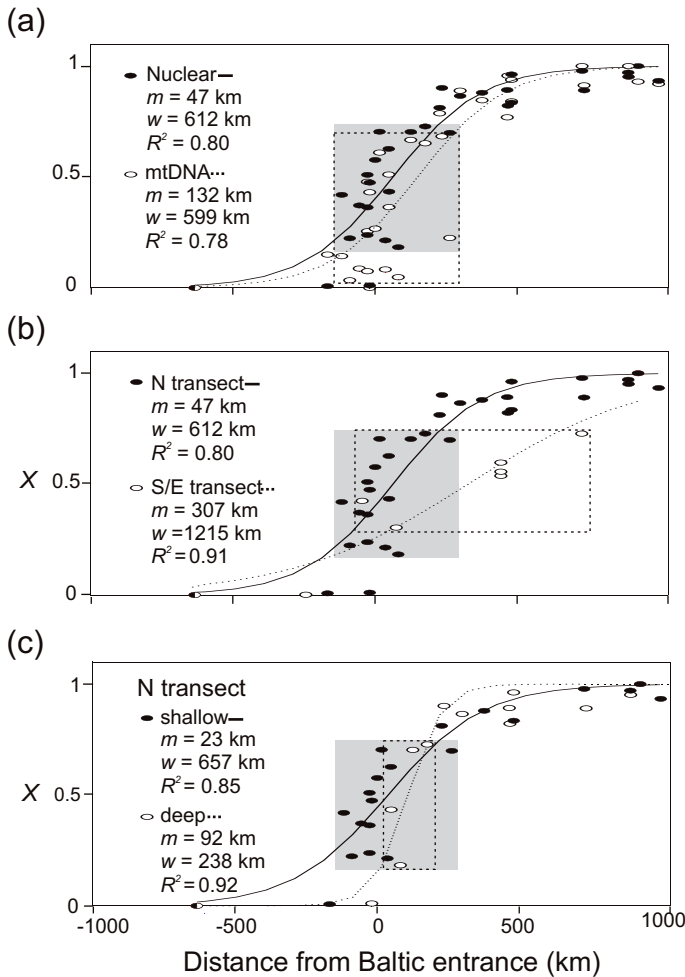


Figure 6 a-c. Comparison of genetic transitions along transects from the North Sea to the Northern Baltic in different subsets of the *Macoma* data –along different coasts, at different depths, and in different genomes (IV). – (a) Nuclear allozyme loci vs. mtDNA along the N transect only (both shallow and deep samples). – (b) N transect (Öresund-Swedish coast) vs. the S/E transect (both shallow and deep samples). – (c) Shallow vs. deep sample sites along the N transect only. Dispersion of the transition zone samples in the compared transitions of each panel is illustrated by the boxes drawn around them. Goodness-of-fit to the fitted logistic curve are given (R^2).

from the Baltic transition zone. Firstly, the influence of the Atlantic type genome is stronger and reaches further into the Baltic along the southern-eastern Baltic coast than along the northern coast (Fig. 6b). Secondly, the Atlantic influence is stronger in deep bottom samples than in adjacent nearshore sites (Fig. 6c). Thirdly, the genotypic equilibria are stronger in nearshore than in deeper-water samples, indicating more influence of larval mixing at these sites. (Fig. 4a). Actually, the disequilibria in the deeper samples of the Southern Bal-

tic basin are mostly as low as in the Northern Baltic hybrid swarm. At the same time, these samples exhibit the most regular geographical cline in allele frequencies among the studied transects (Fig. 6a).

The geographical patterns of Atlantic genomic contribution within the Baltic Sea and (in) the degree of local interbreeding in the Baltic entrance are evidently connected to the large-scale Coriolis-driven circulation pattern of the basin, and to the fate of the saline North Sea water entering the turbulent Öresund Strait (IV). Howev-

er, assuming that the secondary contact of Pacific and Atlantic *Macoma* types in the Baltic Sea started several millennia ago, and no further input of pure Pacific subspecies has occurred since the suggested Early Holocene trans-Arctic invasion, natural selection favouring the Pacific type genome (or amalgamation of Pacific and Atlantic type genomes) in environments like the marginal seas must be invoked to explain the persistence of the Pacific genomic components in the Baltic Sea. Interestingly, Jahn & Theede (1997) found that individuals from such Baltic *Macoma* populations that (based on data of the present work) likely had a large share of Pacific genome were better able to tolerate hydrogen sulphide than the Baltic and North Sea populations with a low Pacific contribution. Hydrogen sulphide frequently occurs in deep areas of the Northern Baltic Sea, and is also increasingly found in shallow habitats under thick mats of drifting filamentous algae that eutrophication brings about. It inhibits cell respiration by binding to cytochrome c oxidase in mitochondria (Nicholls & Kim 1982). Hypothetically, better performance of Pacific mtDNA in Baltic physical conditions, coupled with cytonuclear coadaptation, could even account for the maintenance of Pacific type alleles in face of the continuous gene flow from the adjacent Atlantic subspecies in the North Sea – Kattegat area.

5. CONCLUSIONS

In this thesis I have explored the phylogeography and patterns of genetic structure arisen from secondary contacts and hybridization in two bivalve species that inhabit the North European marginal seas. The main implication of the work for marine biogeography is confirmation of the hypothesis that trans-Arctic paleoinvasions from the Pacific Ocean have pervasively influenced the identities of bivalve populations in the Baltic, White and Barents seas. Hybridization and introgression between the resident Atlantic and invading Pacific lineages of *Macoma* clams have created three genetically distinct hybrid swarms in those NE Atlantic marine regions that were cleared of life by the last glaciation. From a global perspective, these populations represent the most wide-spread instance of hybrid swarms so far documented in marine animals. Generally, these hybrid swarms manifest the potential of introgressive hybridization to give rise to new evolutionarily and ecologically significant units in marine animals within a geologically short time frame.

The high genetic diversity within local geographical populations of *Macoma* and between localities in the Baltic Sea stands in contrast to the generally low genetic diversity of Baltic organisms (Johannesson & André 2006). Introgressive hybridization can be viewed as a shortcut to increased regional and local genetic diversity. As genetic diversity is regarded as a prerequisite for adaptation to a changing environment, the question arises whether the multitude of genotypic combinations brought about by introgressive hybridization of *Macoma* in the marginal seas has born any significance for their past adaptation to these extreme and novel environments (Lewontin & Birch 1966). An example of hybrid ad-

vantage in colonization of novel habitats documented in real time is provided by freshwater sculpins in the river Rhein: after an anthropogenic secondary contact of deep phylogeographic lineages in the 1990's, hybrid sculpins have invaded habitats that were left uncolonized by pure lineages (Nolte et al. 2005). In case of a more ancient secondary contact (like that of *Macoma* lineages), hybrid advantage is impossible to demonstrate based on distributional evidence only. However, it is notable that a postglacial (natural) secondary contact of deep phylogeographic lineages of sculpins – in depth comparable to the meeting *Macoma* lineages – has taken place in the Baltic Sea (Kontula & Väinölä 2001), and that sculpin populations with a mixed ancestry inhabit exactly the brackish-water Baltic coastal habitats that certainly are extreme for a fish of freshwater origin (Kontula & Väinölä 2001).

In the framework of this thesis, the hypothesis of hybrid advantage in marginal *Macoma* populations was thus supported only by the circumstantial evidence provided by the apparent long term persistence of the hybrid swarms in face of potential contact with one of the parental forms. An alternative explanation for persistence of the hybrid status of the marginal sea populations would be superior fitness of some individual Pacific alleles and subsequent hitchhiking of large portions of the Pacific genome with them. Testing the hybrid advantage hypothesis experimentally could involve exposing pure subspecies and their hybrid offspring to physical settings typical of the marginal seas, i.e. low salinity and temperature and presence of hydrogen sulphide, and measuring their performance. Potentially, the genetic diversity of the hybrid swarms could have a role in resilience of the marginal bivalve populations in face of the global climate change. Hopefully, the

roles of natural selection and local adaptation vs hydrography in maintenance of the hybrid swarms and genetic clines can some day be assessed by comparing the allozyme data presented in this thesis with data from most plausibly neutral nuclear markers like microsatellites or subspecies-specific AFLP loci.

The findings of this thesis emphasize the status of the Baltic Sea as a biogeographically complex ecosystem. Previously, the view was based on the coexistence of *species* originating from different biogeographical provinces and from marine and fresh-water environments. The observations on *Macoma* and on the largely analogous *Mytilus* extend the phenomenon of biogeographical mixing into the intraspecific and genomic levels by the amalgamation of the genetically highly distinct Pacific and Atlantic subspecies. Another indication of a mixed evolutionary ancestry has been found in the Baltic population of the extinct Atlantic sturgeon (*Acipenser*) (Tiedemann et al. 2007). With the genetic affinities of the North European marginal sea populations of many trans-Atlantic and trans-Arctic species still unexplored, it remains unclear just how common the pattern of mixed ancestry actually is.

Furthermore, our characterization of the marginal *Macoma* populations as globally unique evolutionary units that have formed after a trans-Arctic connection independent of human activity stresses the fact that irreplaceable genetic diversity exists in these sea areas, and that they should be managed accordingly. Under the recently promoted view of species as genotypic clusters that remain distinct in the face of hybridization and gene flow from other genotypic clusters (Mallet 2007), the *Macoma* hybrid swarms in the Baltic, Barents and White seas could each be regarded as an endemic (evolved in situ) hy-

brid species, arisen through synthesis of old diversity. Lacking knowledge on how strongly (if at all) selection favours the Pacific genome in the marginal sea environments, we are not able to reconstruct the course of this synthesis or the colonization sequence of the Pacific and Atlantic *M. balthica* subspecies in the North European marginal seas. Anyway, I envision that a replacement of a pure resident *M. b. rubra* by its amalgamation into later invading *M. b. balthica* would only be plausible if selection strongly favoured the Pacific type genome; otherwise, a primary postglacial colonization by a relatively pure *M. b. balthica* and subsequent introgression of the *M. b. rubra* -genome would be the simplest adequate scenario.

More generally, the scenarios of historical geographic isolation and subsequent secondary contact that we propose as explanations of the phylogeographic patterns of both *Macoma* and *Cerastoderma* are

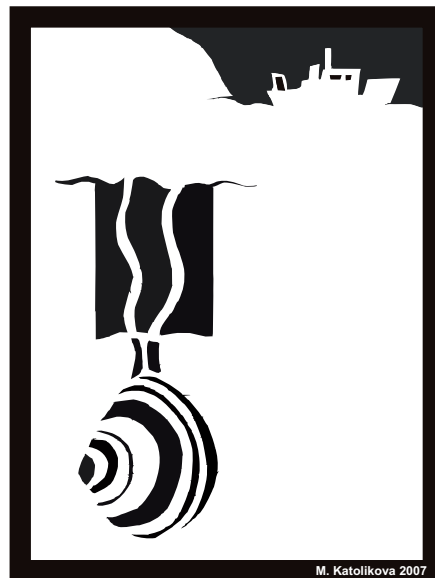
in concordance with the emerging insight from marine phylogeography that deep genetic divergences or breaks often occur in marine species with high dispersal potential (see *Introduction*). Like in many other species inhabiting areas affected by Pleistocene glaciations, the lineage splits in *Cerastoderma* and *Macoma* were inferred to predate the last glacial maximum, implicating the limited power of even repeated natural habitat disturbance to drive to extinction or shuffle much of the intraspecific genetic diversity. This inferred persistence of distinct evolutionary lineages through multiple glacial episodes offers a sharp contrast to the rapid extinctions of local lineages that have taken place in many species because of over-exploitation, anthropogenic habitat destruction (Sala & Knowlton 2006) or genetic swamping by cryptic anthropogenic invasions (Rhymer & Simberloff 1996, Mooney & Cleland 2001, Grosholz 2002).

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