JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE
243

Jenni Kesäniemi

Variation in Developmental Mode and Its Effects on Divergence and Maintenance of Populations

JYVÄSKYLÄN YLIOPISTO

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 243

Jenni Kesäniemi

Variation in Developmental Mode and Its Effects on Divergence and Maintenance of Populations

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston vanhassa juhlasalissa S212
elokuun 10. päivänä 2012 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
in auditorium S212, on August 10, 2012 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2012

Variation in Developmental Mode and Its Effects on Divergence and Maintenance of Populations

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE 243

Jenni Kesäniemi

Variation in Developmental Mode and Its Effects on Divergence and Maintenance of Populations

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2012

Editors
Jari Haimi
Department of Biological and Environmental Science, University of Jyväskylä
Pekka Olsbo, Ville Korkiakangas
Publishing Unit, University Library of Jyväskylä

Jyväskylä Studies in Biological and Environmental Science
Editorial Board

Jari Haimi, Anssi Lensu, Timo Marjomäki, Varpu Marjomäki
Department of Biological and Environmental Science, University of Jyväskylä

URN:ISBN:978-951-39-4819-1
ISBN 978-951-39-4819-1 (PDF)

ISBN 978-951-39-4818-4 (nid.)
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä 2012

ABSTRACT

Kesäniemi, Jenni
Variation in developmental mode and its effects on divergence and maintenance of populations
Jyväskylä: University of Jyväskylä, 2012, 47 p.
(Jyväskylä Studies in Biological and Environmental Science
ISSN 1456-9701; 243)
ISBN 978-951-39-4818-4 (nid.)
ISBN 978-951-39-4819-1 (PDF)
Yhteenveto: Kehitysmuotojen variaatio ja sen populaatiogeneettiset seuraukset
Diss.

For invertebrates with complex life cycles in which one or more larval stages precede the adult stage, developmental mode is a key life-history characteristic. A variety of different larval developmental modes are seen among marine invertebrate species and the different modes typically have differing dispersal potential, and this consequently affects population-level gene flow and genetic differentiation, especially in species were adults are sessile. The spionid polychaete *Pygosio elegans* can produce small free swimming larvae feeding in the plankton, or benthic larvae feeding on nurse eggs while brooded in egg capsules in the maternal tube. Support for poecilogony in *P. elegans* was found using DNA sequence data and phylogenetic analyses: divergence in the COI gene was low and haplotypes were shared among populations with different larval modes. In population genetic analyses with newly developed genetic markers, genetic variation and effective population size were found to be higher in populations were the planktonic larval mode predominates, compared to populations with benthic larvae. Populations with benthic larvae were also more prone to the effects of genetic drift and were temporally unstable. Isolation by distance pattern and significant genetic structure was seen among most population pairs in Europe, despite larval mode. These results and the high estimated local recruitment rates suggest that the larval dispersal may not be tightly correlated with the developmental mode in this species and that factors other than dispersal may be affecting the genetic structure seen. On a small geographical scale, no habitat characteristics were found to significantly affect the observed genetic structure, but on a larger scale, the developmental mode may be associated with geography or environmental factors. Because of the observed polymorphism in developmental mode seen in *P. elegans*, it is a good model species for research on the mechanisms and consequences of life history variation.

Keywords: Developmental mode; dispersal; microsatellite; poecilogony; population genetics; *Pygospio elegans*.

Jenni Kesäniemi, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland

Author's address Jenni Kesäniemi
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland
jenni.kesaniemi@jyu.fi

Supervisors PhD K. Emily Knott
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland

Professor Damhnait McHugh
Department of Biology
Colgate University
Hamilton, NY 13346, USA

Reviewers Professor Michael Hart
Department of Biological Sciences
Simon Fraser University
8888 University Drive
Burnaby, British Columbia
Canada

PhD Raisa Nikula
University of Otago
Department of Zoology
340 Great King Street
Dunebin, New Zealand

Opponent Professor Kerstin Johannesson
University of Gothenburg
Biological and Environmental Sciences
Tjärnö Marine Biological Laboratory
SE 452 96 Strömstad Sweden

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-V. I have been involved in the planning and execution of all studies. I have collected the genetic data in all of the studies, except in IV, when this work was shared with M. Mustonen. I have been responsible for all data analyses. I am the primary author of all papers, which were written in collaboration with all authors.

I Kesäniemi, J.E., Rawson, P.D., Lindsay, S.M. & Knott, K.E. 2012. Phylogenetic analysis of cryptic speciation in the polychaete *Pygospio elegans*. *Ecology and Evolution* 2: 994–1007.

II Kesäniemi, J.E., Boström, C. & Knott, K.E. 2012. New genetic markers reveal population genetic structure at different spatial scales in the opportunistic polychaete *Pygospio elegans*. *Hydrobiologia* 691: 213–223.

III Kesäniemi, J.E., Geuverink, E. & Knott, K.E. 2012. Polymorphism in developmental mode and its effects on population genetic structure of a spionid polychaete *Pygospio elegans*. *Integrative and Comparative Biology* 52(1): 181–196.

IV Kesäniemi, J.E., Mustonen, M., Boström, C., Hansen, B.W. & Knott, K.E. 2012. Temporal dynamics and effective population size in a polychaete with different larval modes. Manuscript.

V Kesäniemi, J.E., Hansen, B.W., Banta, G.T. & Knott, K.E. 2012. Genetic patchiness of a polychaete in a heterogeneous estuarine landscape. Manuscript.

1 INTRODUCTION

1.1 Diversity of marine life history strategies

The study of life history evolution aims at understanding the diversity of adaptive strategies that optimize the survival and reproduction of organisms in different environments (Stearns 1992). A wide diversity of developmental strategies have evolved in marine species, and in many marine phyla, there can be multiple larval stages between the embryo and adult. For invertebrates with complex life cycles, larval type, or developmental mode of the larvae, is a key life-history characteristic. In the literature, developmental modes of larvae have been categorized based on different criteria, e.g. embryology, nutritional mode or site of development (Thorson 1950, Mileikovsky 1971, Jablonski & Lutz 1983, Wilson 1991, McEdward & Janies 1993, Levin & Bridges 1995, Raff & Byrne 2006). In general, if larval development occurs in the water column it is called planktonic development, whereas benthic development takes place on the seafloor or within the sediment. If the development is benthic, the larvae can be free crawling or more commonly, they can be encapsulated in gelatinous masses, capsules or cocoons. These structures are usually attached to the sea floor, plants, on the body of the adult (e.g. some sea stars) or, in tube building species (e.g. some polychaetes), capsules can be laid within the tube. If developmental mode is categorized based on nutritional needs, larvae that feed in the plankton are called planktotrophic. This kind of development is common and widespread in marine invertebrate phyla, as is spawning of gametes into the water column. Facultative planktotrophic larvae do not need to feed (but are able to) in order to reach metamorphosis (Levin & Bridges 1995). If the parent provides nutrition for the developing larvae it can occur via large yolky eggs (lecithotrophy) or the developing larvae can feed on extra embryonic nutritional eggs or their siblings (adelphophagy). Adelphophagy, seen in some nemerteans, polychaetes and molluscs, is considered to be rarer than lecithotrophy (see Levin & Bridges 1995). Maternal food provisioning (especially adelphophagy) is often connected to the protection of larvae during

development in egg masses or capsules (for example, in spionid polychaetes, see Blake & Arnofsky 1999; and in gastropods, see Collin 2004, Collin et al. 2007), but not all species with egg capsules have nurse eggs (Thorson 1946, 1950). Indeed, multiple developmental strategies are often mixed within taxa. For example, in some polychaetes, nemerteans and gastropods, a mixed larval strategy with an initial benthic or brooded lecithotrophic phase followed by a planktotrophic free living phase is common (Pechenik 1979). Also, direct development (no larval stage) is known to occur in most marine invertebrate phyla (Levin & Bridges 1995).

1.2 Evolution of different larval developmental modes

Understanding the variation and complexity seen in the developmental modes of marine invertebrates is critical for understanding their evolution. It has been suggested that external fertilization and feeding planktonic (planktotrophic) mode is the ancestral developmental mode of most taxa (Thorson 1950, Strathmann 1978, Levitan 1996, but see McHugh & Rouse 1998), but in polychaetes, internal fertilization and lecithotrophy may be the ancestral mode (Rouse 2000). The potential ancestral developmental mode is important for inferring transitions in developmental modes and the evolutionary pressures that may lead to such transitions. For example, an evolutionary transition from a feeding larva to a non-feeding larva may be more common than one in the opposite direction, since the evolution of morphological structures required for feeding is expected to be complex (but see Rouse 2000). However, phylogenetic inferences and multiple observations of sister species with different developmental modes (Christiansen & Fenchel 1979, Hart et al. 1997, Blake & Arnofsky 1999, Jeffery et al. 2003, Collin 2004, Ellingson & Krug 2006, Raff & Byrne 2006), suggest that transitions in developmental mode happen often and rapidly on an evolutionary time scale. In some groups, a wide range of different developmental modes can be seen even within a taxonomic family: for example in spionid polychaete annelids, the range is from broadcast spawners followed by planktotrophic or lechithotrophic development in the plankton, to brooding on the female or in capsules (with or without a planktonic phase), to viviparity (Hannerz 1956).

The existence of different developmental modes has been connected to the proposed energetic tradeoffs between egg size, developmental time and number of offspring and the risks to the survival of the offspring (Vance 1973, Strathmann 1985, Havenhand 1995, but see Levitan 2000). The prediction is that planktonic feeding larvae develop from small eggs that are nutritionally poor in yolk and therefore less costly to the mother to produce (Thorson 1950), whereas lecithotrophic, non-feeding larvae develop from larger, nutrient rich eggs which require a significant input of maternal resources. The proposed advantages of planktotrophy are high fecundity and low parental protection, but due to high mortality rates in the plankton (Morgan 1995), a transition towards copulation,

internal fertilization and parental protection may increase the survival of the offspring (Strathmann 1993).

The evolution of lecithotrophy is therefore coupled with increased maternal investment leading to selection for larger egg size and shorter developmental time (Strathmann 1985, Havenhand 1993), or alternatively, the brooding of embryos and providing them with extra embryonic nutrition reduces the need for planktotrophy and high fecundity (see Wray 1995). Evolution of different larval modes can be influenced by ecological factors, such as predation, food availability and competition (Strathmann 1993, Krug & Zimmer 2000, Marshall & Keough 2009). Also, factors affecting settlement and juvenile survival are important. For example, selection for shorter developmental time (egg to juvenile) can affect post-settlement demographics (Havenhand 1993) and larval nutrition can affect the success of the juvenile stage (Pechenik 2006, Emlet & Sadro 2006).

1.3 Consequences of developmental mode

1.3.1 Developmental mode, dispersal and genetic variation

The developmental mode of larvae can affect a species at many levels, for example their fecundity, developmental time and dispersal potential. As mentioned, planktonic life stages have high mortality rates because of the numerous risks the larvae face in the plankton, for example predation, poor food availability or changes in abiotic factors such as salinity and temperature (Thorson 1950, Morgan 1995, Pechenik et al. 2004) and the risks are thought to increase with a longer planktonic larval period (Thorson 1950, Rumrill 1990, Pechenik & Levine 2007). In addition to allowing independence from variable external food sources, the shortened developmental time of larvae with maternal food provision (lecithotrophy or adelphophagy) allows for higher survivorship (Thorson 1950, Wray & Raff 1991, Morgan 1995). Encapsulation of larvae could further increase larval survival, since the time spent in the plankton is shortened or eliminated and the larvae are more protected from predation (Pechenik 1979, Strathmann 1985).

For benthic marine invertebrates, the larval developmental mode is connected to the dispersal ability of a species, which in turn has important evolutionary consequences. In these species, the adults are typically sessile or sedentary, and dispersal is expected to occur mainly during the larval stage, but the active or passive movement of adults and larvae through rafting, crawling, or for example with ballast water in ships should not be ignored (Martel & Chia 1991, Watts et al. 1998, Anil et al. 2002, Fraser et al. 2011). Species with planktonic larvae are generally thought to have high dispersal potential, which is expected to lead to high effective gene flow and low genetic structure among populations. In species with benthic or direct developing larvae, the opposite pattern is expected since the larvae are more likely to settle in their natal

population than disperse (Thorson 1950, Palumbi 1994, Bohonak 1999). Because tracking the movements of larvae in the water column is very challenging (Thorrold et al. 2002, Levin 2006), larval dispersal patterns have been commonly studied with indirect methods such as genetic markers. The pattern of higher connectivity in species with planktonic larvae compared to non-dispersive larvae has been found in many population genetic studies of different marine species (Hellberg 1996, Arndt & Smith 1998, Kyle & Boulding 2000, Collin 2001, Dawson et al. 2002, Elligson & Krug 2006), but studies with different patterns are not uncommon (Bowen et al. 2006, Miller & Ayre 2008, Kelly & Palumbi 2010). Also, higher genetic variation is seen in species with planktonic larvae (Foltz et al. 2004, Ellingson & Krug 2006, Binks et al. 2011) and also higher effective population size is often seen, likely due to the higher fecundity and dispersal ability allowing considerable migration into populations.

Understanding the effects of the different dispersal potential of larval developmental modes is an important evolutionary question, for example in terms of speciation. In theory, species with planktonic larvae should have wider geographic ranges, low extinction rates and low speciation rates because of high effective gene flow among populations. In species with non-dispersive larvae, populations are expected to become isolated faster (Palmer & Strathmann 1981, reviewed in Jablonski & Lutz 1983 but see Hart & Marko 2010). When thinking about contemporary populations, the balance between dispersal and local population maintenance is important. Non-dispersive larvae may be selected for in optimal habitats because these larvae settle into the natal population with high probability, but in an unstable or fragmented habitat, dispersal capacity could be beneficial, since offspring would then be able to move to different habitats that might increase the chances of survival and reproduction (Palmer & Strathmann 1981, Havenhand 1995, but see Strathmann et al. 2002).

1.3.2 Developmental mode and population temporal dynamics

Opportunistic life history characteristics, for example small adult size, high fecundity (with planktonic larvae), short life span and an ability to disperse (Grassle & Grassle 1974, Pearson & Rosenberg 1978) can allow individuals of a species to take advantage of habitats with variable conditions. Opportunistic species can quickly colonize new or re-colonize disturbed habitats, leading to fluctuations in population sizes and instability in the genetic structure of marine populations (Whitlack & Zajac 1985, Warwick 1986, Bolam & Fernandes 2002). In addition, high fecundity coupled with high larval mortality may lead to high variation in reproductive success among individuals. If only a small proportion of the population produces the majority of the individuals of the next generation (sweepstakes reproductive success), the genetic structure of the population may vary temporally (Hedgecock 1994, Hedgecock & Pudovkin 2011). Brooding species with benthic developmental modes are expected to have less flexibility in terms of their potential response to a changing environment and be relatively protected from stochastic variation in reproductive success. As a result, species with larval brooding may have increased temporal genetic stability (Lee &

Boulding 2009). However, in a stochastic and often unpredictable marine environment, other factors affect the temporal stability of populations also, for example in small populations, genetic drift may be the main force shaping temporal and spatial genetic patterns on a short time scale (Tessier & Bernatchez 1999, Virgilio & Abbitatti 2006, Lee & Boulding 2009).

1.3.3 Other factors affecting population genetic structure

Recent trends in population genetic studies have included incorporating the effects of historical and environmental factors on population genetic structure to aid the identification of dispersal barriers. Unexpected genetic structure can be caused by patterns of oceanic currents (Sotka et al. 2004, Pineda et al. 2007), and on different scales, different current patterns may either transport larval life stages long distances or promote local larval retention (Knutsen et al. 2004, Fievet et al. 2006, Kenchington et al. 2006, White et al. 2010). Local retention of planktonic larvae can be influenced by habitat characteristics and larval behaviour (Levin 1986, Palumbi 1994, Metaxas 2001, Sponaugle et al. 2002, Swearer et al. 2002). However, population genetic patterns are often created by a complex interplay of developmental mode, population size, connectivity, adaptation and environmental factors and it may be very difficult to separate the effects of individual factors.

1.4 Poecilogony

While closely related species with different developmental modes are common among marine invertebrates (e.g. Blake & Arnofsky 1999, Jeffery et al. 2003, Collin 2004, Ellingson & Krug 2006), it is rare to observe different larval developmental modes within a single species. This within species polymorphism in developmental mode is termed poecilogony (Giard 1905), and it is mainly known in spionid polychaetes and sacoglossan sea slugs (Chia et al. 1996, Blake & Arnofsky 1999, Gibson & Gibson 2004, Ellingson & Krug 2006). It is unclear what causes developmental mode polymorphism, but several different mechanisms could maintain poecilogony. For example, poecilogony could be determined by genetic polymorphism or be an adaptive response to maintaining populations in variable environmental conditions, i.e. as environmentally induced plasticity (see Knott & McHugh 2012).

One well-studied example of a poecilogonous species is the spionid polychaete *Streblospio benedicti*, which lays its embryos in maternal brood sacs attached to the female. In this species, the different larval phenotypes are visible already as differences in egg size among females (Levin 1984) and egg size, as well as other larval traits have been found to be heritable (Levin et al. 1991). Planktotrophic larvae develop from small eggs (60-90μm in diameter) and they are released from the maternal brood sacs at an early stage to feed in the plankton. If the eggs are large (100-200 μm) and yolky, larvae are brooded for a

longer time and then are released as large lecithotrophic larvae (Levin 1984, Blake & Arnofsky 1999, Pernet & McArthur 2006). The two different larval types have different morphology: some larval traits are missing (e.g., the larval bristles, Levin 1984), and some traits have different structure or develop at a later stage of the developmental period in lecithotrophic larvae in comparison to planktotrophic larvae (Gibson et al. 2010, Pernet & McHugh 2010). Despite the morphological and heterochronic differences during the larval period, adults which have developed through the different pathways are morphologically indistinguishable and interfertile (Levin et al. 1991).

In *S. benedicti*, there is evidence for genetic control over the larval phenotype, but in another poecilogonous polychaete, the different larval developmental modes are dependent on the variability of maternal food provisioning to the embryos. The tube building spionid *Boccardia proboscidea* lays its embryos and nurse eggs (non-developing eggs that serve as food for the larvae) into egg capsules inside the maternal tube (Gibson 1997). Three different reproductive strategies are seen: in the absence of nurse eggs, only planktotrophic larvae are produced; if a small amount of nurse eggs are deposited in the capsules, larvae hatch as slightly larger planktotrophic type larvae (rare), but when nurse eggs are plentiful, the capsules can contain both small planktotrophic larvae and a few adelphophagic larvae that feed on nurse eggs and the smaller larvae. The adelphophagic larvae do not disperse after their release from the egg capsules (Gibson 1997, Gibson et al. 1999). Different larval types are seen within a population (Gibson 1997, Oyarzun et al. 2011), and adults that developed from different larval types are interfertile. When such adults interbreed, their offspring develop via the same developmental mode as did the maternal worms (Gibson 1997).

In the sacoglossan sea slug *Alderia willowi*, the eggs are laid in benthic egg masses and there are seasonal changes in the larval developmental mode. Larger eggs and lecithotrophic larvae are produced in the summer, and a switch to smaller egg size and planktotrophic larvae occurs with a drop in temperature and salinity when winter is approaching (Krug 1998, Krug 2007). Interestingly, these changes happen within individuals, whereas seasonal shifts within individuals are rare in other poecilogonous species (Krug 1998).

In the literature, there have been different definitions for poecilogony. In the original description by Giard (1905), poecilogony encompasses variation seen within individuals and among individuals within or between populations. In some cases, the term has been used when describing plasticity in the timing of hatching but without phenotypic differences in the larvae (see Knott & McHugh 2012), where it may not be the appropriate term.

1.5 Aims of this thesis

I study *Pygospio elegans* (Fig. 1), a spionid polychaete annelid with larval polymorphism. First, since poecilogony is rare and since many presumed

poecilogonous species have been proven to be cryptic species with different developmental modes, it is important to test the hypothesis of poecilogony. Using molecular tools, including DNA barcoding and phylogenetic approaches, the poecilogony hypothesis in *P. elegans* was examined (I). Species with within species polymorphism can be used as a model system in studies aiming to understand the evolutionary transitions in developmental mode. However, the first step is to understand how poecilogony affects population maintenance and how populations are connected via gene flow. To study the consequences of the dispersal potential of different larval types and population connectivity in *P. elegans*, new species-specific molecular markers were developed for use in population genetic studies (II). These microsatellite markers were used in population genetic analyses of *P. elegans* at different spatial scales. On a large scale, genetic diversity and genetic structure in and among European *P. elegans* populations with different developmental modes was examined (III). The main questions were to examine if there is a correlation between larval dispersal ability and developmental mode within a poecilogonous species, more specifically, whether the populations with planktonic larvae are more connected and have higher migration rates than the populations that also have benthic larvae (III). The broad sampling scale also allowed us to investigate if the commonly seen pattern of low diversity and genetic isolation in marine populations of the Baltic Sea is observed in *P. elegans* as well (III). In benthic marine invertebrates, larval developmental mode affects not only gene flow, but also the temporal stability of a population. Therefore, temporal genetic stability of *P. elegans* populations differing in developmental mode was studied to clarify the relationship between larval developmental mode and genetic population structure (IV). In the marine environment, the barriers to larval dispersal may not be obvious. Combining data from habitat characteristics and population genetic patterns may reveal more about factors other than developmental mode that affect larval dispersal and population connectivity (V). A poecilogonous species provides an opportunity to investigate the relationships between larval developmental mode, larval dispersal and population genetic patterns, without the influence of possible species-specific behaviours or adaptive differences which accumulate during or after speciation.

FIGURE 1 An adult *Pygospio elegans* worm (female, approximately 10 mm long).

2 MATERIAL AND METHODS

2.1 Study species

Pygospio elegans (Claparède), is a small (max. 15 mm) tube-dwelling spionid polychaete worm. It has a broad geographic distribution, in the N. Atlantic and Pacific Oceans and in the Baltic Sea. *P. elegans* can be found from variety of habitats, ranging from exposed, intertidal sand or mud flats (Morgan et al. 1999, Bolam 2004), seagrass beds (e.g. Boström & Bonsdorff 1997), deeper subtidal areas (Kube & Powilleit 1997), to polluted areas (Anger 1984), but sandy sediments in shallow waters are preferred (Muus 1967, Rasmussen 1973). In the Baltic, *P. elegans* can be the dominant benthic species (Rasmussen 1973) and it is an important prey item for epibenthic predators, e.g. fish (e.g. Mattila 1997). Additionally, if the worm densities are high, *P. elegans* tube beds can have a significant impact on community and physical structure in benthic habitats (e.g. Bolam & Fernandes 2003).

P. elegans can produce different types of larvae following sexual reproduction. After spermatophore transfer and an internal fertilization, the female lays embryos and nutritional nurse eggs into egg capsules within the maternal tube (Söderström 1920, Hannerz 1956, Rasmussen 1973). The genuine eggs (fertilized embryos) are approximately 100µm in diameter with a distinct nucleus, whereas the nurse eggs are visibly smaller (approx. 70µm), orange-coloured yolky eggs that lack a nucleus (Rasmussen 1973). The difference in the egg types is already visible inside the female coelom before egg laying (Rasmussen 1973, pers.obs. Fig. 2A). If the amount of nurse eggs is small relative to the amount of embryos, planktotrophic larvae develop. These are released from the egg capsules at an early stage (3-setigers) to feed in the plankton (Fig. 2B). In the laboratory, the planktonic period can last up to 5 weeks (Anger et al. 1986). If only a few embryos are laid per egg capsule, they are brooded throughout their development and the developing larvae feed on nurse eggs (adelphophagy). These benthic larvae (Fig. 2C) lack a planktonic stage and swimming setae, and are ready to settle soon after their release (at 14-20-setiger stage). In between these extreme types, intermediate forms are

commonly seen (Gudmudsson 1985, pers.obs). *P. elegans* can also reproduce asexually by fragmenting its body (Rasmussen 1953).

Variation in developmental mode in *P. elegans* has been reported both among and within populations (e.g. Hannerz 1956, Rasmussen 1973, Anger 1984, Gudmundsson 1985, Morgan et al. 1999) and the differences among populations can be seasonal or geographical (Muus 1967, Rasmussen 1973, Gudmundsson 1985, Morgan et al. 1999). Anger (1984) and Morgan (1997) have studied if the variation in larval phenotype is affected by environmental variation, but in Anger's (1984) experiments with planktonic populations, changes in rearing temperature or salinity did not affect the larval development mode. Developmental mode was also conserved in experiments done by Morgan (1997) in which nutrient level and worm densities were varied in two populations with different larval developmental mode. The results from these experiments and observations of populations with apparently fixed development (just one larval type) have raised the question of possible cryptic species with different developmental modes within this species. However, within population larval polymorphism and some genetic and morphological evidence (Morgan et al. 1999) suggest poecilogony.

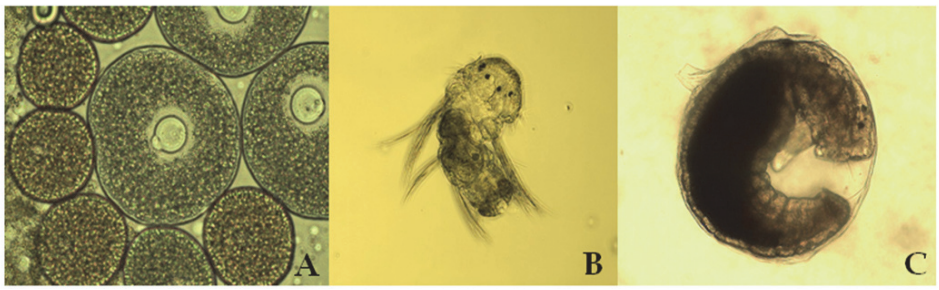


FIGURE 2 *Pygospio elegans* egg types and examples of two different larval modes. A. True eggs with nuclei (100 µm in diameter) and smaller nurse eggs removed from the coelom of a female. B. A planktonic larva after release from the egg capsule (approx. 0.2 mm in length). C. A benthic larva in an egg capsule (width of the egg capsule is approx. 1 mm).

2.2 Sample collecting

P. elegans worms were collected from several locations during years 2008 to 2011. From Europe, sample collecting was done from the Baltic Sea (Finland, Sweden, Denmark, Germany), the Wadden Sea (Netherlands), North Sea (UK), the English Channel (UK, France), the North Atlantic Ocean (Iceland) and the White Sea (Russia). Three locations from the US were also sampled for the phylogenetic study (I) (east coast: two locations in Maine and west coast: Washington). For the temporal sampling, 7 European populations were sampled from the same location 2–3 times during years 2008–2011 (IV).

In the non-tidal Baltic Sea, sediment sampling was done either by scuba diving at 3-5 m depth (Finland), or by wading and shoveling the sediment in less than 1.5 m deep water (Denmark, Sweden). The German sample was collected from a depth of 18 m using a sediment grab operated from a boat. In the mud and sand flats in the Netherlands, France, and UK, partially exposed sediment was sampled in the intertidal zone during low tides. The sediment was immediately sieved (with 0.5 or 1.0 mm sieve) and the *P. elegans* sand tubes were placed on trays with seawater without any sediment. The worms will emerge from their tubes after this disturbance (in most cases, worms were left on trays for up to 24h). Then, the worms were sexed and examined for the presence of gametes or nurse eggs, which can be seen in the coelom through the body wall using a microscope. Sand tubes were examined for egg capsules and if capsules were found, the larvae were examined to determine their developmental mode (embryos can be distinguished from the nurse eggs even during the early stages of their development). Water samples from all the sampling locations were also examined for the presence of planktonic larvae. Worms were preserved in ethanol (70-90 %) until DNA analyses. Also, some live worms were transported to the University of Jyväskylä and were maintained in the laboratory in simple aquaria setup (Anger et al. 1986) where their reproduction could be monitored.

2.3 Molecular methods

2.3.1 Microsatellite loci isolation

For the microsatellite library (I), genomic DNA was extracted from whole individuals using PUREGENE® DNA Purification Kit (Gentra systems). Microsatellite loci were isolated following a modified (Grapputo 2006) FIASCO technique (Zane et al. 2002). Four enrichment libraries were prepared with the following probes: $(CA)_{22}$, $(TA)_{12}$, $(CAG)_{11,}$ $(CATA)_{8}$. PCR amplicons were cloned using the TOPO-TA cloning kit (Invitrogen) and One Shot® TOP10 competent *Escherichia coli* cells. Positive clones were amplified with vector specific primers, sequenced using BigDye Terminator 3.1 reagents and visualized with the ABI PRISM 3130xl (Applied Biosystems). When repeat regions were found in the sequences, primers were then designed to the flanking regions. In total, primers were designed for 17 microsatellite repeat loci using PRIMER3 (Rozen & Skaletsky 2000) and PCR reactions were optimized for the markers.

2.3.2 DNA extractions

DNA was extracted from whole individuals using Qiagen chemicals and protocols from Qiagen's DNeasy Blood and Tissue extraction kit either using the spin columns provided (for small individuals less than 1 cm in length) or

with modifications for use with a KingFisher magnetic processor (Thermo Fisher Scientific) (I, II, III, IV, V).

2.3.3 Sequencing

A 567 or 600 bp fragment of the cytochrome c oxidase subunit I (COI) gene was amplified using species-specific primers (developed by Paul Rawson) in 20 µl reactions containing 1 µl of DNA, 3 mM $MgCl_2$ (Biotools), 200 µM of each dNTP (Fermentas), 0.5 µM of each primer (TAG Copenhagen), 0.1 U of *Taq* polymerase and 1 X PCR Buffer (Biotools). Thermocycling conditions were an initial denaturation step at 94 °C for 2 min, then 35 cycles of 94 °C for 15 s denaturation, annealing at 55 °C for 15 s and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 2 min. For sequencing, the PCR products were treated with Exonuclease I and Shrimp alkaline phosphatase (Fermentas), cycle sequenced in both directions using the BigDye v.3.1 kit, and visualized with an ABI 3130xl Genetic Analyzer and Sequencing Analysis v.5.2 software (all Applied Biosystems). Sequences were corrected by eye and aligned using the ClustalW option of MEGA4 (Tamura et al. 2007) (I).

2.3.4 Microsatellite-PCR and genotyping

5 of the microsatellite loci (Pe6, Pe7, Pe12, Pe13, Pe19) were amplified in 10 µl PCR reactions as follows: 1 µl of DNA, 1X PCR buffer (Biotools), 200 µM of each dNTP (Fermentas), 0.5 µM of the sequence specific primers (1/8 of the forward primer was fluorescently labelled with either 6FAM, NED, VIC or PET, Applied Biosystems), 1.5-3 mM $MgCl_2$ and 0.5 U of *Taq* DNA polymerase (Biotools). Thermocycling conditions were: initial denaturation at 94 °C for 5 min, then 35 cycles of at 94 °C for 30 s (denaturation), primer specific T_a for 30 s (annealing), 72 °C for 30 s (extension), followed by a final extension of 10 min at 72 °C. The 3 other loci (Pe15, Pe17 & Pe18) were amplified using a method described in Schuelke (2000). This method uses three primers, a sequence specific forward primer with M13(-21) tail, a sequence specific reverse primer and universal fluorescently labelled M13(-21) primer (labels 6FAM, NED, VIC or PET, Applied Biosystems). PCR was performed in 10µl reactions with 1µl of DNA, 1X PCR buffer (Biotools), 200 µM of each dNTP (Fermentas), 8 pmol of reverse primer and labeled M13(-21) primer, 2 pmol of the M13(-21) tailed forward primer, 1.5 - 2 mM $MgCl_2$ (Biotools) and 0.5 U of *Taq* DNA polymerase (Biotools). Thermocycling conditions were 94 °C for 5 min, then 30 cycles of 94 °C for 30 s, T_a for 30 s, 72 °C for 30 s, followed by 8 cycles of 94 °C for 30 s, 53 °C for 45 s, 72 °C for 45 s, ending with a final extension of 72 °C for 10 min. All PCR reactions were performed in BioRad C1000 or S1000 thermocycling machines. The PCR products were separated using an ABI PRISM 3130xl and genotyped using GeneMapper v.3.7 software (Applied Biosystems) (II, III, IV, V).

2.4 Statistical analyses

2.4.1 Analyses with the COI sequences (I)

Haplotype and nucleotide diversity were examined using DnaSP 4.0 (Rozas et al. 2003) and the sequence divergence was calculated using MEGA 4 (Tamura et al. 2007). To visualize the relationships among the COI haplotypes, a minimum spanning network was constructed using Arlequin v.3.5.1.2 (Excoffier & Lischer 2010). For the phylogenetic tree reconstruction, maximum likelihood (PhyML 3.0, Guindon & Gascuel 2003) and Bayesian (MrBayes 3.1.2, Ronquist & Huelsenbeck 2003) methods were used. Phylogenetic trees were visualized with FigTree v1.2.2 (http://tree.bio.ed.ac.uk/software/figtree/). Population genetic structure was investigated using AMOVA in Arlequin in which the sequences were grouped according to their sample location (Northern Baltic Sea, Southern Baltic Sea, North Sea + Wadden Sea + English Channel and North Atlantic Ocean). Also, a Bayesian model-based method implemented in BAPS 5.3 (Corander & Tang 2007) was used to examine if the haplotypes grouped together based on their sampling location or larval developmental mode.

Haplotype and nucleotide diversity between populations with different developmental modes were compared using a Mann-Whitney U Test in PASW Statistics 18 (SPSS, Inc., 2009, Chicago, IL, www.spss.com). Neutrality of the sequences were tested with DnaSP 4.0 (Rozas et al. 2003), and population demographic history (past population expansion) was studied by calculating mismatch distributions (the frequencies of observed pairwise differences between haplotypes within a population) and R_2 (Ramos-Onsins & Rozas 2002) and raggedness statistics (Harpending 1994) for each population using DnaSP.

2.4.2 Population genetic analyses with the microsatellite genotype data (II, III, IV, V)

Descriptive population genetic analyses, such as allele frequencies, and observed and expected heterozygosities were calculated with Arlequin v.3.5.1.2 (Excoffier & Lischer 2010). Allelic richness and private alleles were examined with HP-RARE (Kalinowski 2005). FSTAT v.2.9.3.2 (Goudet 2001) was used for calculating inbreeding coefficients and linkage disequilibrium among the loci. Hardy-Weinberg equilibrium was calculated in Arlequin. The presence and frequency of null alleles in the loci were studied using Micro-Checker (Van Oosterhout et al. 2004) and FreeNA (Chapuis & Estoup 2007). The differences in the level of genetic diversity between populations with different developmental modes were compared with the Mann-Whitney U test (III).

Population genetic structure was examined with pairwise comparisons of genetic differentiation. Pairwise F_{ST} was calculated with Arlequin and Jost's D_{est} with DEMEtics (Gerlach et al. 2010) or SMOGD (Crawford 2010). The number of genetic clusters in Europe was estimated with a Bayesian method (Structure v2.3, Pritchard et al. 2000) and with Geneland (Guillot et al. 2005) which

incorporates spatial information of the locations in the analysis (III, V). Isolation by distance was measured by Mantel test and spatial autocorrelation test (III, V) using GenAlEx v.6.4 (Peakall & Smouse 2006). A hierarchical analysis of molecular variance (AMOVA) was used to examine the partitions of genetic variation on different spatial scales, for example among sea areas (III), temporal samples (IV) or on a smaller scale between estuaries (V). The possible genetic isolation of the Baltic Sea populations was estimated with a F_{ST}-based method (III) described in Johannesson & André 2006.

To examine if migration was more effective among the populations with planktonic larvae (III), recent migration rates among our study populations were studied with two Bayesian methods, BayessAss 3 (Wilson & Rannala 2003) and BIMr v.1.0 (Faubet & Gaggiotti 2008).

Temporal genetic stability and the possible relationship with developmental mode and sweepstakes reproductive success in *P. elegans* (IV) was studied by examining the patterns of population genetic structure in time (F_{ST}, AMOVA), and with an individual assignment test (GeneClass 2, Piry et al. 2004). Structure (Pritchard et al. 2000) was used to see if the temporal samples from a population clustered together. The temporal sampling scheme (IV) also allowed the calculation of short term effective population sizes using different temporal methods. Three methods assuming closed populations were used: Moment Based Temporal method (MBT, Waples 1989), a likelihood based method (MLNE, Wang & Whitlock 2003) and TempoFs (Jorde & Ryman 2007). MLNE was also used to calculate N_e and migration rates jointly. A historical effective population size (represented by theta, $\Theta = 4N_e\mu$, where μ is the microsatellite mutation rate), was estimated with Migrate 3.2.6 (Beerli & Felsenstein 2001).

2.4.3 Combined analysis of genetic and environmental data (V)

To investigate population genetic structure and the possible effect of different environmental variables on it, multiple *P. elegans* populations were sampled from a Danish estuary complex with heterogeneous habitat (Isefjord and Roskilde fjord). The effect of the estuary, distance from the mouth of the estuaries, salinity, larval developmental mode, sediment type, the presence of vegetation, worm density and the presence of juveniles (representing a difference in timing of reproduction) were included as variables in the analysis conducted with a Bayesian method in GESTE (Foll & Gaggiotti 2006). This program uses a general linear model to correlate local population F_{ST} values with environmental variables to find the best model explaining the observed genetic structure.

3 RESULTS AND DISCUSSION

3.1 Poecilogony and cryptic speciation

When multiple developmental modes are observed within a species, a question of the presence of cryptic species is raised. This question is relevant, since in some cases, species suggested to be poecilogonous have turned out to be cryptic species with different developmental modes (see Hoagland & Robertson 1988) and cryptic sibling species are commonly overlooked (Knowlton 1993). Based on their observations of developmental mode in different *P. elegans* populations, Hannerz (1956) and Rasmussen (1973) concluded that the variation in development seen represents extremes of a single developmental trajectory. Rasmussen (1973) hypothesized that the variation in development stems from variation in environmental factors, in particular, temperature. However, this hypothesis was refuted in some experiments which have been conducted to determine if larval polymorphism is a plastic response to changing environmental factors. Since neither Anger (1984) nor Morgan (1997) observed changes in the developmental mode in *P. elegans* during their experiments, plasticity in response to environmental variation in this species was not supported. Anger (1984) suggested that cryptic species, each with different developmental modes, comprise the taxon *P. elegans*. Later, using allozyme loci as genetic markers, Morgan and colleagues (1999) studied population genetic structure among 4 *P. elegans* populations differing in developmental mode. Their results suggested poecilogony for *P. elegans*, but indicated significant population genetic structure among some populations (Morgan et al. 1999).

Based on these previous studies, the hypothesis was that *P. elegans* is indeed poecilogonous. In this study, the geographical scope was expanded to include 14 European and 3 North American populations sampled from a variety of environmental conditions in order to increase the chances of detecting possible cryptic species if they existed. Using DNA barcoding approach and phylogenetic and haplotype network analyses of data from the COI gene, further evidence to support the hypothesis of poecilogony in *P.*

elegans was found. First, even though the European samples were from populations with different developmental modes, low mean sequence divergence in the COI sequence (1.7 %) was seen, lower than the within species divergence observed in other poecilogonous species (Schulze et al. 2000, Ellingson & Krug 2006) or the DNA barcoding threshold (Goetze 2003, Hebert et al. 2003, Costa et al. 2007). The COI sequence divergence between European and North American samples was higher (5.3 %), and possible cryptic species in North America cannot be ruled out without a larger scale analysis. Nevertheless, the COI sequence divergence among closely related species is commonly higher than 5 % (Jolly et al. 2005, Blank & Bastrop 2009, Luttikhuizen & Dekker 2010, Carr et al. 2011, Nygren & Pleijel 2011). Despite the low sequence divergence observed in Europe, there were 123 unique haplotypes in the data set of 299 sequences, leading to a large number of low frequency haplotypes.

Secondly, in the phylogenetic analyses, minimum spanning haplotype network analysis (MSN), and in the clustering analysis, the COI haplotypes did not group according to geography or larval developmental mode. In the phylogenetic trees, only a few well-supported groups (based on high bootstrap support) were formed among the European samples due to the low sequence divergence. Haplotypes were also shared among populations with different larval types, a pattern found also in other poecilogonous species (*B. proboscidea*: Gibson et al. 1999, Oyarzun et al. 2011; *A. willowi*: Ellingson & Krug 2006). The two most common haplotypes (comprising 25 % of all individuals) spanned the whole sampling area, and the most common haplotype, EUNA10, was also observed from the east coast of North America. The rest of the North American haplotypes were clearly different from the European sequences (differing by 26 mutational steps in the MSN and with high bootstrap values in the phylogenetic trees). It is acknowledged that in some rare cases, subspecies may not be distinguished with mitochondrial DNA analyses, for example due to recent hybridization events (Nikula et al. 2007).

Demographic analyses suggested that most European populations were in demographic equilibrium, except for signs of recent population expansion in the populations with planktonic larvae from the UK and France. Especially low sequence divergence was seen in the geographically marginal populations with benthic larvae in Iceland and Ängsö, Finland, in the northern Baltic Sea. A higher COI haplotype diversity was seen in the populations with planktonic larvae, and AMOVA analyses indicated population genetic structure on different spatial scales.

Based on these results, it is concluded that *P. elegans* is poecilogonous and that differences in different developmental modes between populations affects their genetic diversity. To clarify the correlation of genetic structure and developmental mode in *P. elegans,* additional population genetic analyses using additional polymorphic genetic markers was then conducted.

3.2 Microsatellite marker isolation

Since there were almost no genetic tools available for *P. elegans* when this study was initiated (2008), new markers were developed for the population genetic analyses. 17 loci containing repeat regions were isolated with the microsatellite library protocol, and after primer design, testing and optimization of the PCR reactions, 12 loci were amplified successfully and 8 of these were polymorphic. The 8 novel microsatellite markers were highly polymorphic and revealed population genetic structure even on a small scale of 100 m (II). Heterozygote deficiency caused deviations from Hardy-Weinberg equilibrium in some loci and low to moderate null allele frequencies were seen in the deviating samples (except in locus Pe12, null allele frequencies were moderate to high). In addition to the possible presence of null alleles, other explanations to HWE deviations in *P. elegans* are inbreeding, asexual reproduction, within population genetic structure (II) and high local recruitment (III). Positive F_{IS} values (caused by heterozygote deficiency) and HWE deviations in population genetic studies of marine species are not uncommon (Addison & Hart 2005, Zhan et al. 2009). Despite these issues, the loci proved to be useful new genetic tools for investigating population genetic patterns in *P. elegans*. The markers could also be tested for use in other, closely related spionid species to increase their utility.

3.3 Developmental mode polymorphism and population genetic structure

3.3.1 Spatial genetic structure

Many studies have found a correlation between larval developmental mode, larval dispersal and population genetic structure, resulting in predictable pattern: species with planktonic larvae are genetically more diverse and show higher population connectivity than species with non-dispersive larvae (Hellberg 1996, Hoskin 1997, Arndt & Smith 1998, Ayre & Hughes 2000, Kyle & Boulding 2000, Collin 2001, Dawson et al. 2002, Ellingson & Krug 2006, Watts & Thorpe 2006, Lee & Boulding 2009, Binks et al. 2011). This pattern is expected to be a general one, and when deviations from the pattern are found (e.g. Johannesson 1988, Taylor & Hellberg 2003, Ayre et al. 2009, Kelly & Palumbi 2010), an explanation is usually provided showing that larval dispersal is independent of developmental mode, for example, through larval behaviour (Warner & Palumbi 2003). Comparisons of population genetic patterns have been made previously only between closely related species differing in developmental mode. A poecilogonous species provides a useful study model when investigating the effect of developmental mode on population genetic structure when populations differ in their developmental mode (as in *P. elegans*). The general pattern should exist at the population level as well as

species level: populations with planktonic larvae were expected to be more diverse genetically and have higher population connectivity. Since the adult *P. elegans* worms live in tubes embedded in the sediment, they are relatively sessile, and the larval stage is assumed to be the dispersing life stage in this species. Planktonic *P. elegans* larvae can live for up to 5 weeks in the plankton (based on laboratory estimates, Anger et al. 1986), whereas benthic larvae are assumed to disperse only short distances (based on their ability to build their own tubes after emergence from brood capsules and their lack of swimming setae).

In the study with 18 *P. elegans* populations from Europe (Table 1, III), higher genetic diversity (expected heterozygosity, allelic richness and gene diversity) was found in the populations with planktonic larvae compared to those populations that also have benthic larvae. However, because the developmental mode may be associated with geography, it may be difficult to determine if the patterns results from developmental mode differences alone. In this study populations with planktonic larvae were more commonly seen in the marine North Sea area, whereas longer brooding was more common in the Baltic Sea, leading to a pattern of lower genetic diversity in the Baltic Sea. In *B. proboscidea*, another poecilogonous spionid polychaete, longer brooding and higher maternal investment is seen at higher latitudes (Oyarzun et al. 2011).

The population pairwise F_{ST} values ranged from 0.001 to 0.170, but significant spatial genetic structure (statistically significant values) were seen between most populations. In the Baltic Sea, genetic structure was seen even among geographically close populations, whereas in the North Sea where the planktonic larval type predominates, some geographically close populations were genetically similar. Nevertheless, significant genetic structure was seen in the North Sea also. Interestingly, the polymorphic Schiermonnikoog population from the Netherlands was more similar to the Danish populations (also polymorphic) than to the other Dutch populations where planktonic larvae predominate (indicated by significant F_{ST} values). The genetic structure in Europe was characterized by an isolation by distance pattern, which is more commonly found in marine species with non-dispersive larvae (Hellberg 1996, Goldson et al. 2001, Duran et al. 2004, Watts & Thorpe 2006). Clustering analyses led to a similar conclusion of isolation by distance: here, geographically close populations clustered together. Also, asymmetric migration rates and high self-recruitment were estimated for most populations, regardless of the larval developmental mode. The results indicate that the planktonic larvae of *P. elegans* may not be superior dispersers, but that the dispersal potential in the different larval modes may be similar (III). The individual assignment test results also supported this conclusion (IV). In fact, many recent studies have suggested that local recruitment of planktonic marine larvae may be more common than thought (Swearer et al. 2002, Warner & Cowen 2002, Almany et al. 2007). The proposed correlation between larval type, dispersal potential and population genetic structure is not clearly supported in *P. elegans*, suggesting that the larval type of a species cannot be used to predict population connectivity if larval dispersal is not tightly correlated with larval

type. Similar findings have been seen in other studies (Porter et al. 2002, Bowen et al. 2006, Marko et al. 2007, Miller & Ayre 2008, Shanks 2009, Zhan et al. 2009, Kelly & Palumbi 2010). Also, the hypothesis of longer planktonic larval duration leading to increased distances of larval migration and therefore stronger population differentiation has been challenged (Weersing & Toonen 2009).

TABLE 1 Information on *Pygospio elegans* sample locations, observed population developmental modes and observed densities (qualitative observations only) for the populations used in study III.

Region	Country	Location	Developmental mode*	Estimated density
Baltic Sea	Finland	Ängsö	B (A)	Low
		Fårö	(A)	Low
		Hanko	(A)	Low
	Germany	Germany		
	Denmark	Vellerup	I, P, B (A)	Medium
		Herslev	I, B (A)	Low
		Rorvig	I, B, P (A)	Medium
	Sweden	Gullmar fjord		Low
North Sea	Netherlands	Schiermonnikoog	P, I, B	High
		Harlingen	P	High
		Breskens	P	High
	France	Canche Bay	P	Very high
		Somme Bay	P	Very high
	UK	Drum sands	P	High
		Eden estuary		High/medium
		Plym Bay	P	Low/medium
		Ryde sands		Very low
Atlantic Ocean	Iceland	Iceland	B, I	

* Observed larval developmental mode: B = benthic, I = intermediate, P = planktonic, (A) = asexual reproduction

3.3.2 Temporal dynamics and effective population size

Next it was examined whether the hypothesis that temporal genetic stability will be higher in species with brooding than in species with planktonic larvae (Lee & Boulding 2009) would also be relevant for a poecilogonous species with populations differing in developmental mode. In the marine environment, temporal genetic instability is often found in species with planktonic larvae (Heath et al. 2002, Østergaard et al. 2003, Lee & Boulding 2007) and in some cases (Planes & Lenfant 2002, Robainas-Barcia et al. 2005, Florin & Höglund 2007) the instability can be explained by sweepstakes reproductive success (Hedgecock 1994, Hedgecock & Pudovkin 2011), which is caused by high variation in individual reproductive success. Species with high fecundity but

high larval mortality are potentially more affected by individual variation in reproductive success (Thorson 1950, Morgan 1995, Pechenik 1999, Lee & Boulding 2009) than species developing via other modes. Since the planktonic larvae of *P. elegans* have a high mortality rate (Pedersen et al. 2008), populations with this larval developmental mode could be more prone to variation in reproductive success and temporal genetic variation.

In most of the study populations used here, the patterns of genetic diversity (heterozygosity, allelic richness) fluctuated only slightly among the different sampling years, although AMOVA analysis suggested both spatial and temporal genetic structure in *P. elegans* (IV). Contrary to expectations, significant temporal genetic structure (based on F_{ST} and Structure clustering analyses) was found in the Baltic Sea populations where benthic or intermediate larval developmental modes predominate. However, the most striking temporal change was seen in a Dutch population (Schiermonnikoog) with multiple larval types, in which the 2011 sample was very diverse genetically and noticeably different from samples collected in the previous years. Additionally, the estimated effective population sizes were low for all the populations except for the one UK population (IV) with strictly planktonic larvae and high genetic diversity (III). Genetic drift seems to be the most likely explanation for the temporal genetic patterns in these populations. In the Baltic, *P. elegans* density is lower in comparison to the more marine areas (Morgan 1997, Boström & Bonsdorf 1997, 2000), which could also lead to lower N_e and support the proposed role of random genetic drift on the temporal patterns. The Baltic populations also had a lower historical population size (IV). In the Netherlands population, extreme environmental conditions during the harsh winter of 2010/2011 probably reduced the population size, after which the site was probably recolonized by migration from multiple nearby populations. In species with planktonic larvae, gene flow from different sources can be a likely cause of temporal genetic variation (Johnson & Black 1982, 1984, Moberg & Burton 2000).

Signatures of sweepstakes reproductive success may have gone undetected due to the restricted sampling of the strictly planktonic populations and short time span between the samples. However, these results indicate that other factors are more likely to cause the observed temporal instability in *P. elegans*.

3.3.3 Effect on environmental factors on population structure

Although *P. elegans* shows broad environmental tolerances and can be found in a variety of habitats, the distribution of *P. elegans* could be affected by environmental factors, which could also affect developmental mode as originally hypothesized by Hannerz (1956) and Rasmussen (1973). For example, according to previous observations, populations with primarily planktonic larvae are found from marine intertidal mud- or sand-flats, whereas populations with a larger proportion of benthic developmental modes are found in estuarine habitats (I, II, III). The environment also may affect

population genetic structure without any effect on developmental mode. For example, in the Finnish archipelago *P. elegans* is associated with the sea grass *Zostera marina*, and exists in a fragmented *Z. marina* landscape (Boström & Bonsdorff 2000). As a result, *P. elegans* presence and abundance is also patchy in this area. Patchy habitat together with a predominance of benthic larvae and asexual reproduction are likely all involved in creating the significant population genetic structure observed in *P. elegans* in the northern Baltic Sea (spatially, on the scale of 20km [III] and temporally, on the scale of a few years [IV]).

Distribution of *P. elegans* is also patchy in the Danish estuarine complex, Isefjord and Roskilde fjord, which could be due to the heterogeneity of the habitats in the estuaries (Rasmussen 1973, and see Bilton et al. 2002). It was found that the population genetic structure among *P. elegans* populations in this area was not affected by the geography of the estuary complex, i.e. gene flow between the estuaries was not limited. However, many population pairwise F_{ST}/D_{est} comparisons (within and between estuaries) were statistically significant (V). The overall genetic pattern could not be explained by isolation by distance, which was previously seen among *P. elegans* populations on a larger spatial scale (III). However, any significant environmental variables were not identified to help explain the somewhat "chaotic" genetic structure seen in the estuaries. The opportunistic life history characteristics often linked to *P. elegans* (e.g. Desprez et al. 1992, Morgan et al. 1999), fluctuations in population size (pers. obs, Morgan 1997, Bolam & Fernandez 2002, 2003) and genetic drift may have affected the observed genetic patterns in the estuaries, and these may be temporally unstable (IV).

In other studies using a larger geographical scale, genetic structure has been found to be affected by factors other than larval dispersal ability. For example, oceanic current patterns (Galarza et al. 2009, Selkoe et al. 2010, White et al. 2010), temperature (Dionne et al. 2008), salinity (Gaggiotti et al. 2009), habitat fragmentation (Johnson & Black 2006) or even behavioral characteristics (Gaggiotti et al. 2009) can help explain genetic structure. Also, adaptation to local environmental conditions could create population genetic differentiation (Larmuseau et al. 2010, Nissling & Dahlman 2010) and even affect life-history characteristics (Hemmer-Hansen et al. 2007).

Local habitat characteristics can be important for the successful settlement of marine invertebrate larvae and juvenile survival (Turner et al. 1994, Cohen & Pechenik 1999, Qian et al. 2000). Also, the benthic community structure may be affected, for example in terms of competition or predation (Kube & Powilleit 1997, Bolam & Fernandes 2003). To conclude, further analysis with more specific qualitative environmental factors (e.g. sediment grain size and organic material content of the sediment, or species richness) and sampling across a larger spatial scale may reveal more about the importance of different environmental factors on the stability and connectivity of *P. elegans* populations.

4 CONCLUSIONS

Despite the wide range of developmental strategies seen in marine invertebrate species, different larval phenotypes are rarely seen within a single species (poecilogony). Different explanations have been suggested for the presence of such polymorphism, for example, whether there is a genetic basis for the phenotypes, or if the plasticity is environmentally induced (polyphenism) (see Knott & McHugh 2012). Also, a correlation to speciation has been proposed, in which larval polymorphism represents a transient stage in speciation coupled with a transition in developmental mode (Gibson & Gibson 2004, Ellingson & Krug 2006). When considering the evolution of larval developmental modes, it is important to understand the evolutionary forces driving transitions of developmental modes. Poecilogonous species are ideal models for such studies, since although individuals might differ in developmental mode, other differences between them are expected to be minor. This contrasts with studies of sibling species in which in addition to developmental mode differences, considerable differentiation in other traits is expected to have accumulated during speciation. Also, studying the effects of developmental mode variation on population maintenance and connectivity sheds more light on the demographic consequences of poecilogony.

As a first step, using sequencing and phylogenetic methods combined with a broad sampling scale, further support for poecilogony in *P. elegans* was found (I). Different larval phenotypes were found both among and within populations, and intermediate larval forms were commonly seen in the Baltic Sea. The sequence divergence in COI was low, and no clustering based on developmental mode or geographical location was seen.

After developing new species specific polymorphic microsatellite markers for *P. elegans* (II), it was investigated whether the pattern of genetic connectivity is affected by the larval developmental mode in this poecilogonous species (III). In marine invertebrate species with sedentary or sessile adults, a planktonic larval stage is hypothesized to lead to high population connectivity, whereas a non-dispersive larval stage would lead to genetically differentiated populations. However, the pattern of genetic structure in the European *P.*

elegans populations was best explained by an isolation by distance pattern and most of the pairwise comparisons of genetic differentiations were significant (III). In the fragmented sea grass landscape of the northern Baltic Sea, the observation of genetically differentiated *P. elegans* populations was not surprising, since their patchiness is most likely linked to the patchiness of the sea grass landscape they inhabit. Also, benthic larval type and asexual reproduction in a patchy habitat may be favored to maintain local populations (Levin 1984, Pechenik 1999). However, genetic differentiation was observed among some of the planktonic North Sea populations also. Estimations of migration rates indicated high local recruitment despite developmental mode. Together these results suggest that dispersal is limited among *P. elegans* populations, even in the case of planktonic larval production, and the observed genetic patterns may be maintained by the continuous high local recruitment of individuals (III, IV). Recent studies have also suggested that the marine environment may not be as open as previously thought, and local larval recruitment and unexpected population genetic patterns are often seen.

The expected pattern of lower genetic diversity in the *P. elegans* populations with also benthic larvae compared to populations with only planktonic larvae was seen (I, II, III). Temporal genetic instability in the strictly planktonic population due to the high mortality of the planktonic larvae (Pedersen et al. 2008) was expected. However, unstable temporal structure was seen in the populations where benthic or intermediate larval modes predominate (IV). This variation is likely caused by genetic drift and not migration from other populations, since these populations have low effective population size (IV) and high local recruitment rates (III).

In many species, the Baltic Sea populations are genetically isolated and have low genetic diversity (Johannesson & André 2006). This pattern might result from the young age of the area, geographical isolation, low salinity, low temperature and lower oxygen levels compared for example to the North Sea. In *P. elegans*, the distribution of the different larval modes may be connected to geography or environmental variables, since in Europe, longer larval brooding was observed in the brackish Baltic Sea, whereas planktonic populations were predominating in the marine habitat of the North Sea (I, II, III). However, this pattern was not without exceptions (all larval modes were seen in both areas also). Also, genetic structuring was seen between the Baltic and the North Sea populations, but it was not considerably stronger than structure seen among populations within an area (I, III). On a smaller geographical scale, when information on ecological variables and spatial information was added to the analysis of population genetic data, no specific factors which would predict the observed *P. elegans* genetic patterns in the heterogonous habitat of the Danish estuary complex were found (V), but significant patterns may arise if larger scales would be used.

The association of geography and larval mode can be seen in other poecilogonous species also. Selection may favour longer brooding in higher latitudes, for example due to environmental factors affecting developmental rate or food availability (Thorson 1950, Elligson & Krug 2006, O'Connor et al.

2007, Oyarzun et al. 2011). One larval phenotype may be more successful in one environment, and a different larval phenotype in another, and this could lead to populations with different larval types. The existence of populations with multiple larval types is challenging to explain, since if one of the phenotypes had fitness advantage over the others, the other types would be expected to be eventually lost (Levin et al. 1987). Even though the different larval modes affect many population parameters and life history characteristics, the fitness of the mothers producing the different larval types may be similar in the end, as is suggested for *S. benedicti* (Levin & Hugget 1990), where the high fecundity in the planktonic larvae is balanced by the high survival of the lecithotrophic larvae. Also, multiple modes within a population could coexist because of a bet-hedging strategy, an adaptation to surviving and maintaining a population in unpredictable or heterogeneous environments (where plasticity is favoured) (Krug 2009, see also Crean & Marshall 2009). This would allow the maintenance of the local population, but also migration away from the population if the local habitat conditions deteriorate, for example due to high predation pressure or competition (Strathmann et al. 2002), or with seasonal changes (Krug 1998, Krug 2007). However, this kind of bet-hedging plasticity is rare.

In the future, more detailed research on why and with what mechanisms do developmental mode transitions occur could be pursued using *P. elegans* as a model. Gene expression experiments using transcriptome and qPCR techniques allow investigation of whether different genes are involved in the development of the different larvae or if the developmental genes have timing differences in their expression when development proceeds through different modes (see Gibson & Gibson 2004 for heterochrony in *B. proboscidea*). Also, since maternal food provisioning is associated with the different developmental modes in *P. elegans* and other poecilogonous species, maternal influences on the larval developmental modes and the possible differences in production of genuine eggs and nurse eggs should be addressed. In addition, the role of environmental cues on larval polymorphism could be studied further because of the observed geographical pattern in the developmental modes. Laboratory experiments using both polymorphic and fixed populations and multiple worm generations are needed. In addition, mating experiments combining individuals from populations with different larval modes would reveal more about the genetic background and heritability of the larval phenotype. In the future, results from studies like these will improve our understanding of life history evolution in marine species.

Acknowledgements

First and foremost, I would like to thank my supervisor Emily Knott. It has been great to work with you in our small research team. You are probably one of the nicest people I know. Thank you for the scientific influence, and for your inspiration, support and positive thinking! I'd also like to acknowledge my other supervisor Damhnait McHugh for the poecilogony discussions and encouragement. I especially want to thank both of you for organizing the very interesting poecilogony symposium at this year's SICB meeting and inviting me to give a talk there. It was a pleasure to finally meet the other poecilogony people. Also, Anneli Hoikkala, supervisor of my master's thesis, thank you for your support also during my PhD years and for bringing evolutionary genetics to Jyväskylä.

A special thanks goes to all my coauthors outside Jyväskylä (B.W. Hansen, C. Boström, P. Rawson, S. Lindsay, E. Geuverink, G. Banta) for your help and advice with my manuscripts. Of course, I owe the biggest thanks to Emily for shaping my scientific writing skills. Many people have also helped with sample collecting around Europe. Thanks especially to the lovely Benni Hansen, who has hosted our many trips to Denmark and helped us to find the Danish *Pygospio* sites. Thanks also to Christoffer Boström for providing access to the Korpoström field station and Camilla Gustafsson for diving and collecting *Pygospio* in the Finnish archipelago. To the reviewers of my thesis, Michael Hart and Raisa Nikula, many thanks for your comments and questions which have helped me improve this thesis. Also, thanks to Jari Haimi for editing the thesis.

My work was funded by the Jenny and Antti Wihuri Foundation, the BIOINT graduate school and the University of Jyväskylä (through a scholarship granted by the Rector). The DNA lab was supported by the Centre of Excellence in Evolutionary Research and the EU Marie Curie ITN "Speciation". Funding for travelling granted from Societas pro Fauna et Flora Fennica, Nordic Marine Academy, Finnish Concordia Fund, Emil Aaltonen Foundation and BIOINT is also very much appreciated.

I am very grateful for the many opportunities to travel to conferences, workshops and field trips abroad. My fear of airplanes and flying is almost cured! Also, attending workshops in Finland has been great, especially the BIOINT graduate school workshops have been a lot of fun! I would also like to acknowledge the Marie Curie ITN "Speciation" for organizing interesting conferences and for other networking opportunities.

The EKO section at the University has been a nice place to work, and I would like to thank the PhD students and other staff for that. I have had many office mates in the three different offices I've been in during these years, I want to thank all of you for your company, and for sometimes providing the much needed distraction from work stress. All those countless hours in the DNA lab were made better by Jaana Haka, Sari Viinikainen, Anssi Lipponen, Elina Virtanen and Veronica Chevasco. Students Marina Mustonen and Anna Lempiäinen did valuable work in the DNA lab with my worm samples. Marina

also did her bachelor's and master's theses in our group; Marina, thank you for your enthusiastic attitude and good work!

Then, to the lovely flygirls and flyboys, thanks for your company at the University. Especially Tiina, Laura, Hannele, Venera and Nina, thanks for your friendship during these years! Tiina and Laura, thanks for having the same kind of sense as humor as me. I can't tell you how much fun I've had with you both at work and outside work. Miss you! Hannele, you are as crazy as me, thanks for always cheering me up! Venera, I've really enjoyed our time at the same office. I will remember the cakes, fondue trials and our high quality population genetics discussions. Hannele, Mikko, Darren and Nina made the last few months of this thesis project bearable, thanks for dragging me to coffee breaks and then kicking me back to work.

I would also like to thank all my lovely friends outside the science circles. I won't name many names since I don't want to forget anybody, but I would especially like to thank my dear friend Tiina, whom I've known since early childhood. And Jukka, thanks for being in my life, you know that you are important to me. Lastly, warm thoughts go to my parents and Joonas & Elina. Mum and dad, thanks for always believing in me. I look forward to your calls every Sunday!

YHTEENVETO (RÉSUMÉ IN FINNISH)

Kehitysmuotojen variaatio ja sen populaatiogeneettiset seuraukset

Meressä elävillä lajeilla tavataan erityisen paljon erilaisia lisääntymisstrategioita. Esimerkiksi monilla selkärangattomilla pohjaeläinlajeilla aikuinen kehittyy useiden erilaisten toukkavaiheiden kautta, ja näillä lajeilla toukkien kehitysmuoto onkin tärkeä mm. lajin lisääntymismenestykseen, yksilönkehitysaikaan ja levittäytymispotentiaaliin vaikuttava elinkiertopiirre. Meressä elävillä selkärangattomilla yleisin lisääntymisstrategia on luultavasti gameettien vapauttaminen veteen ja sitä seuraava ulkoinen hedelmöitys ja planktinen vedessä vapaasti elävä ja ravintoa etsivä toukka. Jos toukalla ei ole vapaasti uivaa vaihetta, se voi elää meren pohjalla joko vapaana tai suojattuna (ei-planktinen toukka). Ei-planktiset toukat kehittyvät usein ravinteikkaista suurista munasoluista, jolloin toukan ravinnontarve on vähäinen. Toukka voi myös kasvaa naaraan tekemässä suojatussa rakenteessa (kapseli tai suojaava massa), jolloin naaras voi antaa toukalle ulkoista ravintoa (ravintomunia). Tällaista strategiaa on tavattu mm. joillakin madoilla ja nilviäisillä. Meriselkärangattomat voivat myös kehittyä kokonaan ilman toukkamuotoa, ja myös suvuton lisääntyminen on yleistä. Toukkamuotojen monimuotoisuuden tunteminen on keskeinen asia kehitysmuotojen synnyn evolutiivisen taustan ymmärtämisessä. Monilla meressä elävillä läheistä sukua olevilla lajeilla on erilaisia toukkamuotoja, mistä voidaan päätellä että siirtymiä eri toukkamuotojen välillä on tapahtunut useasti ja suhteellisen lyhyessä ajassa. Toukkamuotojen evoluutioon on usein ajateltu liittyvän energeettisiä kompromisseja jälkeläisten määrän, munasolun koon ja kehitysajan suhteen. Myös monet ekologiset tekijät, kuten elinympäristön laatu, predaatioriski, ravinnon saatavuus ja kilpailu, ovat todennäköisesti vaikuttaneet eri toukkamuotojen syntyyn ja niiden nykyiseen esiintymiseen.

Erilaisia toukkamuotoja on havaittu myös samalla lajilla (poecilogonia). Tämänkaltainen monimuotoisuus on harvinaista, ja sitä tavataan vain joillakin kotiloilla (Sacoglossa-kotilot), sekä Spionidae-heimon monisukasmadoilla. Toukkamuotojen polymorfismi voi olla esimerkiksi geneettistä tai ympäristöolojen säätelemää. Tutkimuslajini *Pygospio elegans* (hiekkaputkimato) on rakentamassaan hiekkaputkessa meren pohjassa elävä monisukasmato (Spionidae), joka pystyy lisääntymään erilaisten toukkamuotojen kautta. Sisäisen hedelmöityksen jälkeen *P. elegans* naaras munii alkiot ja ravintomunia kapseleihin hiekkaputkensa sisälle. Jos alkioita on paljon, naaras vapauttaa toukat aikaisessa vaiheessa planktoniin (planktinen toukka). Jos taas alkioita on vain muutama, ne elävät kapseleissa suojattuna ja syövät ravintomunia. Kun nämä toukat vapautuvat kapseleistaan, niillä ei ole planktista kehitysvaihetta, vaan ne ovat valmiita muodonvaihdokseen toukasta juveniileiksi (ei-planktinen muoto). Lajilla tavataan myös välimuotoisia toukkia, joilla on lyhyt planktinen vaihe. Toukkamuodot voivat siis erota toisistaan lukumäärältään ja morfologialtaan sekä ravinnon tarpeen ja planktisen vaiheen pituuden suhteen. Toukkamuoto-

jen on havaittu muuntelevan sekä populaatioiden sisällä että niiden välillä, mutta on epävarmaa pystyykö yksi naaras tuottamaan eri toukkamuotoja.

Väitöskirjatutkimukseni tavoitteena oli selvittää kuinka lajinsisäinen variaatio toukkien kehitysmuodoissa vaikuttaa *P. elegans*in populaatiogeneettiseen rakenteeseen, siinä tapahtuviin muutoksiin ja populaatioiden väliseen migraatioon. Eri toukkamuotoja tuottavien *P. elegans* -populaatioiden on aikaisemmin päätelty kuuluvan eri lajeihin, ja koska lajinsisäinen polymorfia kehitysmuodoissa on harvinaista, selvitin väitöskirjatyössäni myös *P. elegans*in lajistatusta molekyyligeneettisin menetelmin. Sekvenssiaineisto ja fylogeneettiset analyysit osoittivat, että laajalta maantieteelliseltä alueelta erilaisista ympäristöoloista kerätyt näytteet kuuluvat hyvin todennäköisesti samaan lajiin, koska ne eivät ryhmittyneet fylogeneettisissä puissa toukkamuodon tai populaation mukaan, ja myös sekvenssien divergenssi oli alhainen. Saman DNA-alueen sekvensointia on käytetty myös tutkittaessa muita kehitysmuotopolymorfiaa omaavia lajeja (*Streblospio benedicti*, *Alderia willowi*).

Seuraavaksi *P. elegans*in genomista eristettiin kahdeksan uutta polymorfista mikrosatelliittimarkkeria populaatiogeneettisiä tutkimuksia varten. Polymorfisen lajin populaatiogenetiikka on mielenkiintoista, sillä erilaisilla toukkamuodoilla on meriympäristössä hyvin erilaiset kohtalot ja ne eroavat mm. kuolleisuuden ja levittäytymispotentiaalin suhteen. Hypoteesin mukaan pohjaeläinlajeilla, joilla on dispersoiva planktinen toukkavaihe, populaatioiden välinen migraatio ja geenivirta johtavat geneettisesti samankaltaisiin monimuotoisiin populaatioihin. Jos lajilla ei ole dispersoivaa toukkavaihetta, populaatioiden välinen migraatio on rajattua ja ne eriytyvät geneettisesti. Näitä hypoteeseja on tutkittu vertaamalla keskenään kehitysmuodoiltaan erilaisia lähisukuisia lajeja, mutta kehitysmuodoiltaan polymorfisen lajin käyttäminen tällaisissa tutkimuksissa on uutta. *P. elegans* onkin hyvä mallilaji tutkittaessa toukkamuodon, levittäytymiskyvyn ja populaatiogeneettisen rakenteen välisiä suhteita, koska yhtä polymorfista lajia tutkittaessa ei tarvitse ottaa huomioon lajiutumiseen liittyviä adaptiivisia eroja. Koska *P. elegans* –lajin aikuiset elävät tekemässään hiekkaputkessa, ne ovat suhteellisen paikallaan pysyviä, ja levittäytymisen oletetaan tapahtuvan pääasiassa toukkavaiheen aikana.

Tutkimusta varten näytteitä kerättiin *P. elegans* –lajista useista populaatioista Euroopasta. Planktisia toukkia tuottavien populaatioiden havaittiin olevan geneettisesti monimuotoisempia verrattuna populaatioihin, joissa oli myös ei-planktisia toukkia. Maantieteellinen sijainti saattaa vaikuttaa toukkamuodon esiintymiseen tällä lajilla, sillä Pohjanmeren alueella havaittiin yleisesti planktisia toukkia, kun taas Itämeressä ei-planktisten toukkien ja välimuotojen osuus oli suurempi. Suomen saaristossa *P. elegans* esiintyy pääasiassa meriajokasniityillä *(Zostera marina)*, ja koska saariston niityt ovat fragmentoituneita, tämä vaikuttaa myös *P. elegans*in esiintymiseen. Tutkimuksessa havaittiinkin Itämeressä lähekkäin olevien populaatioiden olevan geneettisesti eriytyneitä. Geneettisesti eriytyneitä populaatioita havaittiin myös Pohjanmeren alueella, jossa planktinen toukkamuoto on yleisin. Lajin populaatiorakenne näyttää riippuvan populaatioiden välisistä maantieteellisistä etäisyyksistä, jolloin geneettinen

eriytyminen kasvaa etäisyyden kasvaessa, riippumatta toukkakehitysmuodosta. Kun populaatioiden välistä migraation voimakkuutta analysoitiin, havaittiin paikallisista populaatioista ulospäin suuntautuvan migraation olevan vähäistä toukkamuodosta riippumatta. Nämä tulokset viittaavat siihen, että erilaisilla *P. elegans*in toukkamuodoilla ei ole suurta eroa levittäytymiskyvyssä, eikä planktinen toukkamuoto välttämättä johda voimakkaampaan geenivirtaan ja populaatioiden geneettiseen samankaltaisuuteen. Muita populaatiogeneettiseen rakenteeseen vaikuttavia tekijöitä voivat olla mm. lajin historiaan ja leviämiseen liittyvät tekijät, käyttäytyminen tai esimerkiksi habitaatti ja merivirrat. Tutkimme ympäristötekijöiden vaikutusta populaatiogeneettisen rakenteeseen pienellä mittakaavalla Tanskan Själlantin saaren vuonojen heterogeenisessä elinympäristössä, mutta tutkimuksessa ei havaittu minkään yksittäisen tekijän (esimerkiksi sedimenttityypin tai veden suolapitoisuuden) vaikuttavan populaatioiden eriytymiseen.

Toukan kehitysmuoto voi vaikuttaa myös populaatioiden ajalliseen vakauteen, ja geneettisen populaatiorakenteen nopeaa ajallista muutosta on havaittu useimmiten lajeilla, joilla on planktisia toukkia. Planktisten toukkien korkea kuolleisuus voi johtaa epätasaiseen lisääntymismenestykseen naaraiden välillä, ja geneettisesti eriytyneisiin ikäluokkiin. Vastoin odotuksia Itämeren populaatioista eri vuosina kerättyjen *P. elegans* –lajin näytteiden havaittiin olevan geneettisesti erilaisia, vaikka ei-planktiset ja välimuotoiset toukat ovat näissä populaatioissa yleisiä, kun taas vain planktisia toukkia tuottava Pohjanmeren populaatio oli ajallisesti muuttumaton. Toukkamuoto vaikutti mahdollisesti myös efektiiviseen populaatiokokoon, koska se estimoitiin huomattavasti pienemmäksi populaatioissa joissa oli ei-planktisia toukkia. Pieni (efektiivinen) populaatiokoko ja alhainen migraationopeus populaatioiden välillä viittaavat siihen, että ajalliset muutokset populaatiogeneettisessä rakenteessa johtuvat tällä lajilla geneettisestä satunnaisajautumisesta.

Tulevaisuudessa *P. elegans* -lajilla voidaan tutkia tarkemmin erilaisia toukkamuotoja, esimerkiksi geenien ilmentymisen tasolla (transkriptomiikka), ja eri toukkamuotoja tuottavien naaraiden vertailu voi tuoda lisätietoa naaraan vaikutuksesta toukkamuotoon. Lajit, jotka ovat kehitysmuotojen suhteen polymorfisia, ovat erityisen hyviä malleja tutkimuksissa, joissa halutaan ymmärtää kehitysmuotojen sekä niiden muuttumisen evolutiivista taustaa.

REFERENCES

Addison J.A. & Hart M.W. 2005. Spawning, copulation and inbreeding coefficients in marine invertebrates. *Biol. Lett.* 1: 450–453.

Almany G.R., Berumen M.L., Thorrold S.R., Planes S. & Jones G.P. 2007. Local replenishment of coral reef fish populations in a marine reserve. *Science* 316: 742–744.

Anil A.C., Venkat K., Sawant S.S., Dileepkumar M., Dhargalkar V.K., Ramaiah N., Harkantra S.N. & Ansari Z.A. 2002. Marine bioinvasion: concern for ecology and shipping. *Curr. Sci.* 83: 214–218.

Anger V. 1984. Reproduction in *Pygospio elegans* (Spionidae) in relation to its geographical origin and to environmental conditions: a preliminary report. *Forts. Zool.* 29: 45–51.

Anger K., Anger V. & Hagmeier E. 1986. Laboratory studies on larval growth of *Polydora ligni*, *Polydora ciliata* and *Pygospio elegans* (Polychaeta, Spionidae). *Helgoländer Meeresunters* 40: 277–395.

Arndt A. & Smith M.J. 1998. Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. *Mol. Ecol.* 7: 1053–1064.

Ayre, D.J. & Hughes P.T. 2000. Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution* 54: 1590–1605.

Ayre D.J., Minchinton T.E. & Perrin C. 2009. Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? *Mol. Ecol.* 18: 1887–1903.

Beerli P. & Felsenstein J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. *Proc. Natl. Acad. Sci. USA*. 98: 4563–4568.

Bilton D.T., Paula J. & Bishop D.D. 2002. Dispersal, genetic differentiation and speciation in estuarine organisms. *Est. Coast. Shelf. Sci*. 55: 937-952.

Binks R.M., Evans J.P., Prince R.J. & Kennington W.J. 2011. Fine-scale patterns of genetic divergence within and between morphologically variable subspecies of the sea urchin *Heliocidaris erythrogramma* (Echinometridae). *Biol. J. Linn. Soc.* 103: 578–592.

Blake J.A. & Arnofsky P.L. 1999. Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. *Hydrobiologia* 402: 57–106.

Blank M. & Bastrop R. 2009. Phylogeny of mud worm genus *Marenzelleria* (Polychaeta, Spionidae) inferred from mitochondrial DNA sequences. *Zool. Scr.* 38: 313–321.

Bohonak A.J. 1999. Dispersal, gene flow, and population structure. *Quart. Rev. Biol.* 74: 21–45.

Bolam S.G. 2004. Population structure and reproductive biology of *Pygospio elegans* (Polychaeta:Spionidae) on an intertidal sandflat, Firth of Forth, Scotland. *Invertebr. Biol*. 123: 260–268.

Bolam S.G. & Fernandes T.F. 2002. Dense aggregations of tube-building polychaetes: response to small-scale disturbances. *J. Exp. Mar. Biol. Ecol.* 269: 197–222.

Bolam S.G. & Fernandes T.F. 2003. Dense aggregations of *Pygospio elegans* (Claparede): effect on macrofaunal community structure and sediments. *J. Sea Res.* 49: 171–185.

Boström C. & Bonsdorff E. 1997. Community structure and spatial variation of benthic invertebrates associated with *Zostera marina* (L.) beds in the northern Baltic Sea. *J. Sea Res.* 37: 153–166.

Boström C. & Bonsdorff E. 2000. Zoobenthic community establishment and habitat complexity - the importance of seagrass shoot density, morphology and physical disturbance for faunal recruitment. *Mar. Ecol. Prog. Ser.* 205: 123–138.

Bowen B.W., Bass A.L., Muss A., Carlin J. & Robertson D.R. 2006. Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. *Mar. Biol.* 149: 899–913.

Carr C.M., Hardy M.S., Brown M.T., Macdonald A.T. & Hebert P.D.N. 2011. A Tri-Oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. *PLoS ONE* 6:e22232.

Chapuis M.P. & Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. *Mol. Biol. Evol.* 24: 621–631.

Chia F.-S., Gibson G. & Qian P.Y. 1996. Poecilogony as a reproductive strategy of marine invertebrates. *Oceanol. Acta* 19: 203–208.

Christiansen F.B. & Fenchel T.M. 1979. Evolution of marine invertebrate reproductive patterns. *Theor. Pop. Biol.* 16: 267–282.

Cohen R.A. & Pechenik J.A. 1999. Relationship between sediment organic content, metamorphosis, and postlarval performance in the deposit-feeding polychaete *Capitella* sp. I. *J. Exp. Mar. Biol. Ecol.* 240: 1–18.

Collin R. 2001. The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). *Mol. Ecol.* 10: 2249–2262.

Collin R. 2004. Phylogenetic effects, the loss of complex characters, and the evolution of development in calyptraeid gastropods. *Evolution* 58: 1488–1502.

Collin R., Chaparro O.R, Winkler F. & Veliz D. 2007. Molecular phylogenetic and embryological evidence that feeding larvae have been reacquired in a marine gastropod. *Biol. Bull.* 212: 83–92.

Corander J. & Tang J. 2007. Bayesian analysis of population structure based on linked molecular information. *Math. Biosci.* 205: 19–31.

Costa F.O., deWaard J.R., Boutillier J., Ratnasingham S., Dooh T.R., Hajibabaei M. & Hebert D.P.N. 2007. Biological identifications through DNA barcodes: the case of the Crustacea. *Can. J. Fish Aquat. Sci.* 64: 272–295.

Crawford N.G. 2010. SMOGD: software for the measurement of genetic diversity. *Mol. Ecol. Resour.* 10: 556–557.

Crean A.J. & Marshall D.J. 2009. Coping with environmental uncertainty: dynamic bet hedging as a maternal effect. *Philos. Trans. R. Soc. B* 364: 1087–1096.

Dawson M.N., Louie K.D., Barlow M., Jacobs D.K. & Swift C.C. 2002. Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. *Mol. Ecol.* 11: 1065–1075.

Desprez M., Rybarczyk H., Wilson G.J., Ducrotoy P.J., Seuer F., Olivesi R. & Elkaim B. 1992. Biological impact of eutrophication in the Bay of Somme and the induction and impact of anoxia. *Neth. J. Sea Res.* 30: 149–159.

Dionne M., Caron F., Dodson J.J. & Bernatchez L. 2008. Landscape genetics and hierarchical genetic structure in Atlantic salmon: the interaction of gene flow and local adaptation. *Mol. Ecol.* 17: 2382–2396.

Duran M., Pascual M., Estoup A. & Turon X. 2004. Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. *Mol. Ecol.* 13: 511–522.

Ellingson R. A. & Krug J.P. 2006. Evolution of poecilogony from planktotrophy: cryptic speciation, phylogeography, and larval development in the gastropod genus *Alderia*. *Evolution* 601: 2293–2310.

Emlet R.B. & Sadro S. 2006. Linking stages of life history: how larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*). *Integr. Comp. Biol.* 46: 334–346.

Excoffier L. & Lischer H.E.L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux andWindows. *Mol. Ecol. Resour.* 10: 564–567.

Faubet P. & Gaggiotti O.E. 2008. A new Bayesian method to identify the environmental factors that influence recent migration. *Genetics* 178: 1491–1504.

Fievet V., Touzet P., Arnaud J. & Cuguen J. 2006. Spatial analysis of nuclear and cytoplasmic DNA diversity in wild sea beet (*Beta vulgaris ssp. maritima*) populations: do marine currents shape the genetic structure? *Mol. Ecol.* 16: 1847–1864.

Florin A. & Höglund J. 2007. Absence of population structure of turbot (*Psetta maxima*) in the Baltic Sea. *Mol. Ecol*. 16: 115–126.

Foll M. & Gaggiotti O.E. 2006. Identifying the environmental factors that determine the genetic structure of populations. *Genetics* 174: 875–891.

Fotlz D.W., Hrincevich A.W. & Rocha-Olivares A. 2004. Apparent selection intensity for the cytochrome oxidase subunit I gene varies with mode of reproduction in echinoderms. *Genetica* 122: 115–125.

Fraser C.I., Nikula R. & Waters J.M. 2011. Oceanic rafting by a coastal community. *Proc. R. Soc. B*. 278: 649–655.

Gaggiotti O.E., Bekkevold D., Jørgensen H.B.H., Foll M., Carvalho G.R., André C. & Ruzznate D.E. 2009. Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of

natural populations: atlantic herring as a case study. *Evolution* 63-11: 2939–2951.

Galarza J.A., Carreras-Carbonell J., Macpherson E. et al. 2009. The influence of oceanographic fronts and early-life-history traits on connectivity among littoral fish species. *Proc. Natl. Acad. Sci. USA*. 106: 1473–1478.

Gerlach G., Jueterbock A., Kraemer P., Deppermann J. & Harmand P. 2010. Calculations of population differentiation based on G(ST) and D: forget G(ST) but not all of statistics!. *Mol. Ecol.* 19: 3845–52.

Giard A. 1905. La poecilogony. *Compte-Rendu des Séances du Sixième Congrès International de Zoologie Berne*. 1904: 617–646.

Gibson G.D. 1997. Variable development in the spionid *Boccardia proboscidea* (Polychaeta) is linked to nurse egg production and larval trophic mode. *Invertebr. Biol.* 116: 213–226.

Gibson G.D. & Gibson J.F. 2004. Heterochrony and the evolution of poecilogony: generating larval diversity. *Evolution* 58: 2704–2717.

Gibson G., MacDonald K. & Dufton M. 2010. Morhogenesis and phenotypic divergence in two developmental morphs of *Streblospio benedicti* (Annelida, Spionidae). *Invertebr. Biol.* 129: 328–343.

Gibson G., Paterson G.I., Taylor H. & Woolridge H. 1999. Molecular and morphological evidence of a single species, *Boccardia proboscidea* (Polychaeta: Spionidae) with multiple developmental modes. *Mar. Biol.* 134: 743–751.

Goetze E. 2003. Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. *Proc. R. Soc. Lond. B* 270: 2321–2331.

Goldson A.J., Hughes R.N. & Gliddon C.J. 2001. Population genetic consequences of larval dispersal mode and hydrography: a case study with bryozoans. *Mar. Biol.* 138: 1037–1042.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www.unil.ch/izea/softwares/fstat.html.

Grapputo A. 2006. Development and characterization of microsatellite markers in the Colorado potato beetle, *Leptinotarsa decemlineata. Mol. Ecol. Notes* 6: 1177–1179.

Grassle J.F. & Grassle J.P. 1974. Opportunistic life histories and genetic systems in marine benthic polychaetes. *J. Mar. Res.* 32: 253–284.

Gudmundsson H. 1985. Life history patterns of polychaete species of the family Spionidae. *J. Mar. Bio. Assoc. UK* 65: 93–111.

Guillot G., Mortier F. & Estoup A. 2005. Geneland: A program for landscape genetics. *Mol. Ecol. Notes* 5: 712–715.

Guindon S. & Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52: 696–704.

Hannerz L. 1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil and Poecilochaetidae N. fam. in the Gullmar Fjord (Sweden). *Zool. Bidr. Upps.* 31:1–204.

Harpending H. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum. Biol*. 66: 591–600.

Hart M.W. & Marko P.B. 2010. It's about time: divergence, demography, and the evolution of developmental modes in marine invertebrates. *Integ. Comp. Biol*. 50: 643–661.

Hart M.W., Byrne M. & Smith M.J. 1997. Molecular phylogenetic analysis of life-history evolution in asterinid starfish. *Evolution* 51: 1848–1861.

Havenhand J.N. 1993. Egg to juvenile period, generation time, and the evolution of larval type in marine invertebrates. *Mar. Ecol. Prog. Ser.* 97: 247–260.

Havenhand J.N. 1995. Evolutionary ecology of larval types. In: McEdward L. (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton Florida. pp.79–122.

Heath D.D., Busch C., Kelly J. & Atagi D.Y. 2002. Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). *Mol. Ecol.* 11: 197–214.

Hebert P.D.N., Ratnasingham S. & deWaard J.R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond*. B 270:S96–S99.

Hedgecock D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont A.R. (ed) Genetic and Evolution of Aquatic Organisms. Chapman & Hall, London, UK. pp. 122–134.

Hedgecock D. & Pudovkin A.I. 2011. Sweepstakes reproductive success in highly fecund marine fish and shellfish: A review and commentary. *B. Mar. Sci*. 87: 971–1002.

Hellberg M.E. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. *Evolution* 50: 1167–1175.

Hemmer-Hansen J., Nielsen E.E., Grønkjær P. & Loeschcke V. 2007. Evolutionary mechanisms shaping the genetic populations structure of marine fishes; lessons from the European flounder (*Platichthys flesus* L.). *Mol. Ecol.* 16: 3104–3118.

Hoagland K.E. & Robertson R. 1988. An assessment of poecilogony in marine invertebrates: phenomenon or fantasy? *Biol. Bull*. 174: 109–125.

Hoskin M.G. 1997. Effects of contrasting modes of larval development on the genetic structures of populations of three species of prosobranch gastropods. *Mar. Biol*. 127: 647–656.

Jablonski D. & Lutz R.A. 1983. Larval ecology of marine benthic invertebrates. Paleobiological implications. *Biol. Rev.* 58: 21–89.

Jeffery C.H., Emlet R.B. & Littlewood D.T.J. 2003. Phylogeny and evolution of development mode in temnopleurid echinoids. *Mol. Phylogenet. Evol*. 28: 99–118.

Johannesson K. 1988. The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)? *Mar. Biol.* 99: 507–513.

Johannesson K. & André C. 2006. Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. *Mol. Ecol.* 15: 2013–2029.

Johnson M.S. & Black R. 1982 Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. *Mar. Biol*. 70: 157–164.

Johnson M.S. & Black R. 1984. Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution* 38: 1371–1383.

Johnson M.S. & Black R. 2006. Islands increase genetic subdivision and disrupt patterns of connectivity of intertidal snails in a complex archipelago. *Evolution* 60: 2498–2506.

Jolly M.T, Jollivet D., Gentil F., Thi´ebaut E. & Viard F. 2005. Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the north coast of France. *Heredity* 94: 23–32.

Jorde P.E. & Ryman N. 2007. Unbiased estimator for genetic drift and effective population size. *Genetics* 177: 927–935.

Kalinowski S.T. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol. Ecol. Notes* 5: 187–189.

Kelly R.P. & Palumbi S.R. 2010. Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. *PLoS ONE* 5:e8594.

Kenchington E.L., Patwary M.U., Zouros E. & Bird C.J. 2006. Genetic differentiation in relation to marine landscape in a broadcast-spawning bivalve mollusc (*Placopecten magellanicus*). *Mol. Ecol.* 15: 1781–1796.

Knott K.E. & McHugh D. 2012. Introduction to symposium: poecilogony- a window on larval evolutionary transitions in marine invertebrates. *Integ. Comp. Biol.* doi: 10.1093/icb/ics037

Knowlton N. 1993. Sibling species in the sea. *Annu. Rev. Ecol. Syst.* 24: 189–216.

Knutsen H., André C., Jorde P.E., Skogen M.D., Thuróczy E. & Stenseth N.C. 2004. Transport of North Sea cod larvae into the Skagerrak coastal populations. *Proc. R. Soc. Lond. B* 271: 1337–1344.

Krug P.J. 1998. Poecilogony in an estuarine opisthobranch: planktotrophy, lecithotrophy, and mixed clutches in a population of the sacoglossan *Alderia modesta*. *Mar. Biol.* 132: 483–494.

Krug P.J. 2007. Poecilogony and larval ecology in the gastropod genus *Alderia*. *Am. Malacol. Bull.* 23: 99–111.

Krug P.J. 2009. Not my "type": larval dispersal dimorphism and bet-hedging in opisthobranch life histories. *Biol. Bull.* 216: 355–372.

Krug P.J. & Zimmer R.K. 2000. Developmental dimorphism and expression of chemosensory-mediated behavior: habitat selection by a specialist marine herbivore. *J. Exp. Biol.* 203: 234–244.

Kube J. & Powilleit M. 1997. Factors controlling the distribution of *Marenzelleria* cf. *viridis*, *Pygospio elegans* and *Steblospio shrubsoli* (Polychaeta: Spionidae)

in the southern Baltic Sea, with special attention for the response to an event of hypoxia. *Aquat. Ecol*. 31: 187–198.

Kyle C.J. & Boulding E.G. 2000. Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. *Mar. Biol.* 137: 835–845.

Larmuseau M.H.D., Vancampenhout K., Raeymaekers J.A.M., Van Houdt J.K.J. & Volckaert F.A.M. 2010. Differential modes of selection on the rhodopsin gene in coastal Baltic and North Sea populations of the sand goby, *Pomatoschistus minutes*. *Mol. Ecol.* 19: 2256–2268.

Lee H.J. & Boulding E.G . 2007. Mitochondrial DNA variation in space and time in the northeastern Pacific gastropod, *Littorina keenae. Mol. Ecol.* 16: 3084–3103.

Lee H.J. & Boulding E.G. 2009. Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on variation at one mitochondrial and two nuclear DNA markers. *Mol. Ecol.* 18: 2165–2184.

Levin L. 1984. Multiple patterns of development in *Streblospio benedicti* Webster (Spionidae) from three coasts of North America. *Biol. Bull*. 166:494–508.

Levin L.A. 1986. The influence of tides on larval availability in shallow waters overlying a mudflat. *B. Mar. Sci.* 39: 224–233.

Levin L.A. 2006. Recent progress in understanding larval dispersal: new directions and digressions. *Integr. Comp. Biol.* 46: 282–297.

Levin L.A. & Bridges S.T. 1995. Pattern and diversity in reproduction and development. In: McEdward L. (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton Florida. pp.1–48.

Levin L.A. & Hugget D.V. 1990. Implications of alternative reproductive modes for seasonality and demography in an estuarine polychaete. *Ecology* 71: 2191–2208.

Levin L.A., Zhu J. & Creed E. 1991. The genetic basis of life-history characteristics in a polychaete exhibiting planktotrophy and lecithotrophy. *Evolution* 45: 380–397.

Levin L.A., Caswell H., DePatra K.D. & Creed E.L. 1987. Demographic consequences of larval development mode: planktotrophy vs. lecithotrophy in *Streblospio benedicti*. *Ecology* 68: 1877–1886.

Levitan D.R. 1996. Effects of gamete traits on fertilisation in the sea and the evolution of sexual dimorphism. *Nature* 382: 153–155.

Levitan D.R. 2000. Optimal egg size in marine invertebrates: theory and phylogenetic analysis of the critical relationship between egg size and developmental time in echinoids. *Am. Nat.* 156: 175–192.

Luttikhuizen P.C. & Dekker R. 2010. Pseudo-cryptic species *Arenicola defodiens* and (Polychaeta: Arenicolidae) in Wadden Sea, North Sea and Skagerrak: morphological and molecular variation. *J. Sea Res.* 63: 17–23.

Marko P.B., Rogers-Bennett L. & Dennis A.B. 2007. MtDNA population structure and gene flow in lingcod (*Ophiodon elongatus*): limited connectivity despite long-lived pelagic larvae. *Mar. Biol.* 150: 1301–1311.

Marshall D.J. & Keough M.J. 2009. Does interspecific competition affect offspring provisioning? *Ecology* 90: 487–495.

Martel A. & Chia F.S. 1991. Drifting and dispersal of small bivalves and gastrods with direct development. *J. Exp. Mar. Biol. Ecol.* 150: 131–147.

Mattila J. 1997. The importance of shelter, disturbance and prey interactions for predation rates of tube-building polychaetes (*Pygospio elegans* (Claparede)) and free-living tubificid oligochaetes. *J. Exp. Mar. Biol. Ecol.* 218: 215–228.

McEdward L.R. & Janies A.D. 1993. Life cycle evolution in asteroids: what is a larva? *Biol. Bull*. 184: 255–268.

McHugh D. & Rouse G.W. 1998. Life history evolution of marine invertebrates: new views from phylogenetic systematics. *Trends. Ecol. Evol.* 13: 182–186.

Metaxas A. 2001. Behaviour in flow: perspectives on the distribution and dispersion of meroplanktonic larvae in the water column. *Can. J. Fish. Aquat. Sci.* 58: 86–98.

Mileikovsky S.A. 1971. Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. *Mar. Biol.* 10: 193–213.

Miller K.J. & Ayre D.J. 2008. Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. *J. Anim. Ecol.* 77: 713–24.

Moberg P.E. & Burton R.S. 2000. Genetic heterogeneity among adult and recruit red sea urchins, *Strongylocentrotus franciscanus*. *Mar. Biol.* 136: 773–784.

Morgan T.S. 1995. Life and death in the plankton: larval mortality and adaptation. In: McEdward L. (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton Florida. pp.279–322.

Morgan T.S. 1997. The formation and dynamics of *Pygospio elegans* tube-beds in the Somme Bay, France. Dissertation, University of Southampton. (UoS Thesis Supply).

Morgan T.S., Rogers D.A., Paterson L.G.J., Hawkins E.L. & Sheader M. 1999. Evidence for poecilogony in *Pygospio elegans* (Polychaeta: Spionidae). *Mar. Ecol. Prog. Ser.* 178: 121–132.

Muus B.J. 1967. The fauna of Danish estuaries and lagoons. Distribution and ecology of dominating species in the shallow reaches of the mesohaline zone. *Meddr Danm. Fisk.- og Havunders.* 5: 3–316.

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.

Nissling A. & Dahlman G. 2010. Fecundity of flounder, *Pleuronectes flesus*, in the Baltic Sea—Reproductive strategies in two sympatric populations. *J. Sea Res.* 64: 190–198.

Nygren A. & Pleijel F. 2011. From one to ten in a single stroke—resolving the European *Eumida sanguine* (Phyllodocidae, Annelida) species complex. *Mol. Phylogenet. Evol.* 58: 132–141.

O'Connor M.I., Bruno J.F., Gaines S.D., Halpern B.S., Lester S.E., Kinlan B.P. et al. 2007. Temperature control of larval dispersal and the implications for

marine ecology, evolution, and conservation. *Proc. Natl. Acad. Sci. USA*. 104: 1266–1271.

Østergaard S., Hansen M.M., Loeschcke V. & Nielsen E.E. 2003. Long-term temporal changes of genetic composition in brown trout (*Salmo trutta* L.) populations inhabiting an unstable environment. *Mol. Ecol.* 12: 3123–3135.

Oyarzun F.X., Mahon R.A., Swalla J.B. & Halanych M.K. 2011. Phylogeography and reproductive variation of the poecilogonous polychaete *Boccardia proboscidea* (Annelida: Spionidae) along the West Coast of North America. *Evol. Dev.* 13: 489–503.

Palmer A.R. & Strathmann R.R. 1981. Scale of dispersal in varying environments and its implications for life histories of marine invertebrates. *Oecologia* 48: 308–318.

Palumbi S.R. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annu. Rev. Ecol. Syst.* 25: 547–572.

Peakall R. & Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* 6: 288–295.

Pearson T.H. & Rosenberg R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr. Mar. Biol. Annu. Rev.* 16: 229–311.

Pechenik J.A. 1979. Role of encapsulation in invertebrate life histories. *Am. Nat.* 114: 859–870.

Pechenik J.A. 1999. On the advantages and disadvantages of larval stages in benthic invertebrate life cycles. *Mar. Ecol. Prog. Ser*. 177: 269–297.

Pechenik JA. 2006. Larval experience and latent effects— metamorphosis is not a new beginning. *Integr. Comp. Biol*. 46: 323–333.

Pechenik J.A. & Levine S.H. 2007. Estimates of plantonic larval mortality using the marine gastropods *Crepidula fornicata* and *C. plana. Mar. Ecol. Prog. Ser.* 344: 107–118.

Pechenik J.A. Blanchard M. & Rotjan R. 2004. Susceptibility of larval *Crepidula fornicata* to predation by suspension-feeding adults. *J. Exp. Mar. Biol. Ecol.* 306: 75–94.

Pedersen T.M., Hansen J.L.S., Josefson A.B. & Hansen B.W. 2008. Mortality through ontogeny of soft-bottom marine invertebrates with planktonic larvae. *J. Marine. Syst.* 73: 185–207.

Pernet B. & McArthur L. 2006. Feeding by larvae of two different development modes in *Streblospio benedicti* (Polychaeta: Spionidae). *Mar. Biol.* 149: 803–811.

Pernet B. & McHugh D. 2010. Evolutionary changes in the timing of gut morphogenesis in larvae of the marine annelid *Streblospio benedicti*. *Evol. Dev.* 12: 618-627.

Pineda J., Hare J.A. & Sponaugle S. 2007. Larval transport and dispersal in the coastal ocean and consequences for population connectivity. *Oceanography* 20: 22–39.

Piry S., Alapetite A., Cornuet J.M., Paetkau D., Baudouin L. & Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. *J. Hered*. 95: 536–539.

Planes S. & Lenfant P. 2002. Temporal change in the genetic structure between and within cohorts of a marine fish, *Diplodus sargus,* induced by a large variance in individual reproductive success. *Mol. Ecol.* 11: 1515–1524.

Porter J.S., Ryland S.J. & Carvalho R.G. 2002. Micro- and macrogeographic genetic structure in bryozoans with different larval strategies. *J. Exp. Mar. Biol. Ecol.* 272: 119–130.

Pritchard J.K., Stephens M. & Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Qian P.Y., Rittschof D. & Sreedhar B. 2000. Macrofouling in unidirectional flow: miniature pipes as experimental models for studying the interaction of flow and surface characteristics on the attachment of barnacle, bryozoan and polychaete larvae. *Mar. Ecol. Prog. Ser.* 207: 109–121.

Raff R.A. & Byrne M. 2006. The active evolutionary lives of echinoderm larvae. *Heredity* 9: 244–252.

Ramos-Onsins S.E. & Rozas J. 2002. Statistical properties of new neutrality tests against population growth. *Mol. Biol. Evol*. 19: 2092–2100.

Rasmussen E. 1953. Asexual reproduction in *Pygospio elegans* Claparede (*Polychaeta sedentaria*). *Nature* 171: 1161–1162.

Rasmussen E. 1973. Systematics and ecology of the Isefjord marine fauna (Denmark). *Ophelia* 11: 1–495.

Robainas-Barcia A., Espinosa López G., Hernández D. & García-Machado E. 2005. Temporal variation of the population structure and genetic diversity of *Farfantepenaeus notialis* assessed by allozyme loci. *Mol. Ecol*. 14: 2933–2942.

Ronquist F. & Huelsenbeck P.J. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Rouse G.W. 2000. Polychaetes have evolved feeding larvae numerous times. *B. Mar. Sci.* 67: 391-409.

Rozas J., Sánchez-DelBarrio J.C., Messeguer X. & Rozas R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496–2497.

Rozen S. & Skaletsky H.J. 2000. PRIMER 3 on the WWW for general users and for biologist programmers. In: Bioinformatics Methods and Protocols: Methods in Molecular Biology, Humana Press, Totowa, New Jersey, pp 365–386.

Rumrill S.S. 1990. Natural mortality of marine invertebrate larvae. *Ophelia*. 32: 163–198.

Schuelke M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nat. Biotechnol*. 18: 233–234.

Schulze S.R., Rice A.S., Simon L.J. & Karl A.S. 2000. Evolution of poecilogony and the biogeography of North American populations of the polychaete *Steblospio*. *Evolution* 54: 1247–1259.

Selkoe K.A., Watson J.R., White C., Horin T.B., Iacchei M., Mitarai S., Siegel D.A., Gaines S.D. & Toonen R.J. 2010. Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Mol. Ecol.* 19: 3708–3726.

Shanks A.L. 2009. Pelagic larval duration and dispersal distance revisited. *Biol. Bull.* 216: 373–385.

Söderström A. 1920. Studien uber die Polychaeten familie Spionidae. Dissertation. University of Uppsala, Sweden.

Sotka E.E., Wares J.P., Barth J.A., Grosberg R. & Palumbi S.R. 2004. Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. *Mol. Ecol.* 13: 2143–2156.

Sponaugle S., Cowen R.K., Shanks A., Morgan S.G., Leis J.M., Pineda J., Boehlert G.W., Kingsford M.J., Lindeman K.C., Grimes C., et al. 2002. Predicting self-recruitment in marine populations: Biophysical correlates and mechanisms. *B. Mar. Sci.* 70: 341–375.

Stearns S.C. 1992. The evolution of life histories. Oxford, Oxford University Press.

Strathmann R.R. 1978. The evolution and loss of feeding larval stages in marine invertebrates. *Evolution* 32: 899–906.

Strathmann R.R. 1985. Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. *Ann. Rev. Ecol. Syst.* 16: 339–361.

Strathmann R.R. 1993. Hypotheses on the origins of marine larvae. *Ann. Rev. Ecol. Syst.* 24: 89–117.

Strathmann R.R., Hughes T.P, Kuris A.M., Lindeman K.C., Morgan S.G., Pandolfi J.M. & Warner R.R. 2002. Evolution of local recruitment and its consequences for marine populations. *B. Mar. Sci.* 70: 377–386.

Swearer S.E., Shima J.S., Hellberg M.E., Thorrold S.R., Jones G.P., Robertson D.R., Morgan S.G., Selkoe K.A., Ruiz G.M. & Warner R.R. 2002. Evidence of self-recruitment in demersal marine populations. *B. Mar. Sci.* 70: 251–271.

Tamura K., Dudley J., Nei M. & Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol*. 24: 1596–1599.

Taylor M.S. & Hellberg M.E. 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. *Science* 299: 107–109.

Tessier N. & Bernatchez L. 1999. Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.). *Mol. Ecol.* 8: 169–179.

Thorrold S.R., Jones G.P., Hellberg M.E., Burton R.S., Swearer S.E., Neigel J.E., Morgan S.G. & Warner R.R. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. *B. Mar. Sci.* 70: S291–308.

Thorson G. 1946. Reproduction and larval development of Danish marine bottom invertebrates. *Medd Dan Fisk- Havunders* 4: 1-523.

Thorson G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* 25: 1–45.

Turner E.J., Zimmer-Faust M.A., Palmer M.A., Luckenbach M. & Pentcheff N.D. 1994. Settlement of oyster *Crassostrea virginica* larvae: effects of water flow and a water-soluble chemical cue. *Limnol. Oceanogr.* 39: 1579–1593.

Vance R.R. 1973. On reproductive strategies in marine benthic invertebrates. *Am. Nat.* 107: 339–352.

Van Oosterhout C., Hutchinson W.F., Wills D.P.M. & Shipley P. 2004. Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4: 535–538.

Virgilio M. & Abbiati M. 2006. Temporal changes in the genetic structure of intertidal populations of *Hediste diversicolor* (Polychaeta: Nereididae). *J. Sea Res.* 56: 53–58.

Wang J. & Whitlock M.C. 2003. Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* 163: 429–446.

Waples R.S. 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* 121: 379–391.

Warner R.R. & Cowen R.K. 2002. Open vs. closed marine populations: synthesis and analysis of the evidence. *Bull. Mar. Sci.* 70: 245–296.

Warner R.R. & Palumbi S.R. 2003. Larvae retention: genes or oceanography? *Science* 300: 1658.

Watts P.C. & Thorpe J.P. 2006. Influence of contrasting larval developmental types upon the population-genetic structure of cheilostome bryozoans. *Mar. Biol.* 149: 1093–1101.

Watts P.C., Thorpe J.P. & Taylor P.D. 1998. Natural and anthropogenic dispersal mechanisms in the marine environment: a study using cheilostome Bryozoa. *Phil. Trans. R Soc. Lond. B Biol. Sci.* 353: 453–464.

Warwick R.M. 1986. A new method for detecting pollution effects on marine macrobenthic communities. *Mar. Biol.* 92: 557–562.

Weersing K. & Toonen J.R. 2009. Population genetics, larval dispersal, and connectivity in marine systems. *Mar. Ecol. Prog. Ser.* 393: 1–12.

White C., Selkoe K.A., Watson J., Siegel D.A., Zacherl D.C. & Toonen R.J. 2010. Ocean currents help explain population genetic structure. *Proc. R. Soc. B* 277: 1685–1694.

Whitlatch R.B. & Zajac R.N. 1985. Biotic interactions among estuarine infaunal opportunistic species. *Mar. Ecol. Prog. Ser.* 21: 299–311.

Wilson W. H. 1991. Sexual reproductive modes in polychaetes: classification and diversity. *B. Mar. Sci.* 48: 500–516.

Wilson G.A. & Rannala B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* 163: 1177–1191.

Wray G.A. 1995. Evolution of larvae and developmental modes. In: McEdward L. (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton, Florida. pp. 413–448.

Wray G.A. & Raff R.A. 1991. The evolution of developmental strategy in marine invertebrates. *Trends Ecol. Evol.* 6: 45–50.

Zane L., Bargelloni L. & Patarnello T. 2002. Strategies for microsatellite isolation: a review. *Mol. Ecol.* 11: 1–16.

Zhan A., Hu J., Hu X., Zhou Z., Hui M., Wang S., Peng W., Wang M. & Bao Z. 2009. Fine-scale population genetic structure of Zhikong Scallop (*Chlamys farreri*): do local marine currents drive geographical differentiation? *Mar. Biotechnol*. 11: 223–235.

ORIGINAL PAPERS

I

PHYLOGENETIC ANALYSIS OF CRYPTIC SPECIATION IN THE POLYCHAETE *PYGOSPIO ELEGANS*

by

Jenni Kesäniemi, Sara M. Lindsay, Paul D. Rawson & K. Emily Knott 2012
Ecology and Evolution 2: 994–1007.

Ecology and Evolution

Open Access

Phylogenetic analysis of cryptic speciation in the polychaete *Pygospio elegans*

J. E. Kesäniemi[1], P. D. Rawson[2], S. M. Lindsay[2] & K. E. Knott[1]

[1]Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014, Finland
[2]School of Marine Sciences, University of Maine, Orono, Maine 04469-5751

Keywords
COI, developmental mode, larvae, population structure.

Correspondence
Jenni E. Kesäniemi, Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014, Finland.
Tel: +358 40 805 3872;
E-mail: jenni.kesaniemi@jyu.fi

Funded by a grant from the Jenny and Antti Wihuri Foundation and the Centre of Excellence in Evolutionary Research (University of Jyväskylä). K. E. K. acknowledges fruitful discussions with members of the Marie Curie Initial Training Network Speciation funded by the European Union.

Received: 26 October 2011; Revised: 16 January 2012; Accepted: 19 January 2012

***Ecology and Evolution* 2012; 2(5): 994–1007**

doi: 10.1002/ece3.226

Abstract

Development in marine invertebrate species can take place through a variety of modes and larval forms, but within a species, developmental mode is typically uniform. Poecilogony refers to the presence of more than one mode of development within a single species. True poecilogony is rare, however, and in some cases, apparent poecilogony is actually the result of variation in development mode among recently diverged cryptic species. We used a phylogenetic approach to examine whether poecilogony in the marine polychaete worm, *Pygospio elegans*, is the result of cryptic speciation. Populations of worms identified as *P. elegans* express a variety of developmental modes including planktonic, brooded, and intermediate larvae; these modes are found both within and among populations. We examined sequence variation among partial mitochondrial cytochrome c oxidase subunit I sequences obtained for 279 individual worms sampled across broad geographic and environmental scales. Despite a large number of unique haplotypes (121 haplotypes from 279 individuals), sequence divergence among European samples was low (1.7%) with most of the sequence variation observed within populations, relative to the variation among regions. More importantly, we observed common haplotypes that were widespread among the populations we sampled, and the two most common haplotypes were shared between populations differing in developmental mode. Thus, our results support an earlier conclusion of poecilogony in *P elegans*. In addition, predominantly planktonic populations had a larger number of population-specific low-frequency haplotypes. This finding is largely consistent with interspecies comparisons showing high diversity for species with planktonic developmental modes in contrast to low diversity in species with brooded developmental modes.

Introduction

Most marine invertebrates have complex life cycles and show a diverse range of larval developmental modes. Developmental mode is often defined as discrete categories describing characteristics of larvae, or larval types (Levin and Bridges 1995). For example, larvae can be planktonic (pelagic) or benthic, feeding or nonfeeding, brooded or free-living, and a combination of multiple descriptors is often necessary for a complete definition of developmental mode (e.g., McEdward and Janies 1993; Collin 2003; Raff and Byrne 2006). Developmental mode is an important aspect of invertebrate life histories, with wide-ranging consequences affecting, for example, development time, mortality, and dispersal potential (Levin and Bridges 1995). Understanding the consequences and evolution of different developmental modes is, on one hand, aided by our tendency to categorize it as discrete types. On the other hand, such definitions may also lead us to overlook intermediate or facultatively varying forms that do not fit definitions of discrete developmental modes (Allen and Pernet 2007).

Many different developmental modes may be observed within genera or larger taxonomic groups, but typically only one developmental mode exists within a single species. In rare cases, species may express two or more development modes. The term poecilogony (Giard 1905 cited in Krug

2009) has been used to describe such developmental mode polymorphism. In poecilogonous species, multiple developmental modes are observed, either within or among different populations of a single species. True poecilogony has been documented within spionid worms (e.g., *Streblospio benedicti*, Levin 1984, and *Boccardia proboscidia*, Gibson 1997; Oyarzun et al. 2011) and in sacoglossan sea slugs (reviewed in Krug 2007, 2009). However, in a number of cases, what were originally described as poecilogonous species have turned out to be morphologically cryptic species with species-specific developmental modes (see Hoagland and Robertson 1988). The rarity of true poecilogony has led some authors to suggest that there are costs associated with polymorphic development and that poecilogony is a transient stage of speciation co-occurring with developmental mode transitions (Gibson and Gibson 2004; Ellingson and Krug 2006). Alternatively, poecilogony might be an advantageous plastic response, and a potential bet-hedging strategy, to enhance offspring success in the face of changing environmental conditions (Krug 2007).

One possible poecilogonous species is *Pygospio elegans* Claparède, a small, sedentary, tube-building spionid polychaete worm, widely distributed in the northern hemisphere (Muus 1967; Anger 1984). After internal fertilization (Hannerz 1956), females deposit embryos and yolky nurse eggs in capsules inside the maternal tube. Different larvae emerge from the capsules depending on the relative number of embryos and nurse eggs laid by the mother; there are no initial differences in embryo size (Söderström 1920; Hannerz 1956; Rasmussen 1973; Anger et al. 1986; Blake and Arnofsky 1999, pers. obs.). Here, we define planktonic larvae as those that emerge when they are 3-setigers long (typically >20 embryos laid per capsule with few or no nurse eggs). The larvae develop long swimming setae and actively swim and feed in the water column (Hannerz 1956). Brooded larvae, on the other hand, do not have swimming setae and remain inside the capsules for a longer period subsisting only on nurse eggs (typically one to two embryos laid per capsule, Fig. 1). These larvae lack a pelagic phase during development and metamorphose into juveniles soon after their emergence from the capsules at 14–20 setigers. An intermediate type of larva also occurs (4–10 embryos laid per capsule; Hannerz 1956, pers. obs.). After emergence at approximately 10 setigers, these larvae have a short pelagic phase. Despite their differences, all larval types metamorphose into morphologically and ecologically identical adults.

Monitoring reproduction in *P. elegans* is laborious and has been done exhaustively in only a few populations. There have been some observations of different larval forms simultaneously within a single population (Rasmussen 1973; Gudmundsson 1985, pers. obs.), providing some evidence that *P. elegans* is a true poecilogonous species. However, whether or not a single individual can produce multiple larval types is not clear (but, see Fig. 30 in Rasmussen 1973). Hannerz (1956) and Rasmussen (1973) hypothesized that developmental mode polymorphism in *P. elegans* is in fact variation within a single developmental mode, reflecting plastic responses to environmental variation. This hypothesis was based on observations that in some populations different larvae are produced seasonally. However, neither simultaneous nor seasonal production of different larvae in a single population is universal. More commonly, among population differences in developmental mode are noted, and some populations have even been considered "fixed" for a particular developmental mode since no other modes have been observed during repeated sampling from these populations (Anger 1984; Morgan et al. 1999; Bolam 2004, pers. obs.). The presence of "fixed" populations differing in developmental mode raises suspicion that cryptic species may be present. This suspicion was strengthened when Anger (1984) found that experimental exposure of worms from several "fixed" populations to different salinities and temperatures did not induce a change in developmental mode. No correlations between other environmental variables and developmental mode have been noted in the literature, but few experimental tests have been performed. Changes in density and food supply did not induce changes in developmental mode in *P. elegans* collected from Somme Bay, France (Morgan 1997), but in North America, low density has apparently increased the frequency of asexual reproduction in *P. elegans* (Wilson 1983).

To clarify the species status of *P. elegans* populations, Morgan and colleagues (1999) examined population

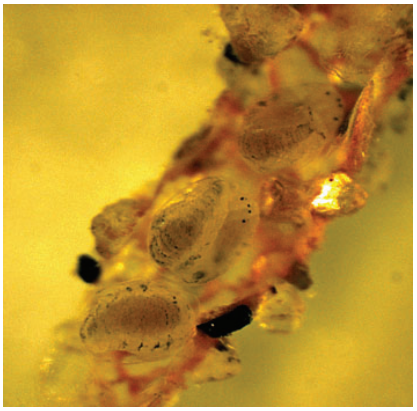


Figure 1. Brooded *Pygospio elegans* larvae in capsules (from Ängsö, Finland). The capsules (approx. 0.5 mm long, each containing one to two larvae) are visible after breaking down the sand tube. Photo credit: Jenni Kesäniemi.

structure among four potentially "fixed" populations in the English Channel differing in developmental mode. They found high genetic similarity and potentially high gene flow among the *P. elegans* populations, and concluded that the species is poecilogonous. Nevertheless, due to the limited scope of their study and the rarity of poecilogony, the question of poecilogony versus cryptic speciation still remains. We addressed this question by surveying variation in a portion of the mitochondrial gene cytochrome c oxidase subunit I using haplotype network and phylogenetic methods, and using a DNA sequence-based criterion advocated in DNA barcoding studies to assess the presence of cryptic species. Our samples covered both a broad geographical area and a range of environmental conditions. For some populations, there were also data available regarding the predominant developmental mode among individuals. The large dataset also allowed us to investigate within-population diversity in our study populations. We hypothesized that *P. elegans* is indeed a poecilogonous species, despite apparent divergence of populations in developmental mode.

Materials and Methods

Sample collection and molecular methods

Adult *P. elegans* were collected between 2007 and 2010 from 14 locations in Europe (Fig. 2) and three locations in the United States (east coast: Maine and west coast: Washington). In Europe, populations from the Baltic Sea (Finland, Germany, Denmark, Sweden), Wadden Sea (the Netherlands, Schiermonnikoog Island), North Sea (Edinburgh, UK), the English Channel (Plymouth, UK, and Somme Bay, France), White Sea (Russia), and the North Atlantic Ocean (Iceland) were sampled (Fig. 2, Table 1). Several colleagues enabled the collecting effort (see Acknowledgements). At most locations, the samples were collected from the shallow intertidal zone (0.1–1 m). The two samples from the Finnish archipelago (Ängsö and Fårö) were collected by scuba from 2–5 m deep water. Samples from Germany were collected from 18-m depth.

At the time of collecting, the adult worms and sand tubes were examined for signs of larvae or egg capsules and then preserved in ethanol (94–99%). Using these observations, and information from previous studies of *P. elegans*' reproduction and development (i.e., Rasmussen 1973; Morgan et al. 1999; Bolam 2004), we characterized the sampling locations by the different larval developmental modes observed (Table 1). This characterization is tentative, since we were unable to survey all populations exhaustively, but represents our best knowledge of the predominant developmental mode in the populations. Additional sampling at the same sites has confirmed our characterization of developmental mode (pers. obs.) but at some sites we have not observed any signs of sexual reproduction and so a predominant developmental mode is not known.

From the European samples, genomic DNA was extracted using the DNeasy Blood and Tissue extraction kit (Qiagen, Germany) and a KingFisher magnetic processor (ThermoScientific, MA, USA). A 600-bp fragment of the cytochrome c oxidase subunit I (COI) gene was amplified using species-specific primers (PeCox1 F 5′ – TAT AGG CCT TTG ATC AGG AAC – 3′, PeCox1 R 5′ – AGG GTC TCC GCC TCC TGT – 3′). Polymerase chain reactions (PCRs) were performed in 20 μL reactions containing 1 μL of the DNA extract, 3 mM $MgCl_2$ (Biotools, Spain), 200 μM of each dNTP (Fermentas, Germany), 0.5 μM of each primer (TAG Copenhagen, Denmark), 0.1 U of Taq polymerase, and 1 X of PCR Buffer (Biotools). Reaction conditions included an initial denaturation step at 94°C for 2 min, then 35 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 45 s, followed by a final extension at 72°C for 2 min. For sequencing, the PCR products were treated with Exonuclease I and Shrimp alkaline phosphatase (Fermentas), cycle sequenced in both directions using the BigDye v.3.1 kit, and visualized with an ABI 3130xl Genetic Analyzer and Sequencing Analysis v.5.2. software (all Applied Biosystems, CA, USA).

DNA extraction, amplification, and sequencing of the North American samples followed similar protocols, but sequencing artifacts at the 5′ end of the resulting sequences reduced the length of high-quality sequence reads for these samples. To be conservative, we analyzed a shorter fragment of the COI gene (567 bp) when North American samples were included. In analyses involving only the European samples, the 600-bp fragment was used.

Haplotype network and phylogenetic analyses

Sequences were aligned using the ClustalW option of MEGA 4 (Tamura et al. 2007). For these analyses, the 567-bp fragment of the COI gene was used and all individuals were included. To examine the relationship between the haplotypes, a minimum spanning network was constructed with Arlequin v.3.5.1.2. (Excoffier and Lischer 2010) and visualized with HapStar (Teacher and Griffiths 2010).

For phylogenetic analyses, a single representative of each haplotype was used. JModeltest (Posada 2008) was used to find the optimal model of sequence evolution for the COI data (selected using the Akaike information criterion, AIC). The general time reversible model with invariant positions and gamma-distributed rates (GTR + I + G) was selected and used in tree reconstruction. Sequence divergence was estimated with MEGA 4 using a gamma shape parameter of 0.637 (according to JModeltest) and the Tamura Nei

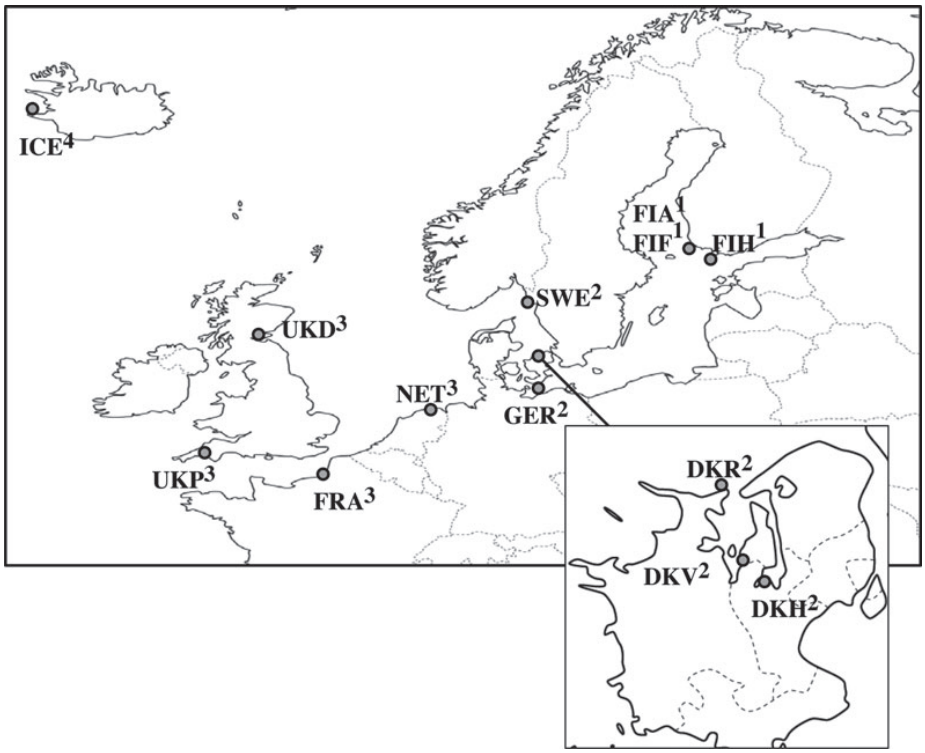


Figure 2. European sampling sites labeled according to their abbreviations in Table 1. Sites FIA (Ängsö) and FIF (Fårö) are located in the Finnish archipelago, approximately 20 km apart. Regional grouping of populations for the hierarchical AMOVA analysis are indicated with numbered superscripts: 1. Northern Baltic Sea: Finland, 2. Southern Baltic Sea: Denmark, Germany, Sweden, 3. North Sea + Wadden Sea + English Channel: UK, France, the Netherlands, and 4. North Atlantic Ocean: Iceland.

substitution model since the GTR model is not available in MEGA 4.

For tree reconstruction, we explored both maximum likelihood and Bayesian analyses. Bayesian analysis was conducted with MrBayes v.3.1.2. (Ronquist and Huelsenbeck 2003). MCMC (Markov Chain Monte Carlo) chains (one cold and three heated chains) were run for 4 million generations, trees were sampled every 100 generations, and 25% of the trees were discarded in the burnin. All parameters were estimated in the analysis. Posterior probabilities were used to assess clade support, with 80% used as the minimum cutoff. Maximum likelihood analysis was conducted with PhyML 3.0. (Guindon and Gascuel 2003). All parameters were estimated in the analysis except the gamma shape parameter, which was set to 0.637 according to the results from JModeltest. Bootstrap analysis with 1000 replicates provided an estimate of clade support, with 70% used as the minimum cutoff. After analysis, trees were rooted along the lineage leading to most of the North American haplotypes (also the longest branch). Trees were visualized using FigTree v.1.2.2. (http://tree.bio.ed.ac.uk/software/figtree/).

Analysis of genetic diversity

Our genetic diversity analyses focused on populations with sufficient sample sizes for making robust estimates, so the

Table 1. Sampling location information, population codes, diversity measurements, and observed larval modes of the populations. N = number of individuals in the genetic analysis, H = haplotype diversity, and π = nucleotide diversity. Box indicates the European populations with sufficient sample size used in diversity and demographic analyses as well as hierarchical analyses of population structure (AMOVA). Groups (regions) defined for AMOVA analysis are shaded.

Region	Location	Code	N	*No. of haplotypes	H	π	Observed larval mode
Europe							
Northern Baltic Sea	Ängsö, Finland	FIA	22	4	0.260	0.0017	Brooded
	Fårö, Finland	FIF	21	10	0.890	0.0129	Not known
	Hanko, Finland	FIH	19	6	0.778	0.0128	Not known
Southern Baltic Sea	Germany	GER	22	16	0.909	0.0118	Not known
	Vellerup, Denmark	DKV	20	12	0.916	0.0141	Brooded, intermediate
	Herslev, Denmark	DKH	20	11	0.916	0.0154	Brooded, intermediate
	Rorvig, Denmark	DKR	21	6	0.710	0.0116	Intermediate, planktonic
	Gullmarfjord, Sweden	SWE	21	5	0.633	0.0080	Not known
Wadden sea	the Netherlands	NET	23	14	0.822	0.0107	Intermediate, planktonic
North sea	Drum sands, UK	UKD	20	19	0.995	0.0130	Planktonic
English Channel	Somme Bay, France	FRA	23	22	0.996	0.0141	Planktonic
	Plym Bay, UK	UKP	24	20	0.975	0.0153	Planktonic
Atlantic Ocean	Iceland	ICE	20	3	0.511	0.0009	Brooded, intermediate
White Sea	Russia	RUS	3	1	0.000	0.0000	Not known
North America							
Atlantic (east)	Lubec, ME	NAE1	7	3	0.733	0.0360	Not known
	Lowe's cove, ME	NAE2	6	3	0.810	0.0210	Not known
Pacific (west)	False Bay, WA	NAW	7	1	0.000	0.0000	Not known

*Based on 600 bp COI fragment in European populations, but based on 567-bp fragment in North American populations.

Russian sample ($n = 3$) and the North American samples ($n = 6$–7) were excluded. In these analyses, the 600-bp fragment of the COI gene was used. Haplotype diversity and nucleotide diversity for each population were calculated with Arlequin v.3.5.1.2. (Excoffier and Lischer 2010), which was also used to estimate population structure (Φ_{ST}) via a hierarchical analysis of molecular variance (AMOVA). In the AMOVA analysis, sequences were grouped according to geographical regions (four groups: Northern Baltic Sea; Southern Baltic Sea; North Sea + Wadden Sea + English Channel; and North Atlantic Ocean; 10,000 permutations). Population structure was also investigated using BAPS 5.3 (Corander and Tang 2007), a Bayesian model-based clustering method that can use sequence data. In these analyses, the maximum number of clusters (K) was set from two to 13, and for each the analysis was run 10 times. In the end, the K with the highest likelihood was chosen to describe the samples.

Exploratory analyses tested whether differences in haplotype and nucleotide diversity measures were evident among the European populations with different developmental mode. Here, planktonic populations (UKP, UKD, FRA, see Table 1) were compared to populations that produce brooded or intermediate type larvae (FIA, DKV, DKH, DKR, NET, ICE). This comparison is contingent on our definition of predominant developmental mode (see Table 1), so populations where developmental mode is not known (FIF, FIH, GER, SWE, and RUS) were excluded. For these comparisons, Mann–Whitney U tests were performed using PASW Statistics 18 (SPSS, Inc., 2009, Chicago, IL, www.spss.com).

To assess if European populations (excluding RUS) have gone through a recent population expansion, Fu's Fs neutrality test was calculated. Fu's test (which is based on the haplotype distribution; Fu 1997) was used because it is thought to be better at revealing signs of population expansion than Tajima's D test (Fu 1997; Schneider and Excoffier 1999). Tajima's D (Tajima 1989) and Fu and Li's F (Fu and Li 1993) were also calculated to test for neutrality of the sequences. Mismatch distributions, the frequencies of observed pairwise differences between haplotypes, were calculated for each European population. Also R_2 (Ramos-Onsins and Rozas 2002) and raggedness statistics (rg, Harpending 1994) with confidence intervals based on coalescent simulations were calculated to detect expansion (10,000 permutations and theta estimated from the data were used in the coalescent simulations). Lower R_2 and rg values are expected for a population growth scenario (Harpending 1994; Ramos-Onsins and Rozas 2002). These analyses were performed in DnaSP 4.0 (Rozas et al. 2003).

Results

Polymorphism and haplotype diversity

A total of 279 *P. elegans* individuals from 14 European locations were sequenced. From this sample, 121 unique

haplotypes of the COI gene fragment (600 bp) were identified. An expanded dataset included 20 additional individuals from three North American locations for a total of 299 sequences, 567 bp in length, with 123 unique haplotypes (GenBank accession numbers JN033571–JN033693). The most common haplotype, EUNA10, was shared by 36 European individuals and was found in all three Danish populations, Iceland, Sweden, and Plym Bay in the UK (English Channel). This haplotype was also observed in worms sampled from both populations on the East Coast of the United States (NAE). The second most common haplotype, EU11, was found in Denmark, Finland, France, the Netherlands, and the White Sea, Russia (35 individuals). Note that both EUNA10 and EU11 were found in populations differing in developmental mode (see Table 1). These two most common haplotypes also were found within the whole sample range in Europe and comprise 25% of all individuals sequenced.

We observed a large number of low frequency haplotypes within locations in Europe. Out of 121 haplotypes, 98 were detected only once in the European dataset (from only one individual of the 279 sequenced). Ninety percent of the haplotypes (109 out of 121) were found in only one population (11 of these were found from more than one individual). Populations from the North Sea, English Channel, and Wadden Sea had the highest percentage of population-specific low-frequency haplotypes. The Baltic Sea populations (Finland, Denmark, Germany, Sweden) shared many haplotypes (seven out of 12 shared haplotypes are found only in the Baltic Sea), and only one haplotype (EU8) was shared exclusively among the three populations in the UK and France. In most populations, haplotype diversity was high (Table 1). However, two European populations had low diversity with most individuals sharing the same haplotype. In Ängsö, Finland, 19 of 22 individuals sampled (86%) shared an identical haplotype (EU6) and in the sample of 20 individuals from Iceland, 13 shared haplotype EU1 and six shared haplotype EUNA10. Overall, populations with predominantly planktonic larvae had higher haplotype diversity than populations that also produced other larval types ($N = 9$, $U = 0.000$, $z = -2.334$, $P = 0.020$). However, nucleotide diversity was not significantly different ($N = 9$, $U = 4.5$, $z = -1.167$, $P = 0.243$). Haplotype diversity in the North American samples was somewhat lower than in most of the European samples (Table 1), but North American sample sizes were also relatively small and so estimates of diversity from these populations may not be reliable.

Mean sequence divergence (Tamura Nei model) within the total European dataset was 1.7%. Divergence between the European and North American haplotypes was noticeably higher: 5.3% (or 6.1% when excluding EUNA10, the haplotype that is shared with the European samples). Mean sequence divergence within the total North American dataset was 3.1%, higher than what we observed from the European sample.

Haplotype network and phylogenetic analyses

Figure 3 shows the minimum spanning haplotype network as calculated in Arlequin. The low sequence divergence among haplotypes is reflected in the network and haplotypes from different populations are intermingled. Arlequin detected many alternative connections among the European haplotypes due to the low level of divergence between them, but graphing all possible alternative connections would have made the network unreadable. The most common haplotype, EUNA10, was found from almost all populations and other linked haplotypes came from Iceland, the North Sea, the English Channel, and the Southern Baltic Sea, but not from Finland. The other common haplotype, EU11, is multiple mutational steps away from EUNA10. Moreover, the other North American haplotypes were not connected closely to EUNA10 and were clearly different from the European sequences. The European haplotype closest to the cluster of North American haplotypes is from the Netherlands (Fig. 3), and alternative connections (also 26 mutational steps to NAW_NAE) are from the UK (two haplotypes from UKP, one from UKD; not shown). Overall, the minimum spanning network included a large number of small nodes depicting the high frequency of singleton haplotypes noted earlier, and some medium frequency haplotypes (observed in two to eight individuals) that were detected in one population only. These singleton and low-frequency haplotypes are widespread throughout the network.

Phylogenetic analyses resulted in similar tree topologies regardless of which tree reconstruction method was used, therefore, only the results from the maximum likelihood analysis are discussed and shown (Fig. 4). As in the haplotype network, there was a clear separation between the European haplotypes and most North American haplotypes (other than EUNA10) and the European clade was well supported by bootstrap analysis (Fig. 4). Within the European clade, there was very little divergence and only a few groups were clearly resolved with high bootstrap support (Fig. 4, dots at supported nodes). The lack of bootstrap support at most nodes indicates limitations of the data for resolving relationships of the *P. elegans* haplotypes. However, this analysis also reveals clusters detected in the haplotype network. For example, one well-supported group contains almost all the Finnish Ängsö haplotypes (3 out of 4; EU6, FIA43, FIA44). Another well-supported group contains individuals from Germany and all of the three Danish populations, even though other haplotypes from these populations were also distributed elsewhere in the phylogenetic tree. In addition, most Swedish (except one) and all Icelandic haplotypes were included in a

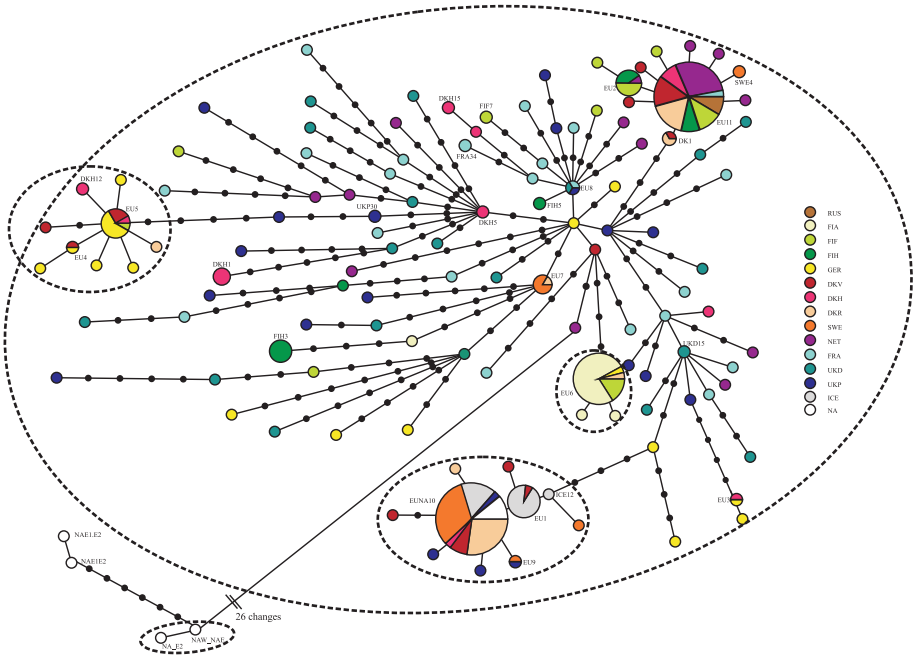


Figure 3. Haplotype network of the 123 COI haplotypes detected in *P. elegans*. Circle size is proportional to haplotype frequency and haplotypes with more than one individual are also named. Small black circles represent undetected intermediate haplotypes and lines connecting circles represent one mutational step unless otherwise specified. Circles are colored to represent sampling sites. Haplotypes found from more than one location are colored as pie charts with proportionally sized wedges representing the haplotype frequency in each population. Ovals with dashed outlines encircle clusters in the haplotype network which were also detected in phylogenetic analyses with strong (70% or greater) bootstrap support.

well-supported group, which also included EUNA10, one of the most common haplotypes also sampled from North America. The two most commonly encountered haplotypes (EUNA10 and EU11) did not group together (Fig. 4, asterisks).

Population structure and demographic analyses

For the test of regional subdivision of sequence diversity in Europe (AMOVA), the populations were arranged into four groups according to geographical region (1. Northern Baltic Sea: Finland, 2. Southern Baltic Sea: Denmark, Germany, Sweden, 3. North Sea + Wadden Sea + English Channel: UK, France, the Netherlands, and 4. North Atlantic Ocean: Iceland, see Fig. 2). These results (Table 2) showed that most of the variation was found within populations (69.8%, $P > 0.001$) and that differentiation among the regions was significant although small (accounting for 8% of the molecular variance, $P = 0.001$). Additional significant variation among populations within each region (22.2%, $P > 0.001$) indicated that structure may also be present on smaller spatial scales.

Analysis using the program BAPS detected seven genetic clusters in our data (with the probability of 0.99). Clusters were not based on sampling location, each cluster containing individuals from four to ten sampling locations. Two clusters were strictly Baltic, one containing most of the individuals sampled from the Finnish Ängsö site and the other containing most of the German samples, although German individuals were also placed into four other clusters (Table 3).

Demographic analyses suggested there has been recent population expansion in the populations from the UK and France. In these populations, Fu's *F*s values were negative and significant. Unimodal mismatch distribution curves for these populations also indicate that a recent population expansion

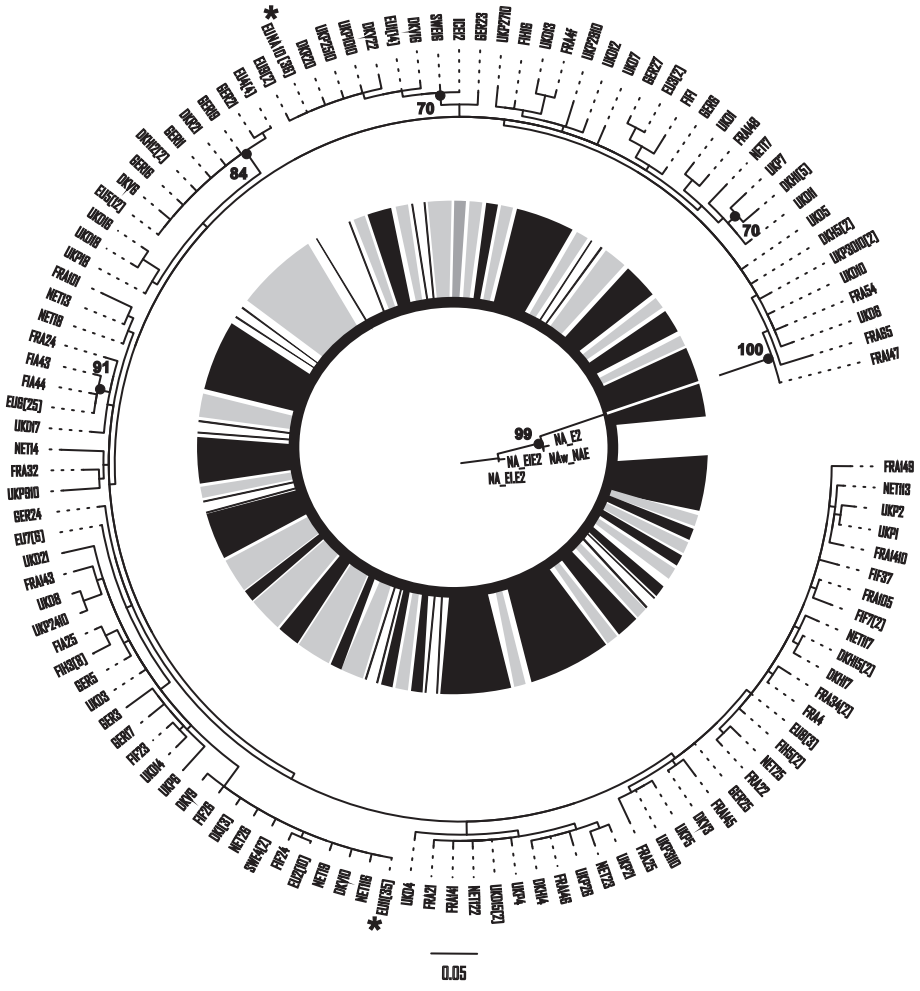


Figure 4. Maximum likelihood tree of COI haplotypes detected in *P. elegans*. Nodes marked with black dots indicate clades resolved with bootstrap values of 70% or higher. Dashed lines connect haplotype names to the branches and should not be interpreted as branch length. Numbers in the brackets following the haplotype name indicate the number of individuals observed with that haplotype. When no number is indicated, the haplotype was sampled only once. Asterisks indicate the two most common haplotypes: EUNA10 and EU11. Central color wheel indicates region of sampling: light gray = Baltic Sea (Northern + Southern); dark gray = Iceland; black = North Sea, English Channel and Wadden Sea; and white = shared European haplotypes. Note, the North American haplotypes in the circular phylogeny are in the center of the color wheel.

Table 2. Analysis of molecular variation results. Four groups (regions) were used in the analysis: Northern Baltic Sea; Southern Baltic Sea; North Sea + Wadden Sea + English Channel; North Atlantic Ocean. Populations included in each region are indicated in Table 1.

Source of variation	Sum of squares	% of variance	Fixation index	*P*-value
Among regions	148.06	8.01	0.080	0.001
Among populations in regions	230.19	22.21	0.241	>0.001
Within populations	864.15	69.78	0.302	>0.001

Table 3. Results from the BAPS genetic clustering analysis. The population code is underlined if 10 or more individuals from that population are in the cluster. If the code is in parentheses, only one to three individuals from that population are observed in the cluster.

	N	Population code	Northern Baltic	Southern Baltic	NS, WS, EC[1]	Atlantic Ocean	White Sea
Cluster 1	27	FIA, FIF (DKR, GER)	25	2			
Cluster 2	19	FIH (GER, UKD, UKP, FIA)	11	3	5		
Cluster 3	57	NET, FIF, DKV, DKR, FIH (DKH, RUS, SWE, UKD)	18	21	15		3
Cluster 4	43	UKD, FRA, DKH, UKP, NET (GER, FIH, FIF)	2	11	30		
Cluster 5	22	GER, DKV (DKH, DKR, FIF)	1	21			
Cluster 6	52	FRA, UKP, UKD, NET, DKH, SWE (GER, FIF, FIH, DKV)	5	14	33		
Cluster 7	59	ICE, SWE, DKR, UKP, DKV (DKH)		32	7	20	

[1]NS = North Sea; WS = Wadden Sea; EC = the English Channel.

may have occurred (see, e.g., Fig. S1). Lower R_2 and rg values were also seen in these populations, but none of these values are significant. For these populations, Tajima's *D* and Fu and Li's neutrality tests were nonsignificant (Table 4), supporting an expansion hypothesis rather than possible selection. Most other populations had bi- or multimodal mismatch curves (characteristics of populations in demographic equilibrium) as well as nonsignificant neutrality test values (Table 4). The one exception is the Finnish Ängsö population which showed negative and significant neutrality test values (Tajima's *D*, Fu and Li). Truncated, left-skewed mismatch distribution curves were seen for the Finnish Ängsö and Iceland populations, indicating that most haplotypes were identical within these populations.

Table 4. Neutrality tests and mismatch distribution parameters. Coalescent simulations (10,000 permutations) were used to asses *P*-values for rg (Raggedness statistics) and R_2.

Population	Fu's *Fs*	Fu and Li's *F*	Tajima's *D*	rg	R_2
FIA	0.093	−3.716*	−2.314**	0.369	0.171
FIF	0.777	−0.477	0.112	0.072	0.129
FIH	4.481	1.055	1.085	0.098	0.179
GER	−4.776	−1.509	−1.017	0.031	0.083
DKV	−0.696	0.539	0.959	0.027	0.166
DKH	0.387	−0.285	−0.141	0.078	0.125
DKR	4.452	−0.527	0.534	0.156	0.151
SWE	3.925	1.143	0.527	0.291	0.157
NET	−2.799	−1.975	−1.048	0.036	0.085
UKD	−11.426***	−1.048	−0.906	0.024	0.089
FRA	−14.231***	−1.486	−0.997	0.022	0.081
UKP	−7.748**	−1.038	−0.708	0.018	0.096
ICE	−0.060	−0.525	−0.090	0.201	0.155

*$P < 0.02$; **$P < 0.01$; ***$P < 0.001$.

Discussion

Our analysis of partial mitochondrial COI sequences from *P. elegans* found little evidence for cryptic species. Sequence divergence was low particularly among European samples (1.7%), which originated from populations with different developmental modes. This modest degree of divergence is lower than within species divergence observed in similar studies of other polychaetes and of other poecilogonous species (discussed further below). In addition, the sequence divergence in European *P. elegans* was lower than threshold values used for delineating "potential" species in the DNA barcoding program (see Goetze 2003; Hebert et al. 2003; Costa et al. 2007; Carr et al. 2011).

However, average sequence divergence between samples from Europe and North America was approximately threefold higher (5.3%). The higher divergence is not surprising given the geographic distance of the samples, but it may indicate a possible cryptic species in North America. Unfortunately, since our collections in North America were limited (three populations, each with six to seven sampled individuals), we are unable to make a strong conclusion about this result, and additional data from unsampled populations in North America and larger sample sizes are needed. However, the degree of divergence among the North American and European haplotypes provides perspective and strengthens our conclusion of poecilogony among European populations.

Several studies have investigated the level of sequence divergence in COI among closely related species of polychaetes. For example, between species divergence in the genus *Arenicola* is on the order of 14% (Luttikhuizen and Dekker 2010) and it is as high as 16% in *Pectoria koreni* (Jolly et al. 2005). Similarly, divergence between species in the *Eumida sanguinea* and *Marenzelleria* species complexes ranges from 6.5% to 18.5% (Nygren and Pleijel 2011) and 11.7% to 21.7% (Blank and Bastrop 2009), respectively. Carr and colleagues (2011) used a COI barcoding approach to survey broadly polychaetes collected from Canadian waters. On average, they detected 16.5% divergence between species, and within-species divergence ranged from 0% to 3.8%.

Divergence in COI sequence also has been used previously to address whether marine invertebrate taxa with observed developmental polymorphism are truly poecilogonous or actually cryptic species. For example, Schulze and colleagues (2000) found evidence for two distinct COI sequence clades among polychaetes in the genus *Streblospio* in North America. Sequence divergence between the two proposed species, a planktotrophic *S. gynobranchiata* and poecilogonous *S. benedicti* was approximately 20% and within-species divergence was ~5%. Both planktotrophic (planktonic) and lecithotrophic (brooded) larvae have been documented within the *S. benedicti* clade. COI sequence data have also been employed to investigate the potential for cryptic species in the marine gastropod genus *Alderia*. Ellingson and Krug (2006) found evidence for two well-supported species-specific sequence clades for *A. willowi* and *A. modesta*, which were 20.6% divergent from one another. Within each clade, sequence divergence was less than 5%. Although the *A. modesta* clade produces only planktonic larvae, slugs in the *A. willowi* clade produce multiple larval types. In this case, both sequence data and morphological evidence (Krug et al. 2007) indicated that *A. willowi* is poecilogonous.

The distribution of genetic variation among *P. elegans* populations provides a second line of support to a conclusion that this species is poecilogonous. We found high haplotype diversity in *P. elegans* in Europe although nucleotide diversity was low. This pattern was consistent with the observed high proportion (90%) of low-frequency haplotypes found in one or a few populations. On the other hand, two haplotypes dominated the sample (25% of individuals). Both of these haplotypes had a broad distribution in many of the European populations we sampled and EUNA10 was also sampled from North America. We also found four haplotypes that were shared among populations characterized by different modes of development, including the most common haplotypes, EUNA10 and EU11. Similar findings were reported for the poecilogonous *A. willowi*, for which shared haplotypes were observed by Ellingson and Krug (2006) between slugs producing different larval types; and also for the polychaete *B. proboscidea*, where there was no association between sequence clades and developmental mode (Gibson et al. 1999; Oyarzun et al. 2011). In our study, we have compared patterns of genetic differentiation among populations which we have characterized by the predominant developmental mode. Ideally, we would have both sequence and developmental mode information for all individuals in our study. Nevertheless, the low levels of sequence divergence and the fact that haplotypes are shared among populations differing in developmental mode and throughout the broad sampling area (Baltic Sea, Wadden Sea, North Sea, and Atlantic Ocean) are inconsistent with a hypothesis of cryptic speciation. Instead, our data support the hypothesis that *P. elegans* populations, particularly those in Europe, are poecilogonous.

However, the significant AMOVA results among regions and among populations within regions indicate that there is genetic structuring at multiple spatial scales within Europe. Our sampled populations cover both a latitudinal and longitudinal gradient and experience different environmental conditions, such as differing temperature extremes, salinities, and substrata, so potential barriers to dispersal may be present between our sampling localities. The AMOVA indicated significant Φ_{ST} among sea regions but it only accounted for 8% of variation in the model. The BAPS-based analysis also suggested the presence of multiple genetic clusters within Europe. On the whole, the clusters did not correspond to our definition of populations by collection locality. The only exceptions were for the haplotypes obtained from Ängsö (Finland), Sweden, and Iceland populations, which were also found in some of the few well-supported clades in our maximum likelihood phylogeny. Overall, these results indicated that the bulk of genetic variation in *P. elegans* resided within populations.

We observed especially low genetic diversity among the worms in the two marginal populations: Ängsö, being located in the inner parts of the Finnish Archipelago, and Iceland, further away from the bulk of the sampled localities in the Atlantic Ocean. Mismatch distribution curves for the sequences from these populations were strongly skewed to the left showing the reduced diversity, which likely reflects limited gene flow to these populations because of their marginal distribution or a recent (re)colonization or other bottleneck-like event. The significant neutrality test values seen in the Ängsö population indicate that selection could also cause this pattern. However, if selection were the cause, we would expect the other two nearby Finnish populations (the distance from FIA to FIF is only 20 km) to show similar results. Alternatively, asexual reproduction could lead to lower diversity. Asexual reproduction has previously been observed in some *P. elegans* populations (e.g., Rasmussen 1953; Hobson and Green 1968; Anger 1984; Lindsay et al. 2007). Wilson (1983) reported that asexual reproduction predominates in False Bay, Washington (west coast United States). In our study, only one *P. elegans*

COI haplotype was found from False Bay, but the sample size was low ($N = 7$) and some possible variation may have gone undetected, so it is impossible to say at present whether an increased frequency of asexual reproduction leads to a greater reduction in genetic diversity.

Several studies have compared genetic diversity among marine invertebrate species with different developmental modes and generally found higher genetic diversity in planktonic-developing species compared to brooded or direct-developing species (Hellberg 1996; Hoskin 1997; Arndt and Smith 1998; Ayre and Hughes 2000; Kyle and Boulding 2000; Collin 2001; Breton et al. 2003; Ellingson and Krug 2006; Lee and Boulding 2009). For example, among snails in the genus *Littorina*, Boulding and colleagues (Kyle and Boulding 2000; Lee and Boulding 2009) observed that genetic diversity is higher in the planktotrophic species *L. scutulata* and *L. plena* than in the direct-developing species *L. sitkana* and *L. subrotundata*. Lee and Boulding (2009) suggested that effective population size (N_e) is larger in planktonic-developing species than in direct-developing species, and as a result, the effects of genetic drift are diminished and a larger number of rare haplotypes are more likely to be retained. In another notable example, Ellingson and Krug (2006) found genetic diversity to be higher in the fully planktotrophic *A. modesta* compared to genetic diversity in the poecilogonous *A. willowi*.

Our analysis with *P. elegans* suggests that the patterns of genetic diversity and development mode may be correlated within a poecilogonous species as well. Haplotype and nucleotide diversity were lowest in Ängsö, Finland population for which brooding is the predominant mode of development, although reduced diversity in this population could also be the result of other demographic factors (see above). In contrast, populations showing a predominantly planktonic developmental mode were more diverse than the Ängsö population as well as those populations showing multiple developmental modes. Fu's *F*s test gave significant negative values for the three populations with predominantly planktonic larvae (UKD, FRA, UKP), indicating an excess of haplotypes, while sequences from these same populations had unimodal mismatch distributions with large numbers of pairwise differences. A pattern of significant Fu's *F*s and nonsignificant Fu and Li's test suggests that the populations may have undergone recent expansion and have high N_e and that background selection is not likely (Rogers and Harpending 1992; Fu 1997). The populations with multiple developmental modes typically had bimodal mismatch distributions with both highly similar and highly differentiated haplotypes.

Although we cannot be certain that our definition of predominant developmental mode for the different populations is correct, our results are interesting because they reflect the general expectations of how developmental mode can influence the genetic diversity within populations, as well as population genetic structure and gene flow among populations of a species (see reviews by Avise et al. 1987; Bohonak 1999; Pechenik 1999). For example, many empirical studies have shown that gene flow is greater for species with pelagic larvae resulting in less genetic structure among populations when compared to species with brooded larvae (e.g., Hellberg 1996; Hoskin 1997; Arndt and Smith 1998; Ayre and Hughes 2000; Collin 2001; Ellingson and Krug 2006). However, the generality of this expectation depends upon dispersal during the pelagic phase. When dispersal is not realized during the pelagic phase, exceptions can occur, and studies highlighting such contrary findings are not uncommon (e.g., Porter et al. 2002; Miller and Ayre 2008; Weersing and Toonen 2009; and see Hellberg et al. 2002 for review).

Our analysis suffers from the lack of individual level data for developmental mode and has only tentatively defined populations as predominantly planktonic, brooding, or both. We cannot rule out the possibility that other developmental modes are predominant at other times of the year or that developmental mode might fluctuate temporally either due to phenotypic plasticity or population turnover. In this regard, it is interesting to note that Morgan and colleagues (1999) found only brooded larvae in the Plym Bay population during repeated sampling in 1997, while in 2010, we observed only planktonic larvae in the same population. In our analyses, the Plym Bay population had high haplotype diversity similar to that seen in other planktonic populations. The change in developmental mode noted in this population may be explained by the ability of *P. elegans* to re-colonize rapidly disturbed areas (Desprez et al. 1992; Kube and Powilleit 1997). For other populations that have been repeatedly sampled, we found no evidence of a change in developmental mode. We observed only planktonic larvae at the Drum Sands and Somme Bay populations, the same mode reported by Bolam (2004) and Morgan et al. (1999), respectively. Like Rasmussen (1973), we found that Danish populations had both planktonic and brooded larvae, as well as intermediate larvae. *Pygospio elegans* from Gullmar Fjord, Sweden, have been previously reported to have intermediate and brooded larvae (Hannerz 1956), but we did not observe reproductive females or larvae during our collection.

Our study is not the first to consider whether *P. elegans* is poecilogonous. Morgan and colleagues (1999) studied four *P. elegans* populations from the English Channel. They found that the Plym Bay and Ryde Sand populations from UK were strictly brooding while the French Somme Bay population and English Swale Bay populations produced only planktonic larvae. Based on allozyme analysis, they observed significant population structure but found no evidence of cryptic species in the English Channel. In our study, we widened the sample area in an attempt to increase the chance of finding cryptic species if such existed in Europe, but our conclusions uphold the previous results even at the broader spatial scale. Although

the previous experimental study by Anger (1984) led her to suggest cryptic species, (since changes in temperature or salinity did not induce changes in developmental mode), we found little evidence to support her view. We feel that alternative explanations for her results should be considered: for example, developmental mode may not be a plastic trait, or if it is, temperature and salinity may not be the appropriate cues to trigger a plastic response.

Conclusion

In this study, we analyzed DNA sequence variation from *P. elegans* sampled from a broad geographic range. Very little sequence divergence was observed among individuals despite variation in developmental mode observed both among and within populations. Using a DNA barcoding criterion based on sequence divergence, there is no evidence for cryptic species in this taxon. In addition, haplotype network and phylogenetic analyses did not point to potentially cryptic species as no clear clustering of haplotypes was resolved among the European samples. Divergence of North American *P. elegans* may warrant further study. Given these results, we conclude that developmental polymorphism in *P. elegans* is likely a true case of poecilogony. These results also confirm the previous population genetic study of Morgan and colleagues (1999) which used genetic methods with lower resolution and had a more restricted geographical scope.

Acknowledgments

This study could not have been done without the help of all of our collaborators: B. W. Hansen, C. Boström, C. Gustafsson, E. Geuverink, R. Bastrop and J. Frankowski, A. Zhadan, G. V. Helgason, and A. Mahon, who enabled the broad range of sampling. A. Lempiäinen and M. Mustonen helped in the DNA laboratory at JYU. D. McHugh provided encouragement and advice. J. E. K. was supported by a grant from the Jenny and Antti Wihuri Foundation. K. E. K. acknowledges fruitful discussions with members of the Marie Curie Initial Training Network Speciation funded by the European Union. This research was also part of the Centre of Excellence in Evolutionary Research at University of Jyväskylä.

References

Allen, J., and B. Pernet. 2007. Intermediate modes of larval development: bridging the gap between planktotrophy and lecithotrophy. Evol. Dev. 9:643–653.

Anger, V. 1984. Reproduction in *Pygospio elegans* (Spionidae) in relation to its geographical origin and to environmental conditions: a preliminary report. Forts Zool. 29:45–51.

Anger, K., V. Anger, and E. Hagmeier. 1986. Laboratory studies on larval growth of *Polydora ligni*, *Polydora ciliata* and *Pygospio elegans* (Polychaeta, Spionidae). Helgoländer Meeresunters 40:277–395.

Arndt, A., and J. M. Smith. 1998. Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. Mol. Ecol. 7:1053–1064.

Avise, J. C., J. Arnold, and M. R. Ball. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18:489–522.

Ayre, D. J., and P. T. Hughes. 2000. Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54:1590–1605.

Blake, J. A., and L. P. Arnofsky. 1999. Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. Hydrobiologia 402:57–106.

Blank, M., and R. Bastrop. 2009. Phylogeny of mud worm genus *Marenzelleria* (Polychaeta, Spionidae) inferred from mitochondrial DNA sequences. Zool. Scr. 38:313–321.

Bohonak, A. J. 1999. Dispersal, gene flow, and population structure. Quart. Rev. Biol. 74:21–45.

Bolam, S. G. 2004. Population structure and reproductive biology of *Pygospio elegans* (Polychaeta: Spionidae) on an intertidal sandflat, Firth of Forth, Scotland. Invertebr. Biol. 123:260–268.

Breton, S., F. Dufresne, G. Desrosier, and U. P. Blier. 2003. Population structure of two northern hemisphere polychaetes, *Neanthes virens* and *Hediste diversicolor* (Nereididae), with different life-history traits. Mar. Biol. 142:707–715.

Carr, C. M., M. S. Hardy, M. T. Brown, A. T. Macdonald, and P. D. N. Hebert. 2011. A Tri-Oceanic perspective: DNA barcoding reveals geographic structure and cryptic diversity in Canadian polychaetes. PLoS One 6:e22232. doi: 10.1371/journal.pone.0022232

Collin, R. 2001. Mode of development, phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae) species. Mol. Ecol. 10:2249–2262.

Collin, R. 2003. World-wide patterns of development in calyptraeid gastropods. Mar. Ecol. Prog. Ser. 247: 103–122.

Corander, J., and J. Tang. 2007. Bayesian analysis of population structure based on linked molecular information. Math Biosci. 205:19–31.

Costa, F. O., J. R. deWaard, J. Boutillier, S. Ratnasingham, T. R. Dooh, M. Hajibabaei, and D. P. N. Hebert. 2007. Biological identifications through DNA barcodes: the case of the Crustacea. Can. J. Fish Aquat. Sci. 64:272–295.

Desprez, M, H. Rybarczyk, G. J. Wilson, P. J. Ducrotoy, F. Seuer, R. Olivesi, and B. Elkaim. 1992. Biological impact of eutrophication in the Bay of Somme and the induction and impact of anoxia. Neth. J. Sea Res. 30:149–159.

Ellingson, R. A., and J. P. Krug. 2006. Evolution of poecilogony from planktotrophy: cryptic speciation, phylogeography, and larval development in the gastropod genus *Alderia*. Evolution 601:2293–2310.

Excoffier, L., and E. H. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics

analyses under Linux and Windows. Mol. Ecol. Resour. 10:564–567.
Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.
Fu, Y.-X., and H. W. Li. 1993. Statistical tests of neutrality of mutations. Genetics 133:693–709.
Gibson, G. D. 1997. Variable development in the spionid *Boccardia proboscidea* (Polychaeta) is linked to nurse egg production and larval trophic mode. Invertebr. Biol. 116:213–226.
Gibson, G. D., and J. F. Gibson. 2004. Heterochrony and the evolution of poecilogony: generating larval diversity. Evolution 58:2704–2717.
Gibson, G., G. I. Paterson, H. Taylor, and H. Woolridge. 1999. Molecular and morphological evidence of a single species, *Boccardia proboscidea* (Polychaeta: Spionidae) with multiple developmental modes. Mar. Biol. 134:743–751.
Goetze, E. 2003. Cryptic speciation on the high seas; global phylogenetics of the copepod family Eucalanidae. Proc. R. Soc. Lond. B 270:2321–2331
Gudmundsson, H. 1985. Life history patterns of polychaete species of the family Spionidae. J. Mar. Bio. Assoc. UK 65:93–111.
Guindon, S., and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52:696–704.
Hannerz, L. 1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil and Poecilochaetidae N. fam. in the Gullmar Fjord (Sweden). Zool. Bidr. Upps. 31:1–204.
Harpending, H. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum. Biol. 66:591–600.
Hebert, P. D. N., S. Ratnasingham, and J. R. deWaard. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lond. B 270:S96–S99.
Hellberg, M. E. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50:1167–1175.
Hellberg, M. E, S. R. Burton, E. J. Neigel, and R. S. Palumbi. 2002. Genetic assessment of connectivity among marine populations. B Mar. Sci. 70:273–290.
Hoagland, K. E., and R. Robertson. 1988. An assessment of poecilogony in marine invertebrates: phenomenon or fantasy? Biol. Bull. 174:109–125.
Hobson, K. D., and H. R. Green. 1968. Asexual and sexual reproduction of *Pygospio elegans* (Polychaeta) in Barnstable Harbor, Massachusetts (Abstracts of papers presented at the marine biological laboratory 1986). Biol. Bull. 135:410.
Hoskin, M. G. 1997. Effects of contrasting modes of larval development on the genetic structures of populations of three species of prosobranch gastropods. Mar. Biol. 127:647–656.
Jolly, M. T, D. Jollivet, F. Gentil, E. Thiébaut, and F. Viard. 2005. Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the north coast of France. Heredity 94:23–32.
Krug, P. J. 2007. Poecilogony and larval ecology in the gastropod genus Alderia. Am. Malacol. Bull. 23:99–111.
Krug, P. J. 2009. Not my "type": larval dispersal dimorphism and bet-hedging in opisthobranch life histories. Biol. Bull. 216:355–372.
Krug, P. J, A. R. Ellingson, R. Burton, and A. Valdés. 2007. A new poecilogonous species of sea slug (Opisthobranchia: Sacoglossa) from California: comparison with the planktonic congener *Alderia modesta* (Lovén, 1844). J. Moll. Stud. 73:29–38.
Kube, J., and M. Powilleit. 1997. Factors controlling the distribution of *Marenzelleria* cf. *viridis*, *Pygospio elegans* and *Steblospio shrubsoli* (Polychaeta: Spionidae) in the southern Baltic Sea, with special attention for the response to an event of hypoxia. Aquat. Ecol. 31:187–198.
Kyle, C. J., and G. E. Boulding. 2000. Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar. Biol. 137:835–845.
Lee, H. J., and G. E. Boulding. 2009. Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on variation at one mitochondrial and two nuclear DNA markers. Mol. Ecol. 18:2165–2184.
Levin, L. 1984. Multiple patterns of development in *Streblospio benedicti* Webster (Spionidae) from three coasts of North America. Biol. Bull. 166:494–508.
Levin, L. A., and S. T. Bridges. 1995. Pattern and diversity in reproduction and development. Pp. 1–48 *in* L. McEdward, ed. Ecology of marine invertebrate larvae. CRC Press, Boca Raton, Florida.
Lindsay, S. M., L. J. Jackson, and Q. S. He. 2007. Anterior regeneration in the spionid polychaetes *Dipolydora quadrilobata* and *Pygospio elegans*. Mar. Biol. 150: 1161–1172.
Luttikhuizen, P. C., and R. Dekker. 2010. Pseudo-cryptic species *Arenicola defodiens* and *Arenicola marina* (Polychaeta: Arenicolidae) in Wadden Sea, North Sea and Skagerrak: morphological and molecular variation. J. Sea Res. 63:17–23.
McEdward, L. R., and A. D. Janies. 1993. Life cycle evolution in asteroids: what is a larva? Biol. Bull. 184:255–268.
Miller, K. J., and J. D. Ayre. 2008. Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. J. Anim. Ecol. 77:713–24.
Morgan, T. S. 1997. The formation and dynamics of *Pygospio elegans* tube-beds in the Somme Bay, France. Dissertation. University of Southampton, United Kingdom. (UoS Thesis Supply).
Morgan, T. S., D. A. Rogers, L. G. J. Paterson, E. L. Hawkins, and M. Sheader. 1999. Evidence for poecilogony in *Pygospio elegans* (Polychaeta: Spionidae). Mar. Ecol. Prog. Ser. 178:121–132.

Muus, B. J. 1967. The fauna of Danish estuaries and lagoons. Distribution and ecology of dominating species in the shallow seaches of the mesohaline zone. Medd Danm Fiskeri-og Havunders 5:1–316.

Nygren, A., and F. Pleijel. 2011. From one to ten in a single stroke—resolving the European *Eumida sanguinea* (Phyllodocidae, Annelida) species complex. Mol. Phylogenet. Evol. 58:132–141.

Oyarzun, F. X., R. A. Mahon, J. B. Swalla, and M. K. Halanych. 2011. Phylogeography and reproductive variation of the poecilogonous polychaete *Boccardia proboscidea* (Annelida: Spionidae) along the West Coast of North America. Evol. Dev. 13:489–503.

Pechenik, J. A. 1999. On the advantages and disadvantages of larval stages in benthic invertebrate life cycles. Mar. Ecol. Prog. Ser. 177:269–297.

Porter, J. S., S. J. Ryland, and R. G. Carvalho. 2002. Micro- and macrogeographic genetic structure in bryozoans with different larval strategies. J. Exp. Mar. Biol. Ecol. 272:119–130.

Posada, D. 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25:1253–1256.

Raff, R. A., and M. Byrne. 2006. The active evolutionary lives of echinoderm larvae. Heredity 9:244–252.

Ramos-Onsins, S. E., and J. Rozas. 2002. Statistical properties of new neutrality tests against population growth. Mol. Biol. Evol. 19:2092–2100.

Rasmussen, E. 1953. Asexual reproduction in *Pygospio elegans* Claparède (*Polychaeta sedentaria*). Nature 171:1161–1162.

Rasmussen, E. 1973. Systematics and ecology of the Isefjord marine fauna (Denmark). Ophelia 11:1–495.

Rogers, A. R, and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9:552–569.

Ronquist, F., and P. J. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Rozas, J., J. C. Sánchez-DelBarrio, X. Messeguer, and R. Rozas. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497.

Schneider, S., and L. Excoffier. 1999. Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089.

Schulze, S. R., A. S. Rice, L. J. Simon, and A. S. Karl. 2000. Evolution of poecilogony and the biogeography of North American populations of the polychaete *Steblospio*. Evolution 54:1247–1259.

Söderström, A. 1920. Studien über die Polychaeten familie Spionidae. Dissertation. University of Uppsala, Sweden.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–593.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24:1596–1599.

Teacher, A. G. F., and J. D. Griffiths. 2010. HapStar: automated haplotype network layout and visualization. Mol. Ecol. Res. 11:151–153.

Weersing, K., and J. R. Toonen. 2009. Population genetics, larval dispersal, and connectivity in marine systems. Mar. Ecol. Prog. Ser. 393:1–12.

Wilson, W. H. 1983. The role of density dependence in a marine infaunal community. Ecology 64:295–306.

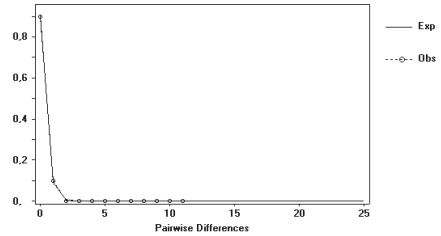
Supporting Information

Additional Supporting Information may be found online on Wiley Online Library.

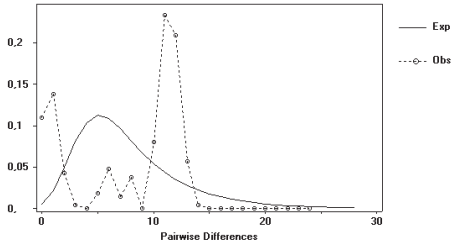
Figure S1. Examples of mismatch distribution curves (analyses for growth-decline scenario): observed pairwise nucleotide site differences and the expected curves for growing or declining population (Rogers and Harpending 1992).

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

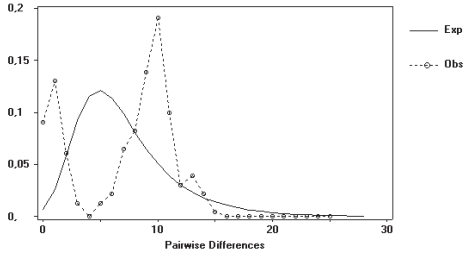
Supplement 1. Examples of mismatch distribution curves (analyses for growth-decline scenario): observed pairwise nucleotide site differences and the expected curves for growing or declining population (Rogers and Harpending 1992).



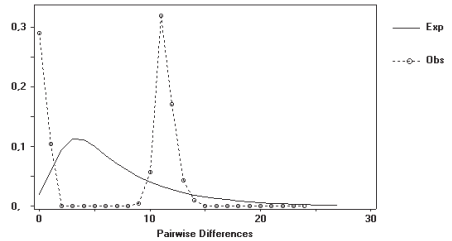
a) Iceland (ICE)



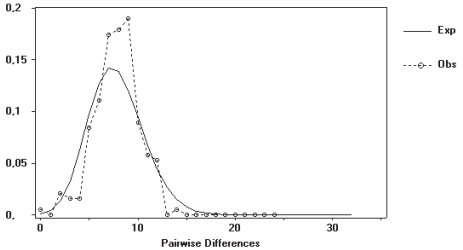
b) Fårö, Finland (FIF)



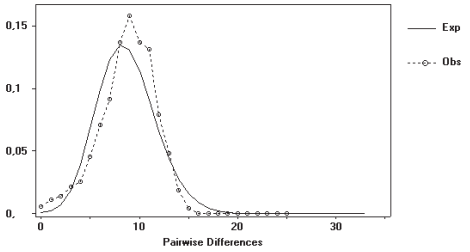
c) Germany (GER)



d) Rorvig, Denmark (DKR)



e) Drum sands, UK (UKD)



f) UKD+UKP+FRA data combined

II

NEW GENETIC MARKERS REVEAL POPULATION GENETIC STRUCTURE AT DIFFERENT SPATIAL SCALES IN THE OPPORTUNISTIC POLYCHAETE *PYGOSPIO ELEGANS*

by

Jenni Kesäniemi, Christoffer Boström & K. Emily Knott 2012

Hydrobiologia 691: 213–223.

Hydrobiologia (2012) 691:213–223
DOI 10.1007/s10750-012-1075-3

PRIMARY RESEARCH PAPER

New genetic markers reveal population genetic structure at different spatial scales in the opportunistic polychaete *Pygospio elegans*

J. E. Kesäniemi · C. Boström · K. E. Knott

Received: 30 November 2011 / Revised: 23 February 2012 / Accepted: 6 March 2012 / Published online: 5 April 2012

Abstract Identifying population genetic structure can shed light on how life history characteristics of opportunistic species affect population turnover and (re)colonization of disturbed habitats. Plasticity in life history traits can be particularly important for opportunistic species. In this study, we investigated population genetic structure of two populations of *Pygospio elegans,* an opportunistic polychaete worm. The populations represented extremes of the range of habitats *P. elegans* exploits: a subtidal brackish site where *P. elegans* is found at lower densities associated with seagrass patches; and a disturbed mudflat in a marine tidal environment where *P. elegans* can reach very high densities with patchy distribution. Eight novel microsatellite loci were isolated from *P. elegans* for the genetic studies. We found higher genetic diversity in the mudflat, which could be due to larger population size, opportunistic behaviour, or the predominantly planktonic larval production of *P. elegans* in this population. No genetic structure was found within the seagrass patch in the Archipelago Sea (SW Finland) where samples were separated by 5–15 m. However, low structure was observed in the Bay of Somme, mudflat (France) where samples were separated by approx. 100 m. When the two locations were compared, high genetic differentiation was observed, indicating restrictions on gene flow between the sea areas. The microsatellite loci were highly polymorphic and proved to be useful tools for investigating the genetic diversity and genetic structure in *P. elegans* at different spatial scales, despite deviations from Hardy–Weinberg expectations at some loci.

Keywords Spionidae · Microsatellite · Spatial genetic structure · Dispersal · Opportunistic · Plasticity

Handling editor: Christian Sturmbauer

J. E. Kesäniemi (✉) · K. E. Knott
Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland
e-mail: jenni.kesaniemi@jyu.fi

C. Boström
Department of Biosciences, Environmental and Marine Biology, Åbo Akademi University, Artillerigatan 6, 20520 Åbo, Finland

Introduction

Opportunistic species are characterized as having small size, early reproduction, high fecundity, strong dispersal ability and a short life span, which enable them to colonize new and disturbed or defaunated habitats rapidly (Pianka, 1970; Grassle & Grassle, 1974; Pearson & Rosenberg, 1978). As a consequence of these life history traits, opportunistic species commonly have unstable populations with fluctuating population sizes (Whitlatch and Zajac, 1985; Warwick, 1986; Bolam and Fernandes, 2002). Together, these characteristics of opportunistic species also can

affect their population genetic structure. For example, such populations may show genetic signatures of rapid population growth, or population bottlenecks when (re)colonized areas are founded by only a few individuals (e.g. Bay et al., 2008). Given these relationships, clarification of population structure can shed light on how an opportunistic life history strategy affects population dynamics.

Among polychaetes, an opportunistic life history strategy is common. In particular, polychaetes living in frequently disturbed habitats, such as sand- and mudflats, show opportunistic characteristics (Grassle & Grassle, 1974). Examples include, e.g. *Streblospio benedicti, Polydora cornuta, Hobsonia florida, Capitella capitata, Syllides verrili* and *Microphthalmus aberrans* (Grassle & Grassle, 1974; Pearson & Rosenberg, 1978). The capability for widespread dispersal via planktonic larvae is common in these opportunistic species, but also local population maintenance without outside larval supply can be important (e.g. through brooded larvae or asexual reproduction: Grassle & Grassle, 1974; Tsutsumi, 1990). Opportunistic species are often tolerant to pollution and can thrive in organically enriched habitats (e.g. Pearson & Rosenberg, 1978; Levin, 1986; Tsutsumi, 1987; Bridges et al., 1994). Probably the best known example of opportunistic polychaete, the *Capitella* spp., appears to reach exceptionally high population densities only in heavily polluted or organically rich areas (Grassle & Grassle, 1974; Tsutsumi, 1987). Some opportunistic species can also be described as invasive (e.g. the polychaete *P. cornuta*; Streftaris & Zenetos, 2006; Karhan et al., 2008). Invasive species often have the same life history characteristics as opportunistic species (see McMahon, 2002), but opportunistic species are thought to be relatively poor competitors (Grassle & Grassle, 1974; Whitlatch & Zajac, 1985), whereas invasive species represent a threat to native species (e.g. Branch & Steffani, 2004).

The spionid polychaete *Pygospio elegans* Claparéde 1863 is often characterized as an opportunistic species (e.g. Desprez et al., 1992; Beukema et al., 1999; Morgan et al., 1999; Bolam & Fernandes, 2002). This species, common in the northern hemisphere, inhabits a wide range of intertidal and subtidal habitats, e.g. mudflats (e.g. Morgan et al., 1999; Bolam, 2004), seagrass beds (Boström & Bonsdorff, 1997), rock crevices (Gudmundsson, 1985) and subtidal areas (Kube & Powilleit, 1997; Fleischer & Zettler, 2008), and even tolerates organically enriched habitats (Anger, 1984). In addition, *P. elegans* thrives in a wide range of salinities, but is most common in sandy, estuarine habitats (Muus, 1967; Morgan 1997; Bolam 2004).

Pygospio elegans displays variation in larval developmental mode, both among and within populations (Hannerz, 1956; Rasmussen, 1973; Morgan et al., 1999; Kesäniemi et al., 2012 in press), which likely contributes to its success as an opportunistic species. After internal fertilization, females deposit true eggs and nurse eggs into egg capsules. Depending on the ratio of true eggs and nurse eggs, either planktotrophic larvae emerge to feed and develop in the plankton; or benthic larvae are brooded, feeding on the nurse eggs provided by the mother. *P. elegans* can also reproduce asexually (Rasmussen, 1953). Although planktonic larvae of *P. elegans* can live up to 4–5 weeks under laboratory conditions (Anger et al., 1986), mortality of these larvae is expected to be very high (Pedersen et al., 2008), so it is unclear how large an effect larval dispersal has on population connectivity in this species.

Adult *P. elegans* are also motile and they will rapidly emerge from their sand tubes and search for new habitat if disturbed (Anger et al., 1986; pers. obs.). Rapid recolonization of disturbed sites by *P. elegans* has also been observed in the field (Desprez et al., 1992; Kube & Powilleit, 1997). *P. elegans* can form dense aggregations of tube mats, and these are often transient patches in frequently disturbed habitats (Morgan, 1997; Morgan et al., 1999; Boström & Bonsdorff, 2000; Bolam & Fernandes, 2002, 2003). However, this is only one extreme of observed *P. elegans* populations. Under less disturbed conditions, for example, in areas where *P. elegans* is explicitly associated with seagrass patches embedded in bare sand, their own patchiness is directly linked to the vegetation mosaic, probably influenced by stabilizing roots (Boström & Bonsdorff, 2000). In seagrasses, *P. elegans* density is lower compared to that of tube mats found in sandy or muddy estuaries lacking vegetation: these vary between 300 and 3,000 ind. m^2 in seagrass patches in the northern Baltic Sea (Boström & Bonsdorff, 1997, 2000), to as high as 400,000 ind. m^2 in a mudflat in northern France, Bay of Somme (Morgan, 1997).

The different extremes of *P. elegans* populations seen in brackish and marine habitats allow

examination of the potential influence of life history characteristics on population genetic structure. Here, we describe isolation of microsatellite markers for this species and we test the markers ability to reveal within population genetic structure in two populations sampled at different spatial scales. The populations differed in several ways. In the subtidal population from Finland, the sediment is stabilized by seagrasses, worm density is low, and brooded larvae and asexual reproduction are common. However, in the mudflat in France, sediments are less stable, worms are found at high density and planktonic larvae are produced, and asexual reproduction has not been observed. We expected that genetic diversity would be lower in Finland than in France, where the unstable environment accentuates the opportunistic life history traits of *P. elegans*, leading to frequent population turnover and where the potential influx of planktonic larvae from other populations is expected to be high. We had no clear expectations of the extent of genetic structure within populations, however, between population structure was expected to be high due to the geographic distance between them.

Materials and methods

Isolation of microsatellite markers

For the microsatellite library, genomic DNA was extracted from whole individuals using the PUREGENE® DNA Purification Kit (Gentra systems). Microsatellite loci were isolated following the FIASCO technique (Zane et al., 2002) with some modifications (Grapputo, 2006). Four enrichment libraries were prepared with the following probes: $(CA)_{22}$, $(TA)_{12}$, $(CAG)_{11}$, $(CATA)_8$. PCR amplicons were cloned using the TOPO-TA cloning kit (Invitrogen) and One Shot® TOP10 competent *Escherichia coli* cells. Positive clones were amplified with vector-specific primers, sequenced using BigDye Terminator 3.1. reagents and visualized with the ABI PRISM 3130xl and Sequencing Analysis v.5.2 software (Applied Biosystems). Primers were designed to the flanking regions of 17 microsatellite repeat loci using PRIMER3 (Rozen & Skaletsky, 2000). Twelve loci were amplified successfully and eight of these were polymorphic.

Study sites and sampling

In Finland, adult *P. elegans* were collected at Ängsö (60.1°N, 21.7°E), a bight located in the Archipelago Sea, northern Baltic Sea. The average ($n = 10$) density of *P. elegans* at this site is 2,800 ind. m^2 (Boström et al., 2006), but densities up to 10,000 ind. m^2 have also been recorded in the northern Baltic Sea (Boström & Bonsdorff, 1997, 2000). The salinity of the site is typically 6–6.5 psu and sediment is fine sand (in vegetation) and sandy gravel in non-vegetated areas (for a general description of the area, see Boström et al., 2006). Samples were collected in August 2009 by SCUBA diving from approximately 4–5 m depth. At this site, and in the northern Baltic Sea area in general, *P. elegans* densities peak in seagrass *Zostera marina* meadows. We therefore chose to sample a *Z. marina* patch (approx. 12 m in diameter). Worms were collected along a transect perpendicular to the shore. The transect crossed the centre of the seagrass patch and contained five sampling plots located approximately 5 m from each other: (1) bare sand (shore side, Sand1), (2) patch edge (shore side, Edge1), (3) patch centre, (4) patch edge (offshore, Edge2) and (5) bare sand (offshore, Sand2) (see Table 3 for sample sizes). At each sampling plot, a non-quantitative sample of approximately 3–4 kg of sediment was collected using a shovel and a bag. The sample was carefully sieved and processed in a boat. Since the density of worms in bare sand was very low, we did not obtain enough material from the sand plot furthest from the shore (Sand2, $N = 3$) and therefore this plot was not included in the genetic analyses. No juveniles or reproducing worms were found. During other collections over 3 years at this site, sexually reproductive females were found only rarely, and all of these produced brooded larvae, however, asexual reproduction also occurs at this site (pers. obs.).

The Bay of Somme (50.23°N, 1.60°E) is an exposed macrotidal estuary in France (max. tidal amplitude of 9–10 m) with water salinity never dropping below 25 psu (Rybarczyk et al., 1993; Morgan, 1997; Morgan et al., 1999). This mudflat supports a high density of *P. elegans* (densities of over 100,000 ind. m^2 are regularly recorded), which seems to be the dominant species in the area (pers.obs). High *P. elegans* density fluctuations have been observed at this site (Desprez et al., 1992; Morgan, 1997; Morgan et al., 1999) and periodic mass mortalities due to

anoxia have also been reported. Also, the sediment in the Bay of Somme can be unstable, since the estuary has two tides per day (Desprez et al., 1992; Rybarczyk et al., 1993; Morgan, 1997). This population was sampled during low tide in March 2010. Three sampling plots were chosen. Plots 1 and 2 were approximately 100 m apart, but both were along the shoreline, approximately 200 m from the shore (Shore1 and Shore2), and Plot 3 (Sea2) was approximately 100 m towards the sea from Plot 2 (Shore2) (see Table 3 for sample sizes). Sediment samples (1–2 kg) were sieved at each plot. Large numbers of juveniles were found, but these were not used in the analysis. Most adult individuals collected contained immature gametes and planktonic larvae were also observed in the egg capsules. At this site, only development of planktonic larvae has been observed previously and asexual reproduction has not been observed (Morgan, 1997; Morgan et al., 1999).

Microsatellite genotyping

From individual adult worms, genomic DNA was extracted using a Kingfisher magnetic processor (Thermo Fisher Scientific) and Qiagen chemistry (DNeasy kit reagents). All amplification reactions were run in Thermo Hybaid MultiBlock System thermocyclers. Three of the loci (Pe15, Pe17 and Pe18) were amplified using the method described by Schuelke (2000). This method uses three primers, a sequence-specific forward primer with M13(−21) sequence tail, a sequence-specific reverse primer and a fluorescently labelled M13(−21) primer (6FAM, NED, VIC or PET, Applied Biosystems). Amplification was performed in 10 µl reactions with 1 µl of DNA, 1× PCR buffer (Biotools), 200 µM of each dNTP (Fermentas), 8 pmol of reverse primer (TAG Copenhagen), 8 pmol of labelled M13(−21) primer (Applied Biosystems), 2 pmol of the M13(−21) tailed forward primer (TAG Copenhagen), 1.5–2 mM of $MgCl_2$ (Biotools) and 0.5 U of *Taq* DNA polymerase (Biotools). Thermocycling conditions were 94 °C for 5 min, then 30 cycles of 94 °C for 30 s, Ta for 30 s (see Table 1), 72 °C for 30 s, followed by 8 cycles of 94 °C for 30 s, 53 °C for 45 s, 72 °C for 45 s, ending with a final extension of 72 °C for 10 min. The other loci (Pe6, Pe7, Pe12, Pe13 and Pe19) were amplified using two sequence-specific primers, forward primer being labelled. Amplifications were also performed in 10 µl reactions as described above, except using 0.5 µM of both primers (1/8 of the forward primer was labelled, Applied Biosystems) and 1.5–3 mM of $MgCl_2$. Thermocycling conditions were as follows: 94 °C for 5 min, then 35 cycles of at 94 °C for 30 s, Ta for 30 s (see Table 1), 72 °C for 30 s, followed by a final extension of 10 min at 72 °C. Products were separated using the ABI PRISM 3130xl and genotyped using GeneMapper v.3.7. software (Applied Biosystems).

Population genetic and statistical analyses

For within population genetic diversity analyses, the number of alleles, gene diversity, and observed and expected heterozygosity at each locus and within a sampling plot were calculated in Fstat v.2.9.3.2. (Goudet, 1995) or Arlequin v.3.5.2.1 (Excoffier & Lischer, 2010). Allelic richness and private allele richness were calculated with HP-RARE 1.1 (Kalinowski, 2005), which uses a rarefaction method that compensates for the unequal sample sizes of the plots. Deviations from Hardy–Weinberg equilibrium (HWE) were assessed with the exact test implemented in Genepop (Raymond & Rousset, 1995) and significance values were adjusted with Bonferroni correction. The presence and frequency of null alleles was checked with Micro-Checker (Van Oosterhout et al., 2004) and FreeNA (Chapuis & Estoup, 2007). Since HWE deviations were present in our data (and since the presence of null alleles can lead to overestimation of F_{ST} and genetic distance, Chapuis & Estoup, 2007), F_{ST} analyses were calculated with both the original dataset (8 loci) and a reduced dataset of six loci (removing Pe12 and Pe15 or Pe13 which had the highest null allele frequencies). Linkage disequilibrium was tested with Genepop and significance values were adjusted with Bonferroni correction. Genetic differentiation was estimated with F_{ST} (calculated in Arlequin) and D_{est} (calculated in SMOGD; Crawford, 2010). F_{ST} was also calculated with FreeNA, which uses the ENA method (described in Chapuis & Estoup, 2007) for correcting the bias introduced by null alleles. Samples (plots) were also grouped by country (Finland and France) and allelic richness (also calculated with HP-RARE), heterozygosity and gene diversity for the two populations were calculated and compared using Fstat.

Table 1 Primer sequences and PCR conditions for the 8 microsatellite loci

Locus name	Repeat	Primer sequence 5′–3′	T_a (°C)	$MgCl_2$ (mM)	Product size (bp)	GenBank accession no.
Pe6	$(CA)_{28}$	F: ACTACGGAAACTGCCTGCAC	54	2	250	GU321899
		R: ATATGGCCACCGAAACCTCT				
Pe7	$(CATA)_{13}$	F: CTCACCCTTTACACCCAAGG	54	3	152	GU321900
		R: AGCGTCTGTTATGGGGTACAG				
Pe12	$(CT)_{38}$	F: ACTCGGTGTTTTCCCTAACG	55	3	212	GU321901
		R: CGACGTGATCAGTTCTGCTG				
Pe13	$(GA)_{23}$	F: CCGGCGTCTCTACACAATAC	58	1.5	176	GU321902
		R: TTCCATTGTGCACGTTCTTT				
Pe15	$(GT)_{10}(GA)_{24}$	F: M13(−21) + TAGTGATCACCCCACATCCA	57	1.5	202	GU321903
		R: AACACACCTTCCCTCACACC				
Pe17	$(TG)_4N_3(TG)_8$	F: M13(21) + CAAATGAGTTGTGGACTAGTAGGG	57	2	172	GU321904
		R: CCCCCTGTGGGCTAGATAG				
Pe18	$(CAA)_4N(CAA)_2$	F: M13(−21) + TGGATACGGTCTCAACCTTTG	57	2	200	GU321905
		R: AGCCATTGCCCAATGATAAC				
Pe19	$(GCAA)_2$ $(GCAGCAA)_4$	F: TATCCAACGCACACCTACCA	54	2	241	GU321906
		R: TTGAGTGATGGTGCGAGGTA				

M13(−21) sequence: 5′-TGT AAA ACG ACG GCC AGT-3′ (18 bp), T_a PCR annealing temperature, *product size* base pairs between the primers in the cloned allele

Results

Characteristics of the microsatellite loci

Details of the microsatellite loci, primers and optimized amplification conditions are shown in Table 1. Polymorphism of the loci ranged widely, with 9–35 alleles per locus (Table 2). In Finland, the number of alleles per locus ranged from 4 to 32 and in France from 8 to 33. In all loci except Pe7, more alleles were found in France than in Finland. Gene diversity was also higher in France in all loci expect Pe7 where values were equal in both countries. No linkage disequilibrium between the loci was detected. Significant departures from HWE after Bonferroni correction were detected in several loci (Table 3). In Finland, significant deviations from HWE were detected in three loci from most (Pe12 and Pe15 in Centre, Edge1 and Edge2, also Pe13 in Edge2), but not all of the sampling plots. In France, the loci Pe13 and

Table 2 Number of alleles and gene diversity per locus in both countries

Locus	N_{aALL}	Ängsö, Finland $N = 90$		Bay of Somme, France $N = 67$	
		N_a	Gene diversity	N_a	Gene diversity
Pe6	9	4	0.201	8	0.372
Pe7	34	32	0.941	27	0.941
Pe12	35	29	0.960	30	0.964
Pe13	28	26	0.954	28	0.962
Pe15	34	27	0.948	33	0.970
Pe17	20	11	0.263	18	0.858
Pe18	12	5	0.523	9	0.610
Pe19	17	8	0.368	13	0.730

N_{aALL} number of alleles across both populations, N_a number of alleles, N number of individuals genotyped

Table 3 Observed heterozygosity (H_o) of each locus across all sampling plots

Locus	Finland H_o				Locus	France H_o		
	Centre ($N = 29$)	Edge1 ($N = 23$)	Sand1 ($N = 9$)	Edge2 ($N = 26$)		Shore1 ($N = 18$)	Shore2 ($N = 17$)	Sea2 ($N = 32$)
Pe6	0.310	0.261	0.000	0.154	Pe6	0.267	0.412	0.375
Pe7	0.828	0.913	0.889	0.769	Pe7	0.833	0.824	0.900
Pe12	*0.913*	*0.588*	0.857	*0.826*	Pe12	*0.667*	*0.563*	*0.444*
Pe13	0.852	0.750	0.750	*0.739*	Pe13	*0.600*	*0.563*	*0.767*
Pe15	*0.553*	*0.565*	0.444	*0.680*	Pe15	0.813	0.750	*0.633*
Pe17	0.310	0.261	0.111	0.231	Pe17	0.750	0.529	*0.581*
Pe18	0.464	0.565	0.778	0.520	Pe18	0.412	0.333	*0.304*
Pe19	0.444	0.429	0.222	0.308	Pe19	0.625	0.588	0.645

Significant HWE deviations (after Bonferroni correction) are shown in italics

Pe12 were problematic, and the plot Sea2 plot showed deviations in three additional loci (Pe 15, Pe17 and Pe18). Analysis with Micro-Checker suggested the presence of null alleles in these sampling plots and loci. FreeNA calculations showed moderate (0.05–0.2) or high (Pe12, Pe17 and Pe18 in Sea2) null allele frequencies in the sampling plots with the HWE deviations.

Population genetic structure

In Finland, there was no difference in observed heterozygosity or gene diversity among the sampling plots. Slightly lower allelic richness and private allele richness was noticed in the Sand1 plot (Table 4). Also, pairwise F_{ST} comparisons among the sampling plots indicated no genetic structure (Table 5). In France,

Table 4 Observed heterozygosity (H_o), gene diversity (H_s), allelic richness and private allelic richness (averaged over loci) among sampling sites within location

Finland						France					
	N	H_o	H_s	Allelic richness[a] (14 genes)	Private allelic[a] richness		*N*	H_o	H_s	Allelic richness[a] (30 genes)	Private allelic[a] richness
Sand1	9	0.506	0.605	5.66	1.23	Shore1	18	0.621	0.757	10.62	2.39
Edge1	23	0.542	0.665	6.48	1.62	Shore2	17	0.570	0.761	10.66	2.41
Centre	29	0.584	0.664	6.58	1.68	Sea2	32	0.581	0.834	12.98	3.35
Edge2	26	0.528	0.617	6.37	1.66						

[a] Calculated with HP-RARE

Table 5 Genetic differentiation (F_{ST}) between pairs of sampling sites within a country (10,000 permutations)

Finland				France		
	Centre	Edge1	Sand1		Shore1	Shore2
Centre	0			Shore1	0	
Edge1	−0.0007/−0.0034			Shore2	0.0142/0.0113	0
Sand1	0.0095/0.0033	0.0113/0.0025		Sea2	0.0007/−0.0021	*0.0218/0.0257*
Edge2	0.0036/0.0047	0.0012/0.0065	0.0110/0.0050			

First value is with 8 loci, second values with dataset of 6 loci (loci Pe12 and Pe15 removed in Finland, Pe12 and Pe13 in France). Italic values are statistically significant ($P \leq 0.01$)

however, low but significant structure was seen between the shore plot 2 and the plot furthest away from the shore (Sea2). Sea2 also had slightly higher allelic richness and private allele number (from HP-RARE, no significance test) compared to the other plots, but no difference in heterozygosity or gene diversity was observed among the sampling plots in France (Table 4). F_{ST} estimates calculated with the original dataset as well as the dataset corrected with ENA for null alleles were similar (data not shown). Also, F_{ST} estimates calculated with the reduced dataset (6 loci) and the original dataset (8 loci) were similar (Table 5).

When data from the sampling plots within each location were combined, we observed significant genetic population structure between Finland and France ($F_{ST} = 0.078$, $P \leq 0.001$, $D_{est} = 0.138$). F_{ST} calculations with the ENA correction for null alleles yielded a similar value ($F_{ST} = 0.076$). Removing the three loci with HWE deviations did not affect the D_{est} or F_{ST} results (data not shown). Comparing the populations with Fstat revealed that the French population had significantly higher allelic richness (France: 7.44, Finland: 6.27, $P = 0.029$) and gene diversity (France: 0.797, Finland: 0.645, $P = 0.033$) than the Finnish population. Furthermore, when allelic richness was estimated with HP-RARE, the French population was also more variable than the Finnish population (allelic richness 20.21 vs. 16.46) and seemed to possess a larger number of private alleles (private allele richness 6.44 vs. 2.69, both calculated with minimum sample size of 110 genes). However, observed heterozygosity (France: 0.590, Finland: 0.549) and F_{IS} estimates (France: 0.260, Finland: 0.149) were not significantly different between the two populations.

Discussion

We isolated new microsatellite loci and investigated their performance in revealing fine scale genetic structure in the opportunistic polychaete *Pygospio elegans* from two populations differing in habitat characteristics: a sheltered seagrass patch in a brackish site and a disturbed mudflat in a marine tidal environment. These different habitats reflect the extremes of habitats where *P. elegans* is found. Life history characteristics of opportunistic species, such as early reproduction, high fecundity, strong dispersal ability and a short life span, have the potential to affect population genetic structure, but the resulting patterns may be habitat-specific and difficult to generalize. Indeed, opportunistic species are expected to benefit from being more plastic, showing population specific life history characteristics according to local conditions (Zajac, 1991). The developmental polymorphism of *P. elegans* is a potentially plastic life history response which differs between the two populations we studied, but developmental polymorphism within these populations has not been observed.

In the relatively stable low density population of Ängsö (Finland), no genetic structure among sampling plots was found (Table 5), but there was slightly lower diversity (allelic richness) in the sand plot compared to the plots sampled from the seagrass patch (Table 4). Here, worms were more abundant within the *Z. marina* patch compared to the ambient bare sand, and no dense tube mats were seen. At the small spatial scale of the seagrass patch (12 m diameter), movement of juveniles and adults within the patch may act to reduce potential genetic structuring. In polychaetes, adults and juveniles can disperse, but time spent dispersing and distances moved are expected to be shorter for adults than for larvae (Levin, 1984). Adult *P. elegans* showing active crawling behaviour have been reported previously, either moving closer to conspecifics or avoiding other species (Wilson, 1983) or possibly avoiding overexploitation of resources in an area (Bolam & Fernandes, 2002). With larval brooding (the only developmental mode observed at Ängsö), fewer offspring are produced, larvae lack a dispersive planktonic stage, and larvae have a higher probability of settling within their native population close to their parents. However, even though we expect the brooded larvae to be poor dispersers, the emerged juveniles and adults of *P. elegans* may still enter the plankton through resuspension/physical disturbance, which has been observed in sandy sites of the Finnish archipelago (Boström et al., 2010; Valanko et al., 2010). If brooding is the predominant developmental mode seen in the fragmented seagrass landscape of the Finnish archipelago, population genetic structure patterns would be expected on a larger spatial scale, e.g. among seagrass patches within a site or between sites (10–50 km). Mitochondrial DNA sequence data has indicated some differentiation between Ängsö and other populations in

the Finnish archipelago (Kesäniemi et al., 2012 in press).

In contrast, the Bay of Somme (France) mudflat experiences hydrodynamic and human mediated disturbance and is optimal for opportunistic species (Desprez et al., 1992). Here, our sampled plots were more distant from each other than the plots in Finland because the mudflat is broad and the population is not restricted by a vegetation mosaic. Given the greater potential for dispersal of planktonic larvae at this site, our samples still represent a fine spatial scale relative to the size of the mudflat. Significant F_{ST} observed between two of our sampling plots: Shore2 and Sea2 (Table 5); indicate that population structure exists at this spatial scale. Many different processes that can lead to development of high density patches could also lead to genetic structuring within *P. elegans.* Although planktonic larvae have the potential for extensive dispersal, Morgan (1997) speculated that gregarious settlement of larval cohorts (siblings) caused by local currents in the Bay of Somme estuary leads to the formation of particularly dense tube mats. We attempted to avoid this potential complication by not sampling from different tube beds, but from an area of uniformly high density. Nevertheless, gregarious settlement is still possible even though no distinct tube bed patches were visible. Dense tube beds can stabilize the sediment on a small scale (Bolam & Fernandes, 2003) and tube beds may resist effective water flow, causing individuals not to be passively transported away from the patch (Morgan, 1997). Higher *P. elegans* juvenile survival within dense tube beds (Morgan, 1997) and possible sweepstakes reproduction (a limited number of parents contributing to the recruitment; Hedgecock, 1994) could facilitate the formation of genetic clusters at a small spatial scale. Sweepstakes reproduction is thought to be more common in changing environments and when species produce large numbers of offspring (Hedgecock, 1994), but may be more relevant for free-spawning invertebrates, which is not the case for *P. elegans*. Unfortunately, we have not sampled at smaller spatial scales in Bay of Somme, so we cannot make any firm conclusions about potential structure within the mudflat at distances <100 m.

Other studies have shown genetic structure in marine organisms on a small spatial scale (e.g. Todd et al., 1998; Tatarenkov et al., 2007; Dupont et al., 2009), but the data are scarce. In the gastropod *Drupella cornus* with planktonic larvae, differences were seen in larval recruitment groups on a fine scale of 80 m (Johnson et al., 1993). Also, in the brooded coral *Seriatopora hystrix,* which has an opportunistic life history strategy, significant population structure is seen on an fine scale (>100 m) due to larval recruitment to their natal reef (Underwood et al., 2007). Porter et al. (2002) found significant genetic structure within *Alcyonidium* bryozoan colonies, even in the species with pelagic larvae. Population genetic studies of species with planktonic larvae conducted at larger spatial scales have shown unexpected population structure contrary to an initial expectation of high gene flow (e.g. Ayre & Hughes, 2000; Weber et al., 2000; Hellberg et al., 2002; Taylor & Hellberg, 2003; Whitaker, 2004). These findings may indicate that genetic structure may be more prevalent at smaller spatial scales in these species as well.

There was significant genetic differentiation between the French and the Finnish study sites, as we expected, and we conclude that gene flow between seas is more restricted than within sea regions. This result was not surprising, given the considerable geographic distance between the countries. The high density French population was observed to be more diverse, which suggest a larger effective population size (e.g. Pechenik, 1999) in France compared to Finland. The predominance of planktonic larvae in this population as well as possible input of larvae from other populations likely contributes to the high diversity we observed. Asexual reproduction (Rasmussen, 1953; Wilson, 1983; Anger, 1984; Gudmundsson, 1985) could lower diversity, and might explain the comparatively lower diversity seen in Finland.

From these analyses, we can conclude that the microsatellite loci that we isolated from *P. elegans* are useful for estimating genetic diversity within and genetic differentiation among populations at different spatial scales. The loci are polymorphic, some with a very large number of alleles, and they are in linkage equilibrium. Some deviations from Hardy–Weinberg expectations were observed, however, particularly in loci Pe12, Pe13 and Pe15. These deviations could be due to technical problems in allele detection, and tests with Micro-Checker and FreeNA indicated a moderate to high probability of null alleles. Nevertheless, since all sampling plots did not show these deviations from HWE, we should consider biological factors that could be involved, in addition to technical problems. In some

cases, the deviation may be due to plot specific reasons. For example, if the sample contains many related individuals due to gregarious settlement of a cohort, it would not be surprising to find a deviation from Hardy–Weinberg proportions. Temporal population structure within the populations or plots could also cause such deviations. In the Bay of Somme population *P. elegans* has at least two reproduction peaks per year (Morgan, 1997), and individuals from different generations could be in our sample. The significant positive F_{IS} estimates seen in both France and Finland can represent deficiency in heterozygosity and therefore also reflect the deviation from HWE. However, potential asexual reproduction in Ängsö could have increased the possibility for inbreeding in this population. Likewise, potential sweepstakes reproduction and reduced water flow in tube beds could increase the possibility for inbreeding in France. High, positive F_{IS} values are not uncommon in population genetic studies of marine invertebrates (Addison & Hart, 2005).

Generalizations of expected population genetic structure in *P. elegans* is difficult since genetic structure can be affected not only by the differences in opportunistic behaviour of the worms but also the predominant developmental mode observed in populations and potential dispersal of both larvae and adults. It is important to take different spatial scales into account as the relevant spatial scale may differ between populations. For opportunistic species and other species with plastic life history characteristics, understanding genetic structure may require more than the traditional analyses of estimating *F* statistics and gene flow from samples taken at a single time point. Temporal analyses and a more complete understanding of how the life history characteristics change with changing environments may be needed.

Acknowledgments The authors would like thank Camilla Gustafsson and Elzemiek Geuverink for help with the collecting. JEK received funding from the Rector of the University of Jyväskylä and this study was done as part of the Centre of Excellence in Evolutionary Research at University of Jyväskylä.

References

Addison, J. A. & M. W. Hart, 2005. Spawning, copulation and inbreeding coefficients in marine invertebrates. Biology Letters 1: 450–453.

Anger, V., 1984. Reproduction in *Pygospio elegans* (Spionidae) in relation to its geographical origin and to environmental conditions: a preliminary report. Fortschritte der Zoologie 29: 45–51.

Anger, K., V. Anger & E. Hagmeier, 1986. Laboratory studies on larval growth of *Polydora ligni*, *Polydora ciliata* and *Pygospio elegans* (Polychaeta, Spionidae). Helgolander Meeresuntersuchungen 40: 277–395.

Ayre, D. J. & T. P. Hughes, 2000. Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54: 1590–1605.

Bay, L. K., M. J. M. Caley & R. H. Crozier, 2008. Meta-population structure in a coral reef fish demonstrated by genetic data on patterns of migration, extinction and re-colonisation. BMC Evolutionary Biology 8: 248–265.

Beukema, J. J., E. C. Flach, R. Dekker & M. Starink, 1999. A longterm study of the recovery of the macrozoobenthos on large defaunated plots on a tidal flat in the Wadden Sea. Journal of Sea Research 42: 235–254.

Bolam, S. G., 2004. Population structure and reproductive biology of *Pygospio elegans* (Polychaeta:Spionidae) on an intertidal sandflat, Firth of Forth, Scotland. Invertebrate Biology 123: 260–268.

Bolam, S. G. & T. F. Fernandes, 2002. Dense aggregations of tube-building polychaetes: response to small-scale disturbances. Journal of Experimental Marine Biology and Ecology 269: 197–222.

Bolam, S. G. & T. F. Fernandes, 2003. Dense aggregations of *Pygospio elegans* (Claparede): Effect on macrofaunal community structure and sediments. Journal of Sea Research 49: 171–185.

Boström, C. & E. Bonsdorff, 1997. Community structure and spatial variation of benthic invertebrates associated with *Zostera marina* (L.) beds in the northern Baltic Sea. Journal of Sea Research 37: 153–166.

Boström, C. & E. Bonsdorff, 2000. Zoobenthic community establishment and habitat complexity - the importance of seagrass shoot density, morphology and physical disturbance for faunal recruitment. Marine Ecology Progress Series 205: 123–138.

Boström, C., K. O'Brien, C. Roos & J. Ekebom, 2006. Environmental variables explaining structural and functional diversity of seagrass macrofauna in an archipelago landscape. Journal of Experimental Marine Biology and Ecology 335: 52–73.

Boström, C., A. Törnroos & E. Bonsdorff, 2010. Invertebrate dispersal and habitat heterogeneity: Expression of biological traits in a seagrass landscape. Journal of Experimental Marine Biology and Ecology 390: 106–117.

Branch, G. M. & C. N. Steffani, 2004. Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). Journal of Experimental Marine Biology and Ecology 300: 189–215.

Bridges, T. S., L. A. Levin, D. Cabera & G. Plaia, 1994. Effects of sediment amended with sewage, algae, or hydrocarbons on growth and reproduction in two opportunistic polychaetes. Journal of Experimental Marine Biology and Ecology 177: 99–119.

Chapuis, M.-P. & A. Estoup, 2007. Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24: 621–631.

Crawford, N. G., 2010. SMOGD: Software for the measurement of genetic diversity. Molecular Ecology Resources 10: 556–557.

Desprez, M., H. Rybarczyk, J. G. Wilson, J. P. Ducrotoy, F. Seuer, R. Olivesi & B. Elkaim, 1992. Biological impact of eutrophication in the Bay of Somme and the induction and impact of anoxia. Netherlands Journal of Sea Research 30: 149–159.

Dupont, L., F. Viard, M. J. Dowell & D. D. Bishop, 2009. Fine- and regional-scale genetic structure of the exotic ascidian *Styela clava* (Tunicata) in southwest England, 50 years after its introduction. Molecular Ecology 18: 442–453.

Excoffier, L. & H. E. L. Lischer, 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567.

Fleischer, D. & M. L. Zettler, 2008. An adjustment of benthic ecological quality assessment to effects of salinity. Marine Pollution Bulletin 58: 351–357.

Goudet, J., 1995. FSTAT (version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86: 485–486.

Grapputo, A., 2006. Development and characterization of microsatellite markers in the Colorado potato beetle, *Leptinotarsa decemlineata*. Molecular Ecology Notes 6: 1177–1179.

Grassle, J. F. & J. P. Grassle, 1974. Opportunistic life histories and genetic systems in marine benthic polychaetes. Journal of Marine Research 32: 253–284.

Gudmundsson, H., 1985. Life history patterns of polychaete species of the family Spionidae. Journal of the Marine Biological Association of the United Kingdom 65: 93–111.

Hannerz, L., 1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil and Poecilochaetidae N. fam. in the Gullmar Fjord (Sweden). Zoologiska Bidrag från Uppsala 31: 1–204.

Hedgecock, D., 1994. Does variance in reproductive success limit effective population size of marine organisms? In Beaumont, A. R. (ed.), Genetics and Evolution of Aquatic Organisms. Chapman & Hall, London: 122–134.

Hellberg, M. E., R. S. Burton, J. E. Neigel & S. R. Palumbi, 2002. Genetic assessment of connectivity among marine populations. Bulletin of Marine Science 70: 273–290.

Johnson, M. S., K. Holborn & R. Black, 1993. Fine-scale patchiness and genetic heterogeneity of recruits of the corralivorous gastropod *Drupella cornus*. Marine Biology 117: 91–96.

Kalinowski, S. T., 2005. HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5: 187–189.

Karhan, S. U., E. Kalkan, N. Simboura, E. Mutlu & M. Bekbölet, 2008. On the Occurrence and Established Populations of the Alien Polychaete *Polydora cornuta* Bosc, 1802 (Polychaeta: Spionidae) in the Sea of Marmara and the Bosphorus Strait (Turkey). Mediterranean Marine Science 9: 5–19.

Kesäniemi, J. E., P. D. Rawson, S. M. Lindsay & K. E. Knott, 2012. Phylogenetic analysis of cryptic speciation in the polychaete *Pygospio elegans*. Ecology and Evolution. doi: 10.1002/ece3.226.

Kube, J. & M. Powilleit, 1997. Factors controlling the distribution of *Marenzelleria* cf. *viridis*, *Pygospio elegans* and *Steblospio shrubsoli* (Polychaeta: Spionidae) in the southern Baltic Sea, with special attention for the response to an event of hypoxia. Aquatic Ecology 31: 187–198.

Levin, L. A., 1984. Life history and dispersal patterns in a dense infaunal polychaete assemblage: Community structure and response to disturbance. Ecology 65: 1185–1200.

Levin, L. A., 1986. Effects of enrichment on reproduction in the opportunistic polychaete *Streblospio benedicti* (Webster): A mesocosm study. Biological Bulletin 171: 143–160.

McMahon, R. F., 2002. Evolutionary and physiological adaptations of aquatic invasive animals: r selection versus resistance. Canadian Journal of Fisheries and Aquatic Sciences 59: 1235–1244.

Morgan, T. S. 1997. The formation and dynamics of *Pygospio elegans* tube-beds in the Somme Bay, France. Dissertation, Southampton University.

Morgan, T. S., A. D. Rogers, G. L. J. Paterson, L. E. Hawkins & M. Sheader, 1999. Evidence for poecilogony in *Pygospio elegans* (Polychaete: Spionidae). Marine Ecology Progress Series 178: 121–132.

Muus, B. J., 1967. The fauna of Danish estuaries and lagoons. Distribution and ecology of dominating species in the shallow reaches of the mesohaline zone. Meddelelser: Danmarks Fiskeri- og Havundersogelser 5: 3–316.

Pearson, T. H. & R. Rosenberg, 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology – An Annual Review, vol 16: 229–311

Pechenik, J. A., 1999. On the advantages and disadvantages of larval stages in benthic invertebrate life cycles. Marine Ecology Progress Series 177: 269–297.

Pedersen, T. M., J. L. S. Hansen, A. B. Josefson & B. W. Hansen, 2008. Mortality through ontogeny of soft-bottom marine invertebrates with planktonic larvae. Journal of Marine Systems 73: 185–207.

Pianka, E. R., 1970. On *r*- and *K*-selection. American Naturalist 104: 592–597.

Porter, J. S., J. S. Ryland & G. R. Carvalho, 2002. Micro- and macrogeographic genetic structure in bryozoans with different larval strategies. Journal of Experimental Marine Biology and Ecology 272: 119–130.

Rasmussen, E., 1953. Asexual reproduction in *Pygospio elegans* Claparède (*Polychaeta sedentaria*). Nature 171: 1161–1162.

Rasmussen, E., 1973. Systematics and ecology of the Isefjord marine fauna (Denmark). Ophelia 11: 1–495.

Raymond, M. & F. Rousset, 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248–249.

Rozen S. & H. J. Skaletsky, 2000. PRIMER 3 on the WWW for general users and for biologist programmers. In Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa: 365–386.

Rybarczyk, H., M. Desprez, J.-P. Ducrotoy, R. Olivesi, R. Delesmont, F. Jarnet & B. Elkaim, 1993. Dynamics of nutrients and faecal bacteria in a macrotidal estuary, The Bay of Somme (France). Netherlands Journal of Aquatic Ecology 27: 395–404.

Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18: 233–234.

Streftaris, N. & A. Zenetos, 2006. Alien marine species in the Mediterranean – the 100 'Worst Invasives' and their impact. Mediterranean Marine Science 7: 87–118.

Tatarenkov, A., R. B. Jönsson, L. Kautsky & K. Johannesson, 2007. Genetic structure of *Fucus vesiculosus* (Phaeophyceae) over spatial scales from 10 m to 800 km. Journal of Phycology 43: 675–685.

Taylor, M. S. & M. E. Hellberg, 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299: 107–109.

Todd, C. D., W. J. Lambert & J. P. Thorpe, 1998. The genetic structure of intertidal populations of two species of nudibranch mollusks with planktotrophic and pelagic lecithotrophic larval stages: are pelagic larvae "for" dispersal? Journal of Experimental Marine Biology and Ecology 118: 1–28.

Tsutsumi, H., 1987. Population dynamics of *Capitella capitata* (Polychaeta; Capitellidae) in an organically polluted cove. Marinr Ecology Progress Series 36: 139–149.

Tsutsumi, H., 1990. Population persistence of Capitella sp. (Polychaeta; Capitellidae) on a mud flat subject to environmental disturbance by organic enrichment. Marine Ecology Progress Series 63: 147–156.

Underwood, J. N., L. D. Smith, M. J. H. Van Oppen & J. P. Gilmour, 2007. Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. Molecular Ecology 16: 771–784.

Valanko, S., A. Norkko & J. Norkko, 2010. Strategies of post-larval dispersal in non-tidal soft-sediment communities. Journal of Experimental Marine Biology and Ecology 384: 51–60.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills & P. Shipley, 2004. Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535–538.

Warwick, R. M., 1986. A new method for detecting pollution effects on marine macrobenthic communities. Marine Biology 92: 557–562.

Weber, L. I., R. G. Hartnoll & J. P. Thorpe, 2000. Genetic divergence and larval dispersal in two spider crabs (Crustacea:Decapoda). Hydrobiologia 420: 211–219.

Whitaker, K., 2004. Non-random mating and population genetic subdivision of two broadcasting corals at Ningaloo Reef, Western Australia. Marine Biology 144: 593–603.

Whitlatch, R. B. & R. N. Zajac, 1985. Biotic interactions among estuarine infaunal opportunistic species. Marine Ecology Progress Series 21: 299–311.

Wilson, W. H., Jr., 1983. The role of density dependence in a marine infaunal community. Ecology 64: 295–306.

Zajac, R. N., 1991. Population ecology of *Polydora ligni* (Polychaeta: Spionidae): I. Seasonal variation in population characteristics and reproductive activity. Marine Ecology Progress Series 77: 197–206.

Zane, L., L. Bargelloni & T. Patarnello, 2002. Strategies for microsatellite isolation: A review. Molecular Ecology 11: 1–16.

III

POLYMORPHISM IN DEVELOPMENTAL MODE AND ITS EFFECTS ON POPULATION GENETIC STRUCTURE OF A SPIONID POLYCHAETE *PYGOSPIO ELEGANS*

by

Jenni Kesäniemi, Elzemiek Geuverink & K. Emily Knott

Integrative and Comparative Biology 52(1): 181–196.

Integrative and Comparative Biology

Integrative and Comparative Biology, volume 52, number 1, pp. 181–196
doi:10.1093/icb/ics064

Society for Integrative and Comparative Biology

SYMPOSIUM

Polymorphism in Developmental Mode and Its Effect on Population Genetic Structure of a Spionid Polychaete, *Pygospio elegans*

Jenni E. Kesäniemi,[1,*] Elzemiek Geuverink[*,†] and K. Emily Knott[*]

[*]Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014, Finland;
[†]Evolutionary Genetics, University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands

From the symposium "Poecilogony as a Window on Larval Evolution: Polymorphism of Developmental Mode within Marine Invertebrate Species" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2012 at Charleston, South Carolina.

[1]E-mail: jenni.kesaniemi@jyu.fi

Synopsis Population genetic structure of sedentary marine species is expected to be shaped mainly by the dispersal ability of their larvae. Long-lived planktonic larvae can connect populations through migration and gene flow, whereas species with nondispersive benthic or direct-developing larvae are expected to have genetically differentiated populations. Poecilogonous species producing different larval types are ideal when studying the effect of developmental mode on population genetic structure and connectivity. In the spionid polychaete *Pygospio elegans*, different larval types have been observed between, and sometimes also within, populations. We used microsatellite markers to study population structure of European *P. elegans* from the Baltic Sea (BS) and North Sea (NS). We found that populations with planktonic larvae had higher genetic diversity than did populations with benthic larvae. However, this pattern may not be related to developmental mode, since in *P. elegans*, developmental mode may be associated with geography. Benthic larvae were more commonly seen in the brackish BS and planktonic larvae were predominant in the NS, although both larval types also are found from both areas. Significant isolation-by-distance (IBD) was found overall and within regions. Most of the pair-wise F_{ST} comparisons among populations were significant, although some geographically close populations with planktonic larvae were found to be genetically similar. However, these results, together with the pattern of IBD, autocorrelation within populations, as well as high estimated local recruitment, suggest that dispersal is limited in populations with planktonic larvae as well as in those with benthic larvae. The decrease in salinity between the NS and BS causes a barrier to gene flow in many marine species. In *P. elegans*, low, but significant, differentiation was detected between the NS and BS (3.34% in AMOVA), but no clear transition zone was observed, indicating that larvae are not hampered by the change in salinity.

Introduction

Marine invertebrates are known for their diverse life-history characteristics and developmental modes (e.g., Mileikovsky 1971; Wilson 1991; Blake and Arnofsky 1999; Ellingson and Krug 2006; Raff and Byrne 2006). Developmental mode includes the dispersal potential of larvae, whether larvae are planktonic, benthic, or brooded, and the larvae may be the primary dispersal stage for invertebrates that are sedentary or sessile as adults. Differences in the dispersal abilities of larvae can affect effective population size, population stability, gene flow, and genetic structure, as well as rate of speciation (e.g., Hart and Marko 2010). As a result, species with planktonic, dispersive larvae are expected to have effective gene flow over a broader geographic area and large panmictic populations. Direct-developing or benthic larvae are expected to have less ability to disperse, and species with these types of larvae are expected have low gene flow and genetically differentiated populations (Palumbi 1994; Bohonak 1999). Empirical studies are often in agreement with these expectations (Hellberg 1996; Arndt and Smith 1998; Kyle and Boulding 2000; Collin 2001; Dawson et al. 2002; Ellingson and Krug 2006), but there are also an increasing number of studies which reach opposite conclusions (Sotka et al. 2004; Bowen et al. 2006;

Advanced Access publication May 10, 2012

Kenchington et al. 2006; Marko et al. 2007; Miller and Ayre 2008; Shanks 2009; Weersing and Toonen 2009; Zhan et al. 2009; Kelly and Palumbi 2010; and see Hellberg 2009). The unexpected results emphasize that other factors, such as environmental tolerances or larval behavior, can affect population genetic structure and suggest that the ability of larvae to disperse may not be the main determinant of population connectivity and genetic structure in marine species. Nevertheless, the ability of larvae to disperse is still considered the most important factor affecting population stability and connectivity of marine species.

Barriers to dispersal and gene flow in the marine environment may not be immediately obvious. Typically, barriers are inferred after examining patterns of genetic structure among populations. More recently, attempts have been made to explicitly combine models of oceanographic conditions together with genetic analysis to understand influences on gene flow (e.g., Selkoe et al. 2010). Combining physical and genetic data in studies of gene flow provide a powerful way of identifying dispersal barriers and to evaluate whether generalizations of the outcomes of larval dispersal are correct. Since dispersal of marine invertebrate larvae is difficult, or impossible, to track directly (Thorrold et al. 2002; Levin 2006), a generalization of potential capability for dispersal based on developmental mode is still commonplace.

Natural barriers to dispersal may exist in transitional zones where environmental variables, such as salinity, are known to change significantly. For example, the Baltic Sea (BS) is a marginal environment for marine species because of its isolation and low salinity. The BS was formed after the last glacial period (10,000–8000 years ago) and during its young history its salinity has fluctuated, allowing marine species to become established there (Zillen et al. 2008; Pereyra et al. 2009). Nowadays, the BS is an ecologically unique, large brackish sea with limited water exchange with the marine North Sea (NS). As a result, there is a salinity gradient from the NS (>30 psu) through the Kattegat (20 psu), Belt Sea (15–18 psu), and Baltic proper (8–10 psu) to the Northern BS (2–3 psu). In addition to low salinity, the BS has very weak tides, lower water temperature, and lower oxygen levels compared to the NS (Helcom 2003). The drastic environmental change seen between the BS and NS may be a restricting factor for successful migration of larvae between these areas. Species with populations spanning this zone (NS, BS, see Fig. 1) provide a way to assess the

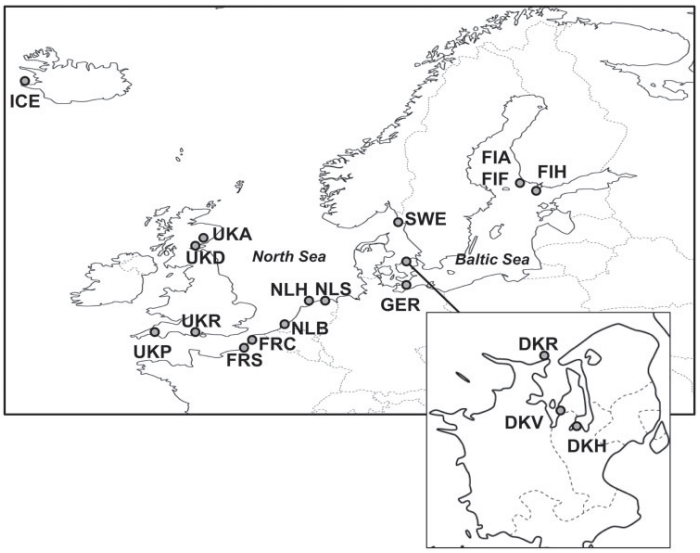


Fig. 1 Map of the sites at which *Pygospio elegans* was sampled (sites are labeled according to their abbreviations in Table 1). Sites FIA and FIF are located in the Finnish archipelago, ~20 km apart.

relative roles of environmental barriers and potential for larval dispersal to affect gene flow and the genetic structure of populations.

Despite the young geological history of the BS, many Baltic populations have diverged significantly from marine NS populations of the same species. Johannesson and André (2006) conducted a meta-analysis of genetic studies comparing Baltic populations to NS/NE Atlantic populations of the same species. They found that among the 29 species studied, the majority of Baltic populations were statistically less genetically variable and genetically more differentiated from their Atlantic populations. BS populations could be differentiated due to geographic isolation, possible genetic bottlenecks (Nilsson et al. 2001; Härkönen et al. 2005), or adaptation to a different environment (Hemmer-Hansen et al. 2007; Larmuseau et al. 2010; Nissling and Dahlman 2010).

We study the spionid polychaete worm, *Pygospio elegans*, which is found in the NS and BS, exposed to a wide range of salinities. This species is an ideal subject for investigating the relative roles of salinity barriers and developmental mode in determining patterns of population genetics because *P. elegans* is poecilogonous, or polymorphic in developmental mode. *Pygospio elegans* can produce different larval types depending on the number of embryos relative to the number of nurse eggs (a nutritional source) laid by the mother to egg capsules that she broods inside her sand tube (Söderström 1920; Hannerz 1956; Rasmussen 1973; Blake and Arnofsky 1999; J. E. Kesäniemi, personal observation). Planktonic larvae emerge from egg capsules containing a large number of embryos (>20/capsule) and few nurse eggs. At emergence, these larvae are approximately three chaetigers long and have long swimming chaetae. Their pelagic period can last up to 4–5 weeks (Anger et al. 1986) and they actively swim and feed in the plankton (Rasmussen 1973). If only a few embryos (one to two) are laid into the capsules, these will be brooded throughout their development. These benthic larvae feed on the nurse eggs provided by the mother, lack long swimming chaetae and are ready to metamorphose into juveniles soon after emerging from the capsules at the 14–20 chaetiger stage. Intermediate larvae with fewer than 10 larvae per capsule, an intermediate brooding period, and short pelagic phase have also been observed in some populations. Dispersal of larvae is expected to be the primary route for gene flow and to be correlated with developmental mode in *P. elegans*. Adults are motile and will leave their tubes if they are disturbed (J. E. Kesäniemi and K. E. Knott, personal observation), but they quickly build new sand tubes in the sediment (e.g., Mattila 1997) and so are not expected to contribute significantly to dispersal.

Variation in developmental mode in *P. elegans* has been observed both within and among populations, arising from among individual differences rather than variation within the broods of a single individual (Hannerz 1956; Rasmussen 1973; Anger 1984; Gudmundsson 1985; Morgan et al. 1999; J. E. Kesäniemi and E. Geuverink, personal observation). Variation within a population can be seasonal (Hannerz 1956; Rasmussen 1973), but different larval types are also known to occur simultaneously (Rasmussen 1973; Gudmundsson 1985; J. E. Kesäniemi, personal observation). Some populations of *P. elegans* have been reported to be fixed for one developmental mode (either planktonic or benthic) (Anger 1984; Morgan et al. 1999; Bolam 2004), but adults metamorphosing from all larval types are morphologically identical. In addition, studies of allozymes (Morgan et al. 1999) and DNA sequences of the cytochrome c oxidase I gene (Kesäniemi et al. 2012b) provided evidence that *P. elegans* populations with different developmental modes belong to one poecilogonous species.

We examined genetic diversity and population genetic structure in European populations of *P. elegans* with different developmental modes. Our study includes samples over a broad geographic scale, from the Atlantic Ocean (Iceland), through the English Channel and NS to the BS. We wanted to investigate if the transition zone caused by a decrease in salinity between the NS and BS affects population genetic structure in this poecilogonous species. We expected low diversity and low gene flow between populations in the Baltic where benthic larvae are more common. In contrast, we expected high diversity and high effective gene flow among NS populations in which planktonic larvae predominate. We hypothesized that a distinct transition would be visible between these seas, as has been found for many species that span this transition zone (Johannesson and André 2006).

Material and methods

Sampling

We sampled adult *P. elegans* from 18 populations in Europe (Fig. 1). Almost all sampling was conducted during late winter, spring, or early summer of 2010: exceptions included the Swedish sample, which was collected in summer of 2008, and samples from Iceland and Germany, which were collected in 2009. In the nontidal BS, sediment containing *P. elegans* was collected either by scuba diving

Table 1 Sampling localities, population codes, number of samples genotyped (*n*), observed developmental modes, and estimated density of *Pygospio elegans* (qualitative observations only)

Region	Country	Location	Lat	Long	Population code	*n*	Developmental mode[a]	Estimated density
Baltic Sea	Finland	Ängsö	60.107	21.709	FIA	42	B (A)	Low
		Fårö	59.925	21.772	FIF	39	(A)	Low
		Hanko	59.827	21.772	FIH	40	(A)	Low
	Germany	Germany	54.199	11.840	GER	30		
	Denmark	Vellerup	55.737	11.868	DKV	43	B, I (A)	Medium
		Herslev	55.678	11.987	DKH	42	B, I (A)	Low
		Rorvig	55.965	11.785	DKR	40	I, P (A)	Medium
	Sweden	Gullmar fjord	58.261	11.464	SWE	42		Low
North Sea	Netherlands	Schiermonnikoog	53.472	6.177	NLS	46	B, I, P	Medium/high
		Harlingen	53.167	5.415	NLH	43	P	High
		Breskens	51.386	3.604	NLB	45	P	Medium/high
	France	Canche Bay	50.547	1.598	FRC	43	P	Very high
		Somme Bay	50.227	1.606	FRS	62	P	Very high
	UK	Drum sands	55.994	−3.336	UKD	49	P	High
		Eden estuary	56.365	−2.823	UKA	46		Medium/high
		Plym Bay	50.378	−4.103	UKP	33	P	Low/medium
		Ryde sands	50.734	−1.150	UKR	7		Very low
Atlantic Ocean	Iceland	Iceland	63.978	−22.395	ICE	42	B, I	

[a]Observed larval developmental mode: B = benthic, I = intermediate, P = planktonic, (A) = asexual reproduction.

at 5 m depth (FIA and FIF) or by wading and shoveling the sediment in <1.5 m deep water (FIH and all Danish samples). The German sample was collected from a depth of 18 m using a sediment grab operated from a boat. In the Netherlands, France, and UK, partially exposed sediment was sampled in the intertidal zone during low tides. All of the samples from the Netherlands, UK, and France are called ''NS samples,'' whereas those from Sweden, Denmark, Germany, and Finland are called ''BS samples'' (Table 1).

Collected sediment was immediately and quickly sieved on location, and sand tubes of *P. elegans* were removed. *Pygospio elegans* emerge from their tubes after disturbance (J. E. Kesäniemi and K. E. Knott, personal observation) and collected tubes were left in seawater in trays without sediment for up to 24 h to allow for such emergence. Afterward, the worms were preserved in ethanol (94–99%) until DNA analysis. During this handling, adults were sexed and examined for the presence of gametes or nurse eggs in the coelom. Tubes were also examined for the presence of egg capsules and brooding females. If capsules were found, the larvae were checked to determine their developmental mode. We did not specifically collect plankton samples, but sea water samples from the collection sites (used for collecting and handling the adults) were examined for the presence of swimming larvae in the laboratory. In addition, we noted whether asexual reproduction was present (indicated by regenerating body segments). According to these observations, we defined a predominant developmental mode for most populations (Table 1). These were consistent with previous reports of observed developmental mode for some populations (Denmark: Rasmussen 1973; Somme Bay: Morgan et al. 1999; Drum Sands: Bolam 2004; Breskens: Rossi et al. 2009), but not others (Ryde Sands: Morgan et al. 1999). Hannerz (1956) reported brooded and intermediate larval types for *P. elegans* in Gullmar Fjord, Sweden, but we could not confirm this since we did not observe reproducing individuals or larvae during our collections at this location.

Molecular methods

DNA was extracted using a Kingfisher magnetic processor (Thermo Fisher Scientific) and Qiagen chemicals or, if the individual was <1 cm in length, Qiagen's DNeasy Blood and Tissue extraction kit with spin columns, following the manufacturer's protocol. All samples were genotyped with eight microsatellite loci described in (Kesäniemi et al. 2012a). Three of these loci (Pe15, Pe17, and Pe18) were amplified using a sequence specific forward primer with additional M13(−21) sequence tail and a fluorescently labeled M13(−21) primer (as described by Schuelke 2000). Amplification was performed in 10 µl reactions with 1 µl of DNA, 1× PCR buffer (Biotools), 200 µM of each dNTP (Fermentas), 8 pmol of reverse primer (TAG Copenhagen), 8 pmol of labeled M13(−21) primer (Applied Biosystems), 2 pmol of the M13(−21) tailed forward

primer (TAG Copenhagen), 1.5–2 mM of $MgCl_2$ (1.5 mM for Pe15, 2 mM for Pe18 and Pe17) (Biotools), and 0.5 U of *Taq* DNA polymerase (Biotools). Thermocycling conditions were 94°C for 5 min, then 30 cycles of 94°C for 30 s, T_a for 30 s (57°), 72°C for 30 s, followed by eight cycles of 94°C for 30 s, 53°C for 45 s, 72°C for 45 s, ending with a final extension of 72°C for 10 min. The other loci (Pe6, Pe7, Pe12, Pe13, and Pe19) were amplified using two sequence-specific primers, forward primer being labeled. The 10 µl PCR reactions were as above, but 0.5 µM of both primers (one-eighth of the forward primer was labeled, Applied Biosystems) and 1.5–3 mM of $MgCl_2$ (1.5 mM for Pe13, 2 mM for Pe6 and Pe19, 3 mM for Pe7 and Pe12) were used. Thermocycling conditions were: 94°C for 5 min, then 35 cycles of at 94°C for 30 s, T_a for 30 s (54° for Pe6, Pe7, Pe19; 55° for Pe12; 58° for Pe13), 72°C for 30 s, followed by a final extension of 10 min at 72°C. Products were denatured with formamide, separated using an ABI PRISM 3130xl and genotyped using GeneMapper v.3.7 software (all Applied Biosystems).

Analysis

We used Arlequin v. 3.5.1.2 (Excoffier and Lischer 2010) to examine allele frequencies, observed and expected heterozygosity at all loci, and to test conformation to Hardy–Weinberg expectations (HWE). The exact test option was used and *P*-values were adjusted for multiple tests with Bonferroni correction (Rice 1989). Frequencies of null alleles in the loci were estimated with FreeNA (Chapuis and Estoup 2007). Linkage disequilibrium between pairs of loci within populations was tested with Genepop (Raymond and Rousset 1995). Allelic richness and private allelic richness were calculated with HP-Rare (Kalinowski 2005), which uses rarefaction to account for the different sample sizes in the study populations (UKR was excluded from this analysis because of its considerably lower sample size).

To compare genetic diversity between the two regions, the sample locations were divided into two groups (BS and NS; Table 1), excluding the Atlantic population from Iceland. Heterozygosity, gene diversity, and allelic richness in these groups were compared using a Mann–Whitney U-test. Genetic diversity was also compared between the populations that produce planktonic larvae (NLH, NLB, FRC, FRS, UKD, UKP) and the populations that in addition have benthic larvae (FIA, DKV, DKR, DKH, NLS, ICE). Populations for which developmental mode is not known (FIF, FIH, GER, SWE, UKA) were not included in this latter comparison.

Population genetic differentiation was analyzed using population pair-wise F_{ST} calculations using Arlequin (10,000 permutations) and Jost's (2008) pair-wise mean D_{est} calculated in the R package DEMEtics (Gerlach et al. 2010). To determine the proportion of genetic differentiation distributed among areas, a hierarchical analysis of molecular variance (AMOVA) was also conducted with Arlequin (10,000 permutations). In addition, we employed a Bayesian approach to investigate the number of genetic clusters in our sample with the program Structure v.2.3 (Pritchard et al. 2000). In these analyses, we used an admixture model with correlated alleles, and Markov-chain Monte–Carlo (MCMC) sampling with a burn-in of 150,000 followed by 300,000 iterations. Data from six of the microsatellite loci were included: Pe12 and Pe15 were removed due to HWE deviations in most populations. Initially, *K*-values from 3 to 18 were tested, each with two replicates. Afterward, the program was re-run with *K*-values from 4 to 10, each with six replicates. Population information was used as prior information, since it was informative ($r > 1$). Furthermore, the number of genetic clusters and their genetic boundaries were also examined with the R package Geneland (Guillot et al. 2005). In addition to using the genetic data, this program incorporates spatial information of the sampling locations (geographic coordinates) while estimating *K*. A spatial model and correlated frequency model were used, and the presence of null alleles was taken into account (Guillot et al. 2008). Ten independent runs with 200,000 MCMC iterations were performed.

Isolation-by-distance (IBD) was measured by Mantel tests and spatial autocorrelation tests using GenAlEx v.6.4 (Peakall and Smouse 2006). Both tests used the default genetic distance as calculated by GenAlEx in which an individual-by-individual squared distance is calculated based on the multilocus genotype (see Smouse and Peakall 1999). Geographic distances were calculated from latitude and longitude of the sampled population (all individuals in a population had identical geographic coordinates). Mantel tests and autocorrelation tests were performed for the whole data set, as well as for smaller spatial scales: NS; BS; Northern BS (Finnish populations only); and Southern BS (Danish populations + GER + SWE). Additionally we tested the correlation between linearized population pair-wise F_{ST} values (calculated in GenAlEx) with population pair-wise geographic distances (linear distances following water-routes between sampling sites measured in Google Earth 6 [http://www.google.com/earth/index.html]).

To investigate whether there is a genetic break between the BS and the NS we conducted a graphical analysis similar to that performed by Johannesson and André (2006) in their meta-analysis. The analysis is based on genetic differentiation (F_{ST}) between the innermost Baltic population (FIA) and the other populations at increasing geographic distances. For this analysis, following Johannesson and André (2006), the Skagerrak and Kattegat areas were defined to be outside the Baltic area, and we set the German sample to represent the entrance to the BS.

Recent migration rates were examined with two Bayesian methods, BayesAss 3 (Wilson and Rannala 2003) and BIMr v.1.0 (Faubet and Gaggiotti 2008). Since BayesAss assumes relatively low migration rates (a maximum of one-third of the population can be migrants), we also used BIMr (no restrictions on migration) to see if the results of the two methods were similar. For these analyses, the data set was divided into three subsets (to improve performance of the methods). The first data set included the three Finnish populations, the second included Denmark and Sweden, and the third included all of the populations from the Netherlands, UK, and France. For this third data set, some populations were combined, based on the nonsignificant F_{ST} values between them: UKR and UKP ($F_{ST} = 0.0243$); FRC and FRS ($F_{ST} = 0.0041$); and NLB and NLH ($F_{ST} = 0.0048$). NLS was not included in any grouping since it was significantly differentiated from the other populations in this data set. In all BayesAss analyses 1×10^8 iterations of MCMC sampling were run after an initial burnin (1×10^7–5×10^7). Using BIMr, the Finnish data set had 5×10^5 samples and 1×10^6 burnin, and the other data sets were run with 1×10^6 samples and burnin of 1×10^6. In all analyses, at least three runs were conducted for each data set to verify consistent results.

Results

Genetic diversity

A total of 734 *P. elegans* individuals from 18 locations were genotyped. Table 2 shows descriptive statistics for each population and locus. The allelic richness across loci per population ranged from 8.66 to 15.65 (corrected for sample size, UKR excluded) and the number of alleles per locus varied among the populations. Heterozygosity was also high (mean H_E ranging from 0.586 to 0.816 and mean H_O ranging from 0.523 to 0.721). Significant deviations from HWE were observed in six loci in some of the populations and all deviations were caused by heterozygote deficiency. Loci Pe15 and Pe12 proved to deviate from HWE in most populations, so some of the analyses were conducted both with, and without, these loci. Analysis with FreeNA confirmed high null allele frequency for locus Pe12, but moderate or low null allele frequencies for the other loci and populations with HWE deviations. FreeNA implements an ENA correction (Chapuis and Estoup 2007) to samples with null alleles and estimates F_{ST} for both the corrected data and original data. Since F_{ST} estimates were similar either with or without the ENA correction ($F_{ST} = 0.0408$ and $F_{ST(ENA)} = 0.0377$), we assumed that possible null alleles cause no bias to our F_{ST} estimates. Linkage disequilibrium was observed between some pairs of loci (1.8% of all comparisons) in three of the study populations: Sweden (Pe7 × Pe17, Pe15 × Pe17, Pe7 × Pe18, Pe17 × Pe18, Pe7 × Pe13, Pe13 × Pe17), Iceland (Pe7 × Pe13, Pe12 × Pe13), and DKR (Pe15 × P13). Since linkage disequilibrium was not consistent in all populations, we chose not to exclude the loci from the analyses.

When comparing geographic areas, NS populations had significantly higher allelic richness (Md NS = 14.87, BS = 12.35, $N = 16$, $U = 62.00$, $P = 0.002$) and expected heterozygosity (Md NS = 0.801, BS = 0.717, $N = 17$, $U = 62$, $P = 0.012$) than did BS populations. NS populations also had higher observed heterozygosity, gene diversity, and private allelic richness (private alleles are found only from one population), but these results were not statistically significant. Populations with planktonic larvae had higher allelic richness (Md planktonic = 15.19, Md benthic = 12.4, $U = 36$, $N = 12$, $P = 0.004$), expected heterozygosity (Md planktonic = 0.801, Md benthic = 0.717, $N = 12$, $U = 36$, $P = 0.004$) and gene diversity (Md planktonic = 0.740, Md benthic = 0.661, $P = 0.004$) than did populations that also have benthic larvae. Since the populations with different developmental modes are mainly distributed in different geographic areas, the planktonic–benthic groups are similar to those in the NS–BS comparison; however, there are fewer populations in the planktonic–benthic groups, and Iceland and NLS from the Netherlands are included with the populations with benthic larvae.

Population genetic structure

Spatial genetic structure (i.e., significant F_{ST} values among populations) was present among the European *P. elegans* populations, even on a small geographic scale. Overall, pair-wise F_{ST} estimates ranged from 0.001 to 0.170. The geographically

Table 2 Genetic variation in 18 *Pygospio elegans* populations

	Population																	
Locus	UKP	UKR	FRS	FRC	NLB	NLH	NLS	UKA	UKD	SWE	DKR	DKV	DKH	GER	FIH	FIF	FIA	ICE
Pe6																		
N_a	6	2	8	10	7	10	4	10	7	6	3	4	5	8	4	3	3	4
H_E	0.370	0.143	0.394	0.489	0.248	0.354	0.108	0.446	0.377	0.222	0.267	0.274	0.201	0.332	0.074	0.169	0.301	0.239
H_O	0.424	0.143	0.387	0.581	0.266	0.372	0.111	0.478	0.408	0.238	0.307	0.309	0.190	0.333	0.075	0.179	0.341	0.238
Pe7																		
N_a	21	9	29	23	30	27	29	21	26	20	18	21	25	25	26	26	28	19
H_E	0.944	0.923	0.943	0.924	0.945	0.943	0.953	0.941	0.942	0.927	0.917	0.931	0.946	0.964	0.956	0.954	0.949	0.929
H_O	0.818	0.857	0.887	0.930	0.888	0.860	0.869	0.945	0.878	0.881	0.897	0.884	0.738	0.733	0.900	0.872	0.905	0.881
Pe12																		
N_a	20	3	33	23	27	26	26	24	31	18	18	20	18	19	25	23	28	16
H_E	0.949	0.750	0.961	0.944	0.963	0.960	0.974	0.955	0.957	0.937	0.934	0.953	0.944	0.950	0.957	0.924	0.954	0.900
H_O	0.740	0.500	0.593	0.882	0.694	0.639	0.818	0.594	0.800	0.538	0.700	0.777	0.616	0.600	0.919	0.766	0.838	0.684
Pe13																		
N_a	24	11	33	24	31	29	24	27	31	22	27	26	30	26	24	29	30	17
H_E	0.950	0.967	0.959	0.951	0.960	0.957	0.955	0.954	0.963	0.944	0.955	0.948	0.969	0.966	0.944	0.963	0.961	0.862
H_O	0.848	0.714	0.688	0.791	0.755	0.721	0.891	0.791	0.755	0.809	0.976	0.974	0.881	0.700	0.825	0.816	0.809	0.857
Pe15																		
N_a	24	9	35	30	32	33	24	29	31	24	30	31	28	23	24	23	28	15
H_E	0.957	0.934	0.969	0.967	0.970	0.967	0.907	0.962	0.963	0.944	0.946	0.958	0.953	0.956	0.940	0.945	0.955	0.884
H_O	0.697	0.571	0.737	0.750	0.822	0.721	0.666	0.696	0.829	0.571	0.650	0.881	0.667	0.800	0.692	0.622	0.756	0.619
Pe17																		
N_a	14	6	17	16	15	14	9	17	19	8	9	12	6	12	3	6	7	5
H_E	0.902	0.681	0.851	0.827	0.828	0.881	0.590	0.838	0.885	0.763	0.613	0.667	0.553	0.826	0.143	0.271	0.416	0.708
H_O	0.742	0.571	0.508	0.714	0.666	0.558	0.565	0.435	0.612	0.619	0.447	0.561	0.316	0.643	0.150	0.243	0.500	0.536
Pe18																		
N_a	8	2	11	11	7	13	9	11	9	4	5	5	8	6	3	6	6	3
H_E	0.549	0.264	0.620	0.678	0.577	0.736	0.676	0.737	0.669	0.577	0.606	0.684	0.709	0.694	0.488	0.576	0.663	0.390
H_O	0.290	0.000	0.339	0.447	0.429	0.558	0.659	0.533	0.396	0.293	0.589	0.744	0.658	0.348	0.474	0.553	0.714	0.429
Pe19																		
N_a	8	4	13	9	6	10	8	10	9	5	6	3	5	8	5	6	6	2
H_E	0.583	0.659	0.733	0.664	0.653	0.727	0.593	0.646	0.621	0.447	0.257	0.420	0.466	0.518	0.189	0.437	0.516	0.312
H_O	0.515	0.571	0.613	0.674	0.600	0.767	0.435	0.630	0.638	0.512	0.282	0.381	0.452	0.533	0.150	0.487	0.667	0.333
$N_{A(44)}$	13.76	–	15.42	14.60	15.00	15.65	13.67	14.73	15.38	11.36	11.86	12.16	12.65	14.39	11.65	12.54	13.32	8.66
$N_{PA(44)}$	0.21	–	0.68	0.22	0.37	0.24	0.77	0.36	0.35	0.24	0.21	1.18	0.40	0.42	0.28	0.22	0.20	0.14
GD	0.715	0.653	0.774	0.741	0.715	0.780	0.669	0.768	0.738	0.682	0.622	0.678	0.652	0.730	0.557	0.588	0.680	0.628
Mean H_E	0.776	0.665	0.804	0.806	0.768	0.816	0.720	0.810	0.798	0.720	0.687	0.729	0.718	0.776	0.586	0.655	0.715	0.653
Mean H_O	0.635	0.491	0.594	0.721	0.637	0.650	0.627	0.636	0.667	0.558	0.606	0.689	0.565	0.586	0.523	0.567	0.691	0.572
F_{IS}8loci	0.185	0.280	0.259	0.106	0.172	0.206	0.131	0.217	0.165	0.228	0.120	0.056	0.216	0.240	0.109	0.136	0.033	0.125
F_{IS}6loci	0.156	0.228	0.241	0.089	0.151	0.168	0.090	0.169	0.170	0.138	0.033	0.018	0.160	0.238	0.080	0.066	−0.034	0.049

N_a = number of alleles, H_E = expected heterozygosity, H_O = observed heterozygosity (values with significant departures from HWE are underlined), N_A = allelic richness (based on 44 samples), calculated with HP-Rare. N_{AP} = private allelic richness, based on 44 samples, GD = gene diversity, F_{IS} = inbreeding coefficient, calculated using the whole data set (8loci), and with 6loci (Pe12 and Pe15 removed) (significant values underlined).

distant Iceland seemed to be most isolated genetically, having the highest pair-wise F_{ST} values (Table 3). On a small scale (within Finland), the pair-wise F_{ST} values were low (0.008–0.028) but significant, indicating genetic structure between the locations. Among the three Danish populations, the F_{ST} values were also low (from 0.006 to 0.010) and the two sample sites within the fjords were not significantly differentiated from each other, but they were significantly differentiated from DKR, the population sampled from the entrance of the fjords (Fig. 1). Interestingly, the German population seemed to be more differentiated from the other Baltic populations than from the NS populations. Some of the geographically close NS populations with planktonic larvae were observed to be genetically similar (nonsignificant F_{ST} values, Table 3). The mean D_{est} estimations were similar to the results of the pair-wise F_{ST} analysis (data not shown). Most of the nonsignificant D_{est} values were between populations within the NS (Table 3). Unlike the F_{ST} results, the UKR population showed more nonsignificant pair-wise D_{est} values, but this might be due to the lower sample size in UKR.

Geneland suggested the presence of eight genetic populations in Europe. Not surprisingly, Iceland formed its own group. In the NS, four clusters with clear genetic boundaries were identified. The two Scottish populations were clustered together (UKD and UKA), and so were the two English Channel populations (UKP and UKR). The two French populations were grouped with the two Dutch populations which had predominantly planktonic larvae (FRS and FRC + NLB and NLH), and there was a clear boundary with NLS (which formed its own cluster), although the latter is geographically close to NLH. Within the BS there were three genetic clusters, but the boundaries among these were more ambiguous. The Finnish populations formed one cluster, which was clearly separated from the others. The German sample formed its own cluster, but alternatively could have been clustered with the Danish samples. The three Danish populations grouped with high probability with the Swedish population, but alternatively the Swedish sample could also have been clustered with NLS. Similar clustering was seen in the Structure analysis, which estimated the presence of nine genetic clusters. As in the Geneland analysis, Structure assigned geographically close populations together, and both analyses distinguished NLS from the other two Netherlands populations and suggested that the German population differs from the other Baltic populations. However, the likelihood values for different K were

Table 3 Population pair-wise F_{ST} values for *Pygospio elegans* in Europe

Code	UKR	UKP	FRS	FRC	NLB	NLH	NLS	UKA	UKD	SWE	DKR	DKV	DKH	GER	FIH	FIF	FIA	ICE
UKR	0																	
UKP	**0.024**	0																
FRS	0.041	0.016	0															
FRC	0.035	0.017	**0.004**	0														
NLB	0.034	0.017	**0.005**	0.009	0													
NLH	0.046	0.014	**0.002**	0.008	**0.005**	0												
NLS	0.026	0.035	0.047	0.039	0.047	0.048	0											
UKA	0.066	0.026	0.024	0.023	0.029	0.021	0.054	0										
UKD	0.058	0.027	0.029	0.029	0.034	0.023	0.050	≥**0.001**	0									
SWE	0.050	0.024	0.037	0.059	0.031	0.036	0.033	0.050	0.050	0								
DKR	0.045	0.035	0.052	0.038	0.054	0.058	0.019	0.058	0.055	0.033	0							
DKV	0.043	0.033	0.042	0.034	0.046	0.046	0.016	0.049	0.046	0.027	0.010	0						
DKH	0.035	0.031	0.039	0.032	0.043	0.046	0.011	0.041	0.040	0.031	0.009	**0.006**	0					
GER	0.058	0.013	0.016	0.022	0.016	0.013	0.044	0.016	0.024	0.021	0.042	0.033	0.031	0				
FIH	0.071	0.098	0.109	0.098	0.107	0.121	0.048	0.113	0.109	0.083	0.034	0.056	0.042	0.105	0			
FIF	0.039	0.061	0.068	0.061	0.067	0.079	0.025	0.076	0.074	0.053	0.023	0.025	0.017	0.066	0.016	0		
FIA	0.029	0.048	0.060	0.046	0.063	0.069	0.018	0.063	0.061	0.047	0.019	0.020	0.009	0.055	0.028	0.008	0	
ICE	0.106	0.055	0.082	0.091	0.074	0.077	0.102	0.096	0.099	0.059	0.104	0.095	0.101	0.064	0.170	0.131	0.124	0

Note that most of the comparisons are significant and only the F_{ST} comparisons with nonsignificant *P*-values are underlined and bold. Comparisons with nonsignificant Jost D_{est} values are on a gray background (D_{est} values not shown).

very similar, indicating possible problems with the data, likely explained by a pattern of IBD (see below).

According to the AMOVA analysis, most microsatellite variation in European *P. elegans* resides within the populations (93.3%, $P<0.001$). Low, but significant, genetic differentiation was seen between BS and NS groups (3.34%, $P<0.001$), suggesting the presence of variation also on a larger scale. F_{ST} among the NS populations (0.020) was somewhat lower than among the BS populations ($F_{ST}=0.028$), but AMOVA indicated significant within-group structure as well (2.35%, $P<0.001$). A plot of linearized population pair-wise F_{ST} against geographic distance (Fig. 2) indicates that population genetic structure can be explained by a pattern of IBD ($r=0.687$, $P<0.001$). Similarly, Mantel tests of correlations between genetic distance and geographic distance matrices were significant for the whole data set as well within areas: NS, BS, Southern BS (all $P<0.01$); but not within the Northern BS (Finnish populations only). Spatial autocorrelation was positive and significant in only the smallest distance classes estimated for each group (data not shown) also indicating a pattern of IBD.

Using the methods of Johannesson and André (2006), we did not find a strong genetic shift between the BS and NS (Fig. 3). However, we do see increased divergence (F_{ST}) with distance from the northern Baltic, a trajectory resembling a combination of the IBD model and the peripheral-perturbation model in which the Northern Baltic populations are differentiated from all other populations because of their marginal location.

Migration patterns

In Finland, the two methods used to estimate gene flow produced similar results. The benthic FIA population receives the highest amount of migration. The mean migration rates into FIA from FIF were 0.17–0.37 and from FIH 0.10–0.15, but the reverse migration rates from FIA to these populations were lower. BIMr estimated a higher migration rate from FIA to FIF than did BayessAss (0.15 versus 0.03). In Denmark, the results were also fairly consistent between the two methods, showing a high rate of self-recruitment in most populations. Both methods suggested high migration rate from DKR to the inner parts of the fjords (DKV and/or DKH) and migration rate from Sweden to Denmark was low. Among the NS samples, a surprisingly high level of self-recruitment was seen. With BayesAss, the highest outward migration rates were from the French populations, suggesting they are source populations. There were high migration rates from France to the nearby Netherlands sample (NLB and NLH combined) and also to the UK populations across the English Channel and to the northern parts of the UK. However, migration rates in the opposite direction (into France) were very low. On the other hand, BIMr estimated high symmetrical migration rate between France and the planktonic Dutch populations (NLB + NLH), but no migration from France to the other sites. It also estimated zero migration into the UK populations, which is inconsistent with the BayesAss results. The NLS population was estimated to have low migrant proportions and low levels of outward migration to the other populations (using both methods) (Table 4).

Discussion

If a correlation between dispersal ability and larval developmental mode exists, population genetic structure of marine invertebrate species is expected to show a predictable pattern: species with planktonic, dispersive larvae should have larger populations with high genetic diversity and effective gene flow, whereas species with benthic, brooded larvae should have considerable population genetic structure due to limited gene flow among smaller, less diverse populations (e.g., Ellingson and Krug 2006; Lee and Boulding 2009; Binks et al. 2011). We investigated whether the expected correlation between developmental mode, dispersal, and population genetic structure would be predictable for the polychaete *P. elegans*, a poecilogonous species that displays variation in developmental mode primarily among populations, but also within populations. We confirmed our hypothesis that *P. elegans* populations with predominantly planktonic developmental mode had higher genetic diversity than did populations that also had benthic larvae. The same result was found previously using DNA sequence data from the mitochondrial gene cytochrome c oxidase subunit I (Kesäniemi et al. 2012b). However, higher diversity in the populations with planktonic larvae could be explained by factors other than developmental mode.

First, higher genetic diversity in the populations with planktonic larvae could be explained by a larger effective population size (N_e) in these populations. Although we do not have estimates of N_e, all of the populations with predominantly planktonic larvae also appeared to have a high density of worms. Higher N_e could also explain the higher number of private alleles we observed in most of

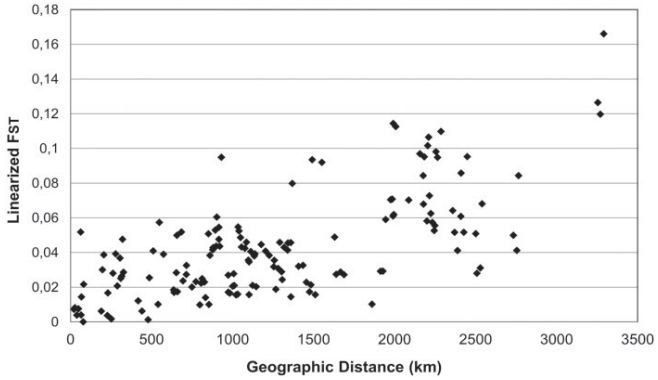


Fig. 2 Scatterplot showing the isolation-by-distance pattern between pair-wise genetic distance (linearized F_{ST}: $F_{ST}/(1-F_{ST})$) and geographic distance (km).

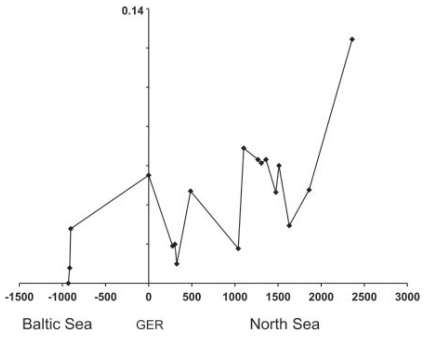


Fig. 3 Genetic differentiation (pair-wise F_{ST} values, *y*-axis) between the innermost Baltic population (FIA) and other populations at increasing distances. The *x*-axis shows distance (km) from the population at the entrance to the Baltic Sea (our GER sample at zero), with negative values going toward the northern Baltic and positive values outside the Baltic through the Kattegat.

the NS populations (although these were not significantly higher). These observations are only qualitative, so a relationship between high density/high N_e and high genetic diversity would need to be addressed with more quantitative methods. However, higher genetic diversity achieved by retention of alleles in large populations has been proposed for other species with planktonic larvae (e.g., Lee and Boulding 2009).

Second, asexual reproduction observed in the Baltic populations of *P. elegans* (Rasmussen 1953; Anger 1984; J. E. Kesäniemi and E. Geuverink, personal observation) could also lower their genetic diversity and complicate interpretations of our findings. Asexual reproduction has not been observed in *P. elegans* in the NS (Morgan et al. 1999; Bolam 2004; K. E. Knott and E. Geuverink, personal observation). The lack of asexual reproduction in the NS could be explained by a higher density of worms in these populations. Wilson (1983) found that in the laboratory, the proportion of asexually reproducing *P. elegans* increased when worms were reared at low densities, and this may also occur in nature. Despite asexual reproduction in some populations, none of the sampled individuals appeared to be clones (multilocus genotypes were not shared); there were, however, significant deviations from HWE and positive F_{IS} values for the loci we studied in most populations, indicating that processes increasing population identity by descent may be common in *P. elegans*. Inbreeding, within population genetic structure (the Wahlund effect) and high local recruitment, in addition to asexual reproduction, could lead to deviations from HWE. These processes may be more common in populations with benthic larvae than in populations with planktonic larvae, but other results (discussed below) indicated that at least high local recruitment was common in most populations. High positive F_{IS} values and deviations from HWE have been noted in other population genetic analyses of marine invertebrates (Addison and Hart 2005; Zhan et al. 2009).

Finally, the difference in genetic diversity could exist because of the association of developmental mode and geographic location. Initial survey of predominant developmental mode indicated that benthic larvae may be more common in the BS (Table 1). However,

Table 4 Migration rates among *Pygospio elegans* populations within three areas analyzed separately (Northern Baltic Sea, Southern Baltic Sea, and North Sea)

To	From				
Northern Baltic Sea					
	FIA	FIF	FIH		
FIA	*0.68/0.53* (0.01/0.13)	0.17/0.37 (0.07/0.14)	0.15/0.10 (0.07/0.07)		
FIF	0.03/0.15 (0.02/0.12)	*0.90/0.79* (0.06/0.12)	0.07/0.06 (0.06/0.05)		
FIH	0.01/0.06 (0.01/0.08)	0.11/0.19 (0.05/0.13)	*0.88/0.75* (0.05/0.01)		
Southern Baltic Sea					
	DKV	DKH	DKR	SWE	
DKV	*0.68/0.51*(0.01/0.08)	0.01/0.18 (0.01/0.08)	0.29/0.22 (0.02/0.08)	0.02/0.09 (0.02/0.05)	
DKH	0.02/0.00 (0.02/−)	*0.70/1.00* (0.02/−)	0.27/0.00 (0.03/−)	0.01/0.00 (0.01/−)	
DKR	0.02/0.00 (0.01/−)	0.02/0.00 (0.01/−)	*0.86/1.00* (0.03/−)	0.10/0.00 (0.03/−)	
SWE	0.11/0.00 (0.03/−)	0.01/0.00 (0.01/−)	0.02/0.00 (0.02/−)	*0.86/1.00* (0.03/−)	
North Sea					
	FRC + FRS	NLB + NLH	NLS	UKA + UKD	UKP + UKR
FRC + FRS	*0.96/0.75* (0.02/0.06)	0.00/0.15 (−/0.06)	0.01/0.01 (0.01/0.01)	0.03/0.05 (0.02/0.03)	0.00/0.04 (−/0.03)
NLB + NLH	0.30/0.14 (0.01/0.06)	*0.67/0.73* (−/0.06)	0.01/0.02 (0.01/0.01)	0.02/0.03 (0.01/0.02)	0.00/0.01 (−/0.05)
NLS	0.04/0.00 (0.02/−)	0.01/0.00 (0.01/−)	*0.93/1.00* (0.02/−)	0.01/0.00 (0.01/−)	0.01/0.00 (0.01/−)
UKA + UKD	0.11/0.00 (0.03/−)	0.00/0.00 (−/−)	0.01/0.00 (0.01/−)	*0.87/1.00* (0.03/−)	0.00/0.00 (0.00/−)
UKP + UKR	0.22/0.00 (0.05/−)	0.01/0.00 (−/−)	0.06/0.00 (0.03/−)	0.04/0.00 (0.03/−)	*0.67/1.00* (0.01/−)

First values are from BayesAss, the second from BIMr and the respective standard deviations are in brackets (SD values <0.00 are marked with a −). The source populations for migration are given in columns and populations receiving migrants are in rows. Migration values along the diagonal axis are the proportions of individuals with local recruitment (underlined and in italics).

there are exceptions; all developmental modes are observed in NLS, and benthic larvae have been reported for UKR and UKP (Morgan et al. 1999). We found lower genetic diversity in the BS populations compared to those in the NS, but the pattern may not be related to developmental mode. Lower diversity in the Baltic is not unexpected, and is known for marine plants, invertebrates (see Johannesson and André 2006), and vertebrates (Nielsen et al. 2003; Säisä et al. 2005; Hemmer-Hansen et al. 2007). Nevertheless, exceptional populations in the NS that also had benthic larvae, (NLS and possibly UKP and UKR), did have somewhat lower genetic diversity than did other NS populations in which only planktonic larvae were found.

In order to tease out the potential factors influencing diversity in this system, additional analyses contrasting populations which differ only in developmental mode, but not in habitat characteristics, density, or propensity for asexual reproduction would be required. A comparison between populations NLS and NLH perhaps provide one such example, but replicate comparisons would be necessary. Alternatively, concomitant analyses of genetic data and environmental data may provide a way to identify the most probable factors influencing genetic patterns (e.g., Case et al. 2005; Banks et al. 2007; Dionne et al. 2008; Gaggiotti et al. 2009).

Given our hypothesis that developmental mode influences the genetic structure of populations, we expected to find significant genetic differentiation only among the Baltic populations with benthic developmental modes. However, significant genetic structure among populations in both the BS and NS were detected (AMOVA). Pair-wise F_{ST} and D_{est} analyses also indicated significant population differentiation between almost all populations (Table 3). In Finland, all three samples were genetically differentiated, despite short geographic distances between their locations (20–100 km). Here *P. elegans* is strongly associated with seagrasses, and its distribution is likely to be patchy due to the highly fragmented distribution of the sea grass *Zostera marina* in the Finnish archipelago (Boström et al. 2006). In a patchy habitat, brooded, nondispersive larvae should be favored as they would maintain local recruitment (Levin 1984; Pechenik 1999). In the Southern Baltic, where populations show multiple developmental modes, genetic differentiation is also seen, but not among all samples. Interestingly,

the German population, sampled from deeper water, is more similar to the NS populations than it is to the Baltic populations and it also has higher diversity than do other Baltic populations.

Only a few *P. elegans* populations were not significantly differentiated according to the pair-wise F_{ST} and D_{est} comparisons, and these included primarily the populations with planktonic larvae in the English Channel and adjacent populations in the Wadden Sea (UKP and UKR; FRS, FRC, NLB and NLH). Nevertheless, there was genetic differentiation between the English Channel (UK) populations and those from the French and Dutch sites. Notably, NLS, from which all larval developmental modes have been found, was significantly differentiated from the nearby population NLH, in which planktonic larvae dominate. Clearly, in *P. elegans*, additional factors other than developmental mode must affect the patterns of genetic structure among populations. In analyses of allozyme data, Morgan et al. (1999) found genetic structure in the English Channel to differ between the French coast and the southern coast of UK, probably because of a hydrographic barrier.

Given the many pair-wise population comparisons showing significant genetic differentiation, long-distance dispersal might not be successful in this species. Planktonic larvae of *P. elegans* are expected to have significant dispersal potential since they can live for 4–5 weeks in the plankton (Anger et al. 1986). However, high larval mortality (Pedersen et al. 2008), or higher-than-expected local recruitment could determine realized dispersal in this species. Our estimates of migration rates revealed surprisingly high estimates of self-recruitment in all populations, including those with primarily planktonic larvae (Table 4). As a result, realized dispersal appears not be tightly correlated with developmental mode and the expected association between larval developmental mode and genetic structure of populations is not clear-cut. In principle, oceanic currents can transport different life-stages over large distances, but currents can also promote local differentiation that can help to explain population genetic patterns (Knutsen et al. 2004; Fievet et al. 2006; Kenchington et al. 2006; White et al. 2010). Local habitat characteristics; such as the estuarine environment, or behavioral factors can also affect retention of the larval stage, as well as population genetic structure (Levin 1986; Palumbi 1994; Metaxas 2001; Sponaugle et al. 2002; Swearer et al. 2002). For example, Bolam (2004) suggested that the *P. elegans* larvae in Drum Sands (UKD) might settle locally because of local hydrodynamic conditions. Further study of larval behavior of *P. elegans* would be worthwhile to pursue in order understand limitations on dispersal in this species.

In our study, we found significant IBD across the entire region (Fig. 2) and within both the BS and the NS, regardless of the larval developmental mode. In previous studies, IBD is more often found in species with nondispersive larvae (Duran et al. 2004). For example, in bryozoans, species with planktonic larvae had lower population genetic structure and low or absent IBD, whereas species with nondispersive larvae showed higher genetic differentiation among populations combined with a pattern of IBD (Goldson et al. 2001; Watts and Thorpe 2006). Hellberg (1996) saw a similar pattern in corals from the coast of California. Together, our results of significant IBD on multiple scales, significant genetic autocorrelation at the smallest geographic distance classes (within populations), and high estimated local recruitment support a conclusion of limited dispersal by planktonic larvae of *P. elegans*. In terms of dispersal, the different developmental modes of this species may actually be very similar.

Focusing our geographic study on the natural environmental transition in salinity between the NS and the BS, we also saw evidence of IBD. *Pygospio elegans* did not show strong differentiation over this transition, whereas genetic differentiation is commonly seen in other species with populations in both areas (Bekkevold et al. 2005; Johannesson and André 2006; O'Leary et al. 2007; Wiemann et al. 2010). Additional sampling between Germany and Finland would provide a more rigorous test of the geographic pattern. However, the result implies that the change in salinity does not impose a barrier to gene flow for *P. elegans*. Johannesson and André (2006) noted that the patterns of loss of diversity among regions and the strength of IBD were not different among species with different dispersal potential. However, Hemmer-Hansen et al. (2007) compared population genetic structure of European flounder (*Platichthys flesus* L.) with plasticity in egg type, a phenomenon uncommonly seen in fish. This species spawns benthic eggs in the northern Baltic, but "normal pelagic eggs" in the southern BS and in the NS. A genetic barrier suggesting restricted gene flow was found for the flounder between these areas and was associated with the difference in developmental mode.

The apparent association of developmental mode with habitats of low salinity may indicate that different developmental modes may be advantageous under different environmental conditions.

Hannerz (1956) and Rasmussen (1973) hypothesized that environmental variation and developmental plasticity would explain larval phenotypic variation in *P. elegans*. However, Anger (1984) did not find plasticity in developmental mode in experiments manipulating temperature and salinity. Environmental variation is known to affect poecilogony in some species, for example, the sea slug *Alderia willowi* (Krug 2007). Also, geographical patterns in poecilogony are not uncommon. In another poecilogonous polychaete, *Boccardia proboscidea*, females at higher latitudes along the western coast of North America invest more in larval nutrition and produce a larger proportion of brooded larvae (Oyarzun et al. 2011).

We examined the role of developmental mode and environment in a broad survey of genetic structure among populations of *P. elegans* extending over a region with extremes of salinity (from the NS to the BS). We found that most genetic variation existed within populations, but that there was significant genetic differentiation at a large spatial scale (across the entire region studied) as well as at moderate scales (within subregions: NS and BS). We observed a significant trend of IBD at both the broadest and regional scales, and the genetic structure of populations did not appear to be affected by the transition zone of changes in salinity, or by the predominant developmental mode of the populations. However, the association of developmental mode and geographic location makes it difficult to separate the effects of these two factors. Our results raise questions about the assumed dispersal potential of the planktonic larvae of *P. elegans*. Local recruitment was estimated to be high regardless of developmental mode or habitat, and could be explained by high mortality rates for planktonic larvae (Pedersen et al. 2008). Alternative explanations of genetic structure in populations with planktonic larvae, such as possible sweepstakes reproductive success (Hedgecock 1994) or temporal Wahlund effects should be investigated.

Acknowledgments

We thank B.W. Hansen, C. Boström, M.T. Jolly, R. Bastrop, and J. Frankowski and G.V. Helgason for their help with collection of the samples. Organization of the symposium was sponsored by the US National Science Foundation (IOS-1157279), The Company of Biologists, Ltd, the American Microscopical Society, and the Society for Integrative and Comparative Biology, including SICB divisions DEDB, DEE, and DIZ.

Funding

This work was supported by the Jenny and Antti Wihuri Foundation (personal grant to J.E.K.) and the Centre of Excellence in Evolutionary Research (at University of Jyväskylä).

References

Addison JA, Hart MW. 2005. Spawning, copulation and inbreeding coefficients in marine invertebrates. Biol Lett 1:450–3.

Anger V. 1984. Reproduction in *Pygospio elegans* (Spionidae) in relation to its geographical origin and to environmental conditions: a preliminary report. Forts Zool 29:45–51.

Anger K, Anger V, Hagmeier E. 1986. Laboratory studies on larval growth of *Polydora ligni*, *Polydora ciliata* and *Pygospio elegans* (Polychaeta, Spionidae). Helgoländer Meeresunters 40:277–395.

Arndt A, Smith MJ. 1998. Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. Mol Ecol 7:1053–64.

Banks SC, Piggott MP, Williamson JE, Bove U, Holbrook NJ, Beheregaray LB. 2007. Oceanic variability and coastal topography shape genetic structure in a long-dispersing sea urchin. Ecology 88:3055–64.

Bekkevold D, Andre C, Dahlgren TG, Clausen LAW, Torstensen E, Mosegaard H, Carvalho GR, Christensen TB, Norlinder E, Ruzzante DE. 2005. Environmental correlates of population differentiation in Atlantic herring. Evolution 59:2656–68.

Binks RM, Evans JP, Prince RJ, Kennington WJ. 2011. Fine-scale patterns of genetic divergence within and between morphologically variable subspecies of the sea urchin *Heliocidaris erythrogramma* (Echinometridae). Biol J Linn Soc 103:578–92.

Blake JA, Arnofsky PL. 1999. Reproduction and larval development of the spioniform Polychaeta with application to systematics and phylogeny. Hydrobiologia 402:57–106.

Bohonak AJ. 1999. Dispersal, gene flow, and population structure. Quart Rev Biol 74:21–45.

Bolam SG. 2004. Population structure and reproductive biology of *Pygospio elegans* (Polychaeta:Spionidae) on an intertidal sandflat, Firth of Forth, Scotland. Invertebr Biol 123:260–8.

Boström C, Jackson EL, Simenstad CA. 2006. Seagrass landscapes and their effect on associated fauna: a review. Est Coast Shelf Sci 68:383–403.

Bowen BW, Bass AL, Muss A, Carlin J, Robertson DR. 2006. Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. Mar Biol 149:899–913.

Case RAJ, Hutchinson WF, Hauser L, Van Oosterhput C, Carvalho GR. 2005. Macro- and micro-geographic variation in pantophysin (*Pan*I) allele frequencies in NE Atlantic cod, *Gadus morhua*. Mar Ecol Prog Ser 301:267–78.

Chapuis M-P, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–31.

Collin R. 2001. Mode of development, phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae) species. Mol Ecol 10:2249–62.

Dawson MN, Louie KD, Barlow M, Jacobs DK, Swift CC. 2002. Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. Mol Ecol 11:1065–75.

Dionne M, Caron F, Dodson JJ, Bernatchez L. 2008. Landscape genetics and hierarchical genetic structure in Atlantic salmon: the interaction of gene flow and local adaptation. Mol Ecol 17:2382–96.

Duran M, Pascual M, Estoup A, Turon X. 2004. Strong population structure in the marine sponge *Crambe crambe* (Poecilosclerida) as revealed by microsatellite markers. Mol Ecol 13:511–22.

Ellingson RA, Krug PJ. 2006. Evolution of poecilogony from planktotrophy: cryptic speciation, phylogeography, and larval development in the gastropod genus *Alderia*. Evolution 601:2293–310.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–7.

Faubet P, Gaggiotti OE. 2008. A new Bayesian method to identify the environmental factors that influence recent migration. Genetics 178:1491–1504.

Fievet V, Touzet P, Arnaud J, Cuguen J. 2006. Spatial analysis of nuclear and cytoplasmic DNA diversity in wild sea beet (*Beta vulgaris ssp. maritima*) populations: do marine currents shape the genetic structure? Mol Ecol 16:1847–64.

Gaggiotti OE, Bekkevold D, Jorgensen HBH, Foll M, Carvalho GR, Andre C, Ruzzante DE. 2009. Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of natural populations: Atlantic herring as a case study. Evolution 63:2939–51.

Gerlach G, Jueterbock A, Kraemer P, Deppermann J, Harmand P. 2010. Calculations of population differentiation based on G(ST) and D: forget G(ST) but not all of statistics!. Mol Ecol 19:3845–52.

Goldson AJ, Hughes RN, Gliddon CJ. 2001. Population genetic consequences of larval dispersal mode and hydrography: a case study with bryozoans. Mar Biol 138:1037–42.

Gudmundsson H. 1985. Life history patterns of polychaete species of the family Spionidae. J Mar Bio Assoc UK 65:93–111.

Guillot G, Mortier F, Estoup A. 2005. Geneland: A program for landscape genetics. Mol Ecol Notes 5:712–5.

Guillot G, Santos F, Estoup A. 2008. Analysing georeferenced population genetics data with Geneland: a new algorithm to deal with null alleles and a friendly graphical user interface. Bioinformatics 24:1406–7.

Hannerz L. 1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil and Poecilochaetidae N. fam. in the Gullmar Fjord (Sweden). Zool Bidr Upps 31:1–204.

Härkönen T, Harding KJ, Goodman SJ, Johannesson K. 2005. Colonization history of the Baltic harbor seals: integrating archaeological, behavioral and genetic data. Mar Mammal Sci 21:695–716.

Hart MW, Marko PB. 2010. It's about time: divergence, demography, and the evolution of developmental modes in marine invertebrates. Integ Comp Biol 50:643–61.

Hedgecock D. 1994. Does variance in reproductive success limit effective population size of marine organisms? In: Beaumont AR, editor. Genetics and evolution of aquatic organisms. London: Chapman & Hall. p. 122–34.

Helcom. 2003. Helsinki commission, The Baltic Marine environment 1999–2002. Baltic Sea Environment Proceedings No. 87.

Hellberg ME. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50:1167–75.

Hellberg ME. 2009. Gene flow and isolation among populations of marine animals. Annu Rev Ecol Evol Syst 40:291–310.

Hemmer-Hansen J, Nielsen EE, Grønkjær P, Loeschcke V. 2007. Evolutionary mechanisms shaping the genetic populations structure of marine fishes; lessons from the European flounder (*Platichthys flesus* L.). Mol Ecol 16:3104–18.

Johannesson K, André C. 2006. Life on the margin: genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. Mol Ecol 15:2013–29.

Jost L. 2008. GST and its relatives do not measure differentiation. Mol Ecol 17:4015–26.

Kalinowski ST. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–9.

Kelly RP, Palumbi SR. 2010. Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. PLoS ONE 5:e8594.

Kenchington EL, Patwary MU, Zouros E, Bird CJ. 2006. Genetic differentiation in relation to marine landscape in a broadcast-spawning bivalve mollusc (*Placopecten magellanicus*). Mol Ecol 15:1781–96.

Kesäniemi JE, Boström C, Knott KE. 2012a. New genetic markers reveal population genetic structure at different spatial scales in the opportunistic polychaete *Pygospio elegans*. Hydrobiologia. (doi:10.1007/s10750-012-1075-3).

Kesäniemi JE, Rawson PD, Lindsay SM, Knott KE. 2012b. Phylogenetic analysis of cryptic speciation in the polychaete *Pygospio elegans*. Ecol Evol. (doi:10.1002/ece3.226).

Knutsen H, André C, Jorde PE, Skogen MD, Thuróczy E, Stenseth NC. 2004. Transport of North Sea cod larvae into the Skagerrak coastal populations. Proc R Soc Lond B 271:1337–44.

Krug PJ. 2007. Poecilogony and larval ecology in the gastropod genus *Alderia*. Am Malacol Bull 23:99–111.

Kyle CJ, Boulding EG. 2000. Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar Biol 137:835–45.

Larmuseau MHD, Vancampenhout K, Raeymaekers JAM, Van Houdt JKJ, Volckaert FAM. 2010. Differential modes of selection on the rhodopsin gene in coastal Baltic and North Sea populations of the sand goby, *Pomatoschistus minutes*. Mol Ecol 19:2256–68.

Lee HJ, Boulding EG. 2009. Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on

variation at one mitochondrial and two nuclear DNA markers. Mol Ecol 18:2165–84.
Levin LA. 1984. Life history and dispersal patterns in a dense infaunal polychaete assemblage: community structure and response to disturbance. Ecology 65:1185–200.
Levin LA. 1986. The influence of tides on larval availability in shallow waters overlying a mudflat. B Mar Sci 39:224–33.
Levin LA. 2006. Recent progress in understanding larval dispersal: new directions and digressions. Integ Comp Biol 46:282–97.
Marko PB, Rogers-Bennett L, Dennis AB. 2007. MtDNA population structure and gene flow in lingcod (*Ophiodon elongatus*): limited connectivity despite longlived pelagic larvae. Mar Biol 150:1301–11.
Mattila J. 1997. The importance of shelter, disturbance and prey interactions for predation rates of tube-building polychaetes (*Pygospio elegans* (Claparede)) and free-living tubificid oligochaetes. J Exp Mar Biol Ecol 218:215–28.
Metaxas A. 2001. Behaviour in flow: perspectives on the distribution and dispersion of meroplanktonic larvae in the water column. Can J Fish Aquat Sci 58:86–98.
Mileikovsky SA. 1971. Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. Mar Biol 10:193–213.
Miller KJ, Ayre DJ. 2008. Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. J Anim Ecol 77:713–24.
Morgan TS, Rogers AD, Paterson GLJ, Hawkins LE, Sheader M. 1999. Evidence for poecilogony in *Pygospio elegans* (Polychaeta: Spionidae). Mar Ecol Prog Ser 178:121–32.
Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Grønkjær P. 2003. Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea, revealed by individual admixture analysis. Mol Ecol 12:1497–508.
Nilsson J, Gross R, Asplund T, Dove O, Jansson H, Kelloniemi J, Kohlmann K, Löytynoja A, Nielsen EE, Paaver T, et al. 2001. Matrilinear phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonization of the Baltic Sea area. Mol Ecol 10:89–102.
Nissling A, Dahlman G. 2010. Fecundity of flounder, *Pleuronectes flesus*, in the Baltic Sea—Reproductive strategies in two sympatric populations. J Sea Res 64:190–8.
O'Leary DB, Coughlan J, Dillane E, McCarthy TV, Cross TF. 2007. Microsatellite variation in cod *Gadus morhua* throughout its geographic range. J Fish Biol 70(Suppl C):310–35.
Oyarzun FX, Mahon AR, Swalla BJ, Halanych KM. 2011. Phylogeography and reproductive variation of the poecilogonous polychaete *Boccardia proboscidea* (Annelida: Spionidae) along the West Coast of North America. Evol Dev 13:489–503.
Palumbi SR. 1994. Genetic divergence, reproductive isolation, and marine speciation. Annu Rev Ecol Syst 25:547–72.
Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–95.
Pechenik JA. 1999. On the advantages and disadvantages of larval stages in benthic invertebrate life cycles. Mar Ecol Prog Ser 177:269–97.
Pedersen TM, Hansen JLS, Josefson AB, Hansen BW. 2008. Mortality through ontogeny of soft-bottom marine invertebrates with planktonic larvae. J Marine Syst 73:185–207.
Pereyra RT, Bergstrom L, Kautsky L, Johannesson K. 2009. Rapid speciation in a newly opened postglacial marine environment, the Baltic Sea. BMC Evol Biol 9:70.
Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–59.
Raff RA, Byrne M. 2006. The active evolutionary lives of echinoderm larvae. Heredity 9:244–52.
Rasmussen E. 1953. Asexual reproduction in *Pygospio elegans* Claparède (*Polychaeta sedentaria*). Nature 171:1161–2.
Rasmussen E. 1973. Systematics and ecology of the Isefjord marine fauna (Denmark). Ophelia 11:1–495.
Raymond M, Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–9.
Rice WR. 1989. Analyzing tables of statistical tests. Evolution 43:223–5.
Rossi F, Vos M, Middelburg JJ. 2009. Species identity, diversity and microbial carbon flow in reassembling macrobenthic communities. Oikos 118:503–12.
Säisä M, Koljonen M-L, Gross R, Nilsson J, Tähtinen J, Koskiniemi J, Vasemägi A. 2005. Population genetic structure and postglacial colonization of Atlantic salmon (*Salmo salar*) in the Baltic Sea area based on microsatellite DNA variation. Can J Fish Aquat Sci 62:1887–904.
Schuelke M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–4.
Selkoe KA, Watson JR, White C, Horin TB, Iacchei M, Mitara S, Siegel DA, Gaines SD, Toonen RJ. 2010. Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. Mol Ecol 19:3708–26.
Shanks AL. 2009. Pelagic larval duration and dispersal distance revisited. Biol Bull 216:373–85.
Smouse PE, Peakall R. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity 82:561–73.
Söderström A. 1920. Studien über die Polychaeten familie Spionidae [dissertation]. Sweden: University of Uppsala.
Sotka EE, Wares JP, Barth JA, Grosberg R, Palumbi SR. 2004. Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. Mol Ecol 13:2143–56.
Sponaugle S, Cowen RK, Shanks A, Morgan SG, Leis JM, Pineda J, Boehlert GW, Kingsford MJ, Lindeman KC, Grimes C, et al. 2002. Predicting self-recruitment in marine populations: Biophysical correlates and mechanisms. B Mar Sci 70:341–75.
Swearer SE, Shima JS, Hellberg ME, Thorrold SR, Jones GP, Robertson DR, Morgan SG, Selkoe KA, Ruiz GM, Warner RR. 2002. Evidence of self-recruitment in demersal marine populations. B Mar Sci 70:251–71.
Thorrold SR, Jones GP, Hellberg ME, Burton RS, Swearer SE, Neigel JE, Morgan SG, Warner RR. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. B Mar Sci 70:S291–308.

Watts PC, Thorpe JP. 2006. Influence of contrasting larval developmental types upon the population-genetic structure of cheilostome bryozoans. Mar Biol 149:1093–101.

Weersing K, Toonen RJ. 2009. Population genetics, larval dispersal, and connectivity in marine systems. Mar Ecol Prog Ser 393:1–12.

White C, Selkoe KA, Watson J, Siegel DA, Zacherl DC, Toonen RJ. 2010. Ocean currents help explain population genetic structure. Proc R Soc B 277:1685–94.

Wiemann A, Andersen LW, Berggren P, Siebert U, Benke H, Teilmann J, Lockyer C, Pawliczka I, Skóra K, Roos A, et al. 2010. Mitochodrial control region and microsatellite analyses on harbor porpoise (*Phocoena phocoena*) unravel population differentiation in the Baltic Sea and adjacent waters. Conserv Genet 11:195–211.

Wilson GA, Rannala B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–91.

Wilson WH. 1983. The role of density dependence in a marine infaunal community. Ecology 64:295–306.

Wilson WH. 1991. Sexual reproductive modes in polychaetes: classification and diversity. B Mar Sci 48:500–16.

Zhan A, Hu J, Hu X, Zhou Z, Hui M, Wang S, Peng W, Wang M, Bao Z. 2009. Fine-scale population genetic structure of Zhikong Scallop (*Chlamys farreri*): do local marine currents drive geographical differentiation? Mar Biotechnol 11:223–35.

Zillen L, Conley DJ, Andren T, Andren E, Björck S. 2008. Past occurrence of hypoxia in the Baltic Sea and the role of climate variability, environmental change and human impact. Earth Sci Rev 91:77–92.

BIOLOGICAL RESEARCH REPORTS FROM THE UNIVERSITY OF JYVÄSKYLÄ

1 RAATIKAINEN, M. & VASARAINEN, A., Damage caused by timothy flies (Amaurosoma spp.) in Finland, pp. 3-8.
SÄRKKÄ, J., The numbers of Tubifex tubifex and its cocoons in relation to the mesh size, pp. 9-13.
ELORANTA, P. & ELORANTA, A., Keurusselän kalastosta ja sen rakenteesta. - On the fish fauna of Lake Keurusselkä, Finnish Lake District, pp. 14-29.
ELORANTA, P. & ELORANTA, A., Kuusveden veden laadusta, kasviplanktonista ja kalastosta. - On the properties of water, phytoplankton and fish fauna of Lake Kuusvesi, Central Finland, pp. 30-47. 47 p. 1975.

2 ELORANTA, V., Effects of different process wastes and main sewer effluents from pulp mills on the growth and production of Ankistrodesmus falcatus var. acicularis (Chlorophyta), pp. 3-33.
ELORANTA, P. & KUNNAS, S., A comparison of littoral periphyton in some lakes of Central Finland, pp. 34-50.
ELORANTA, P., Phytoplankton and primary production in situ in the lakes Jyväsjärvi and North Päijänne in summer 1974, pp. 51-66. 66 p. 1976.

3 RAATIKAINEN, M., HALKKA, O., VASARAINEN, A. & HALKKA, L., Abundance of Philaenus spumarius in relation to types of plant community in the Tvärminne archipelago, southern Finland. 38 p. 1977

4 HAKKARI, L., On the productivity and ecology of zooplankton and its role as food for fish in some lakes in Central Finland. 87 p. 1978.

5 KÄPYLÄ, M., Bionomics of five woodnesting solitary species of bees (Hym., Megachilidae), with emphasis on flower relationships. 89 p. 1978.

6 KANKAALA, P. & SAARI, V., The vascular flora of the Vaarunvuoret hills and its conservation, pp. 3-62.
TÖRMÄLÄ, T. & KOVANEN, J., Growth and ageing of magpie (Pica pica L.) nestlings, pp. 63-77. 77 p. 1979.

7 VIITALA, J., Hair growth patterns in the vole Clethrionomys rufocanus (Sund.), pp. 3-17.
NIEMI, R. & HUHTA, V., Oribatid communities in artificial soil made of sewage sludge and crushed bark, pp. 18-30. 30 p. 1981.

8 TÖRMÄLÄ, T., Structure and dynamics of reserved field ecosystem in central Finland. 58 p. 1981.

9 ELORANTA, V. & KUIVASNIEMI, K., Acute toxicity of two herbicides, glyphosate and 2,4-D, to Selenastrum capricornuturn Printz (Chlorophyta), pp. 3-18.
ELORANTA, P. & KUNNAS, S., Periphyton accumulation and diatom communities on artificial substrates in recipients of pulp mill effluents, pp. 19-33.
ELORANTA, P. & MARJA-AHO, J., Transect studies on the aquatic inacrophyte vegetation of Lake Saimaa in 1980, pp. 35-65. 65 p. 1982.

10 LAKE PÄIJÄNNE SYMPOSIUM. 199 p. 1987.

11 SAARI, V. & OHENOJA, E., A check-list of the larger fungi of Central Finland. 74 p. 1988.

12 KOJOLA, I., Maternal investment in semi-domesticated reindeer (Rangifer t. tarandus L.). 26 p. Yhteenveto 2 p. 1989.

13 MERILÄINEN, J. J., Impact of an acid, polyhumic river on estuarine zoobenthos and vegetation in the Baltic Sea, Finland. 48 p. Yhteenveto 2 p. 1989.

14 LUMME, I., On the clone selection, ectomycorrhizal inoculation of short-rotation willows (Salix spp.) and on the effects of some nutrients sources on soil properties and plant nutrition. 55 p. Yhteenveto 3 p. 1989.

15 KUITUNEN, M., Food, space and time constraints on reproduction in the common treecreeper (Certhia familiaris L.) 22 p. Yhteenveto 2 p. 1989.

16 YLÖNEN, H., Temporal variation of behavioural and demographical processes in cyclic Clethrionomys populations. 35 p. Yhteenveto 2 p. 1989.

17 MIKKONEN, A., Occurrence and properties of proteolytic enzymes in germinating legume seeds. 61 p. Yhteenveto 1 p. 1990.

18 KAINULAINEN, H., Effects of chronic exercise and ageing on regional energy metabolism in heart muscle. 76 p. Yhteenveto 1 p. 1990.

19 LAKSO, MERJA, Sex-specific mouse testosterone 16 "-hydroxylase (cytochrome P450) genes: characterization and genetic and hormonal regulations. 70 p. Yhteenveto 1 p. 1990.

20 SETÄLÄ, HEIKKI, Effects of soil fauna on decomposition and nutrient dynamics in coniferous forest soil. 56 p. Yhteenveto 2 p. 1990.

21 NÄRVÄNEN, ALE, Synthetic peptides as probes for protein interactions and as antigenic epitopes. 90 p. Yhteenveto 2 p. 1990.

22 ECOTOXICOLOGY SEMINAR, 115 p. 1991.

23 ROSSI, ESKO, An index method for environmental risk assessment in wood processing industry. 117 p. Yhteenveto 2 p. 1991.

24 SUHONEN, JUKKA, Predation risk and competition in mixed species tit flocks. 29 p. Yhteenveto 2 p. 1991.

25 SUOMEN MUUTTUVA LUONTO. Mikko Raatikaiselle omistettu juhlakirja. 185 p. 1992.

26 KOSKIVAARA, MARI, Monogeneans and other parasites on the gills of roach (Rutilus rutilus) in Central Finland. Differences between four lakes and the nature of dactylogyrid communities. 30 p. Yhteenveto 2 p. 1992.

27 TASKINEN, JOUNI, On the ecology of two Rhipidocotyle species (Digenea: Bucephalidae) from two Finnish lakes. 31 p. Yhteenveto 2 p. 1992.

28 HUOVILA, ARI, Assembly of hepatitis B surface antigen. 73 p. Yhteenveto 1 p. 1992.

29 SALONEN, VEIKKO, Plant colonization of harvested peat surfaces. 29 p. Yhteenveto 2 p. 1992.

30 JOKINEN, ILMARI, Immunoglobulin production by cultured lymphocytes of patients with rheumatoid arthritis: association with disease severity. 78 p. Yhteenveto 2 p. 1992.
31 PUNNONEN, EEVA-LIISA, Ultrastructural studies on cellular autophagy. Structure of limiting membranes and route of enzyme delivery. 77 p. Yhteenveto 2 p. 1993.
32 HAIMI, JARI, Effects of earthworms on soil processes in coniferous forest soil. 35 p. Yhteenveto 2 p. 1993.
33 ZHAO, GUOCHANG, Ultraviolet radiation induced oxidative stress in cultured human skin fibroblasts and antioxidant protection. 86 p. Yhteenveto 1 p. 1993.
34 RÄTTI, OSMO, Polyterritorial polygyny in the pied flycatcher. 31 p. Yhteenveto 2 p. 1993.
35 MARJOMÄKI, VARPU, Endosomes and lysosomes in cardiomyocytes. A study on morphology and function. 64 p. Yhteenveto 1 p. 1993.
36 KIHLSTRÖM, MARKKU, Myocardial antioxidant enzyme systems in physical exercise and tissue damage. 99 p. Yhteenveto 2 p. 1994.
37 MUOTKA, TIMO, Patterns in northern stream guilds and communities. 24 p. Yhteenveto 2 p. 1994.
38 EFFECT OF FERTILIZATION ON FOREST ECOSYSTEM 218 p. 1994.
39 KERVINEN, JUKKA, Occurrence, catalytic properties, intracellular localization and structure of barley aspartic proteinase. 65 p. Yhteenveto 1 p. 1994.
40 MAPPES, JOHANNA, Maternal care and reproductive tactics in shield bugs. 30 p. Yhteenveto 3 p. 1994.
41 SIIKAMÄKI, PIRKKO, Determinants of clutch-size and reproductive success in the pied flycatcher. 35 p. Yhteenveto 2 p. 1995.
42 MAPPES, TAPIO, Breeding tactics and reproductive success in the bank vole. 28 p. Yhteenveto 3 p. 1995.
43 LAITINEN, MARKKU, Biomonitoring of theresponses of fish to environmental stress. 39 p. Yhteenveto 2 p. 1995.
44 LAPPALAINEN, PEKKA, The dinuclear Cu_A centre of cytochrome oxidase. 52 p. Yhteenveto 1 p. 1995.
45 RINTAMÄKI, PEKKA, Male mating success and female choice in the lekking black grouse. 23 p. Yhteenveto 2 p. 1995.
46 SUURONEN, TIINA, The relationship of oxidative and glycolytic capacity of longissimus dorsi muscle to meat quality when different pig breeds and crossbreeds are compared. 112 p. Yhteenveto 2 p. 1995.
47 KOSKENNIEMI, ESA, The ecological succession and characteristics in small Finnish polyhumic reservoirs. 36 p. Yhteenveto 1 p. 1995.
48 HOVI, MATTI, The lek mating system in the black grouse: the role of sexual selection. 30 p. Yhteenveto 1 p. 1995.
49 MARTTILA, SALLA, Differential expression of aspartic and cycteine proteinases, glutamine synthetase, and a stress protein, HVA1, in germinating barley. 54 p. Yhteenveto 1 p. 1996
50 HUHTA, ESA, Effects of forest fragmentation on reproductive success of birds in boreal forests. 26 p. Yhteenveto 2 p. 1996.
51 OJALA, JOHANNA, Muscle cell differentiation in vitro and effects of antisense oligode-oxyribonucleotides on gene expression of contractile proteins. 157 p. Yhteenveto 2 p.1996.
52 PALOMÄKI, RISTO, Biomass and diversity of macrozoobenthos in the lake littoral in relation to environmental characteristics. 27 p. Yhteenveto 2 p. 1996.
53 PUSENIUS, JYRKI, Intraspecific interactions, space use and reproductive success in the field vole. 28 p. Yhteenveto 2 p. 1996.
54 SALMINEN, JANNE, Effects of harmful chemicals on soil animal communities and decomposition. 28 p. Yhteenveto 2 p. 1996.
55 KOTIAHO, JANNE, Sexual selection and costs of sexual signalling in a wolf spider. 25 p. (96 p.). Yhteenveto 2 p. 1997.
56 KOSKELA, JUHA, Feed intake and growth variability in Salmonids. 27p. (108 p.). Yhteenveto 2 p. 1997.
57 NAARALA, JONNE, Studies in the mechanisms of lead neurotoxicity and oxidative stress in human neuroblastoma cells. 68 p. (126 p.). Yhteenveto 1 p. 1997.
58 AHO, TEIJA, Determinants of breeding performance of the Eurasian treecreeper. 27 p. (130 p.). Yhteenveto 2 p. 1997.
59 HAAPARANTA, AHTI, Cell and tissue changes in perch (Perca fluviatilis) and roach (Rutilus rutilus) in relation to water quality. 43 p. (112 p.). Yhteenveto 3 p. 1997.
60 SOIMASUO, MARKUS, The effects of pulp and paper mill effluents on fish: a biomarker approach. 59 p. (158 p.). Yhteenveto 2 p. 1997.
61 MIKOLA, JUHA, Trophic-level dynamics in microbial-based soil food webs. 31 p. (110 p.). Yhteenveto 1 p. 1997.
62 RAHKONEN, RIITTA, Interactions between a gull tapeworm Diphyllobothrium dendriticum (Cestoda) and trout (Salmo trutta L). 43 p. (69 p.). Yhteenveto 3 p. 1998.
63 KOSKELA, ESA, Reproductive trade-offs in the bank vole. 29 p. (94 p.). Yhteenveto 2 p. 1998.
64 HORNE, TAINA, Evolution of female choice in the bank vole. 22 p. (78 p.). Yhteenveto 2 p. 1998.
65 PIRHONEN, JUHANI, Some effects of cultivation on the smolting of two forms of brown trout (Salmo trutta). 37 p. (97 p.). Yhteenveto 2 p. 1998.
66 LAAKSO, JOUNI, Sensitivity of ecosystem functioning to changes in the structure of soil food webs. 28 p. (151 p.). Yhteenveto 1 p. 1998.
67 NIKULA, TUOMO, Development of radiolabeled monoclonal antibody constructs: capable of transporting high radiation dose into cancer cells. 45 p. (109 p.). Yhteenveto 1 p. 1998.

BIOLOGICAL RESEARCH REPORTS FROM THE UNIVERSITY OF JYVÄSKYLÄ

68 AIRENNE, KARI, Production of recombinant avidins in Escherichia coli and insect cells. 96 p. (136 p.). Yhteenveto 2 p. 1998.
69 LYYTIKÄINEN, TAPANI, Thermal biology of underyearling Lake Inari Arctic Charr Salvelinus alpinus. 34 p. (92 p.). Yhteenveto 1 p. 1998.
70 VIHINEN-RANTA, MAIJA, Canine parvovirus. Endocytic entry and nuclear import. 74 p. (96 p.). Yhteenveto 1 p. 1998.
71 MARTIKAINEN, ESKO, Environmental factors influencing effects of chemicals on soil animals. Studies at population and community levels. 44 p. (137 p.). Yhteenveto 1 p. 1998.
72 AHLROTH, PETRI, Dispersal and life-history differences between waterstrider (Aquarius najas) populations. 36 p. (98 p.). Yhteenveto 1 p. 1999.
73 SIPPONEN, MATTI, The Finnish inland fisheries system. The outcomes of private ownership of fishing rights and of changes in administrative practices. 81 p. (188 p.). Yhteenveto 2 p. 1999.
74 LAMMI, ANTTI, Reproductive success, local adaptation and genetic diversity in small plant populations. 36 p. (107 p.). Yhteenveto 4 p. 1999.
75 NIVA, TEUVO, Ecology of stocked brown trout in boreal lakes. 26 p. (102 p.). Yhteenveto 1 p. 1999.
76 PULKKINEN, KATJA, Transmission of Triaenophorus crassus from copepod first to coregonid second intermediate hosts and effects on intermediate hosts. 45 p. (123 p.). Yhteenveto 3 p. 1999.
77 PARRI, SILJA, Female choice for male drumming characteristics in the wolf spider Hygrolycosa rubrofasciata. 34 p. (108 p.). Yhteenveto 2 p. 1999.

JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

78 VIROLAINEN, KAIJA, Selection of nature reserve networks. - Luonnonsuojelualueiden valinta. 28 p. (87 p.). Yhteenveto 1 p. 1999.
79 SELIN, PIRKKO, Turvevarojen teollinen käyttö ja suopohjan hyödyntäminen Suomessa. - Industrial use of peatlands and the re-use of cut-away areas in Finland. 262 p. Foreword 3 p. Executive summary 9 p. 1999.
80 LEPPÄNEN, HARRI, The fate of resin acids and resin acid-derived compounds in aquatic environment contaminated by chemical wood industry. - Hartsihappojen ja hartsihappoperäisten yhdisteiden ympäristökohtalo kemiallisen puunjalostusteollisuuden likaamissa vesistöissä. 45 p. (149 p.). Yhteenveto 2 p.1999.
81 LINDSTRÖM, LEENA, Evolution of conspicuous warning signals. - Näkyvien varoitussignaalien evoluutio. 44 p. (96 p.). Yhteenveto 3 p. 2000.
82 MATTILA, ELISA, Factors limiting reproductive success in terrestrial orchids. - Kämmeköiden lisääntymismenestystä rajoittavat tekijät. 29 p. (95 p.). Yhteenveto 2 p. 2000.
83 KARELS, AARNO, Ecotoxicity of pulp and paper mill effluents in fish. Responses at biochemical, individual, population and community levels. - Sellu- ja paperiteollisuuden jätevesien ekotoksisuus kaloille. Tutkimus kalojen biokemiallisista, fysiologisista sekä populaatio- ja yhteisövasteista. 68 p. (177 p.). Yhteenveto 1 p. Samenvatting 1 p. 2000.
84 AALTONEN, TUULA, Effects of pulp and paper mill effluents on fish immune defence. - Metsäteollisuuden jätevesien aiheuttamat immunologiset muutokset kaloissa. 62 p. (125 p.). 2000.
85 HELENIUS, MERJA, Aging-associated changes in NF-kappa B signaling. - Ikääntymisen vaikutus NF-kappa B:n signalointiin. 75 p. (143 p.). Yhteenveto 2 p. 2000.
86 HUOVINEN, PIRJO, Ultraviolet radiation in aquatic environments. Underwater UV penetration and responses in algae and zooplankton. - Ultraviolettisäteilyn vedenalainen tunkeutuminen ja sen vaikutukset leviin ja eläinplanktoniin. 52 p. (145 p.). Yhteenveto 2 p. 2000.
87 PÄÄKKÖNEN, JARI-PEKKA, Feeding biology of burbot, *Lota lota* (L.): Adaptation to profundal lifestyle? - Mateen, *Lota lota* (L), ravinnonkäytön erityispiirteet: sopeumia pohjaelämään? 33 p. (79 p.). Yhteenveto 2 p. 2000.
88 LAASONEN, PEKKA, The effects of stream habit restoration on benthic communities in boreal headwater streams. - Koskikunnostuksen vaikutus jokien pohjaeläimistöön. 32 p. (101 p.). Yhteenveto 2 p. 2000.
89 PASONEN, HANNA-LEENA, Pollen competition in silver birch (*Betula pendula* Roth). An evolutionary perspective and implications for commercial seed production. - Siitepölykilpailu koivulla. 41 p. (115 p.). Yhteenveto 2 p. 2000.
90 SALMINEN, ESA, Anaerobic digestion of solid poultry slaughterhouse by-products and wastes. - Siipikarjateurastuksen sivutuotteiden ja jätteiden anaerobinen käsittely. 60 p. (166 p.). Yhteenveto 2 p. 2000.
91 SALO, HARRI, Effects of ultraviolet radiation on the immune system of fish. - Ultraviolettisäteilyn vaikutus kalan immunologiseen puolustusjärjestelmään. 61 p. (109 p.). Yhteenveto 2 p. 2000.
92 MUSTAJÄRVI, KAISA, Genetic and ecological consequences of small population size in *Lychnis viscaria*. - Geneettisten ja ekologisten tekijöiden vaikutus pienten mäkitervakkopopulaatioiden elinkykyyn. 33 p. (124 p.). Yhteenveto 3 p. 2000.

93 TIKKA, PÄIVI, Threatened flora of semi-natural grasslands: preservation and restoration. - Niittykasvillisuuden säilyttäminen ja ennallistaminen. 35 p. (105 p.). Yhteenveto 2 p. 2001.

94 SIITARI, HELI, Ultraviolet sensitivity in birds: consequences on foraging and mate choice. - Lintujen ultraviolettinäön ekologinen merkitys ravinnon- ja puolisonvalinnassa. 31 p. (90 p.). Yhteenveto 2 p. 2001.

95 VERTAINEN, LAURA, Variation in life-history traits and behaviour among wolf spider (*Hygrolycosa rubrofasciata*) populations. - Populaatioiden väliset erot rummuttavan hämähäkin *Hygrolycosa rubrofasciata*) kasvussa ja käyttäytymisessä. 37 p. (117 p.) Yhteenveto 2 p. 2001.

96 HAAPALA, ANTTI, The importance of particulate organic matter to invertebrate communities of boreal woodland streams. Implications for stream restoration. - Hiukkasmaisen orgaanisen aineksen merkitys pohjoisten metsäjokien pohjaeläinyhteisöille - huomioita virtavesien kunnostushankkeisiin. 35 p. (127 p.) Yhteenveto 2 p. 2001.

97 NISSINEN, LIISA, The collagen receptor integrins - differential regulation of their expression and signaling functions. - Kollageeniin sitoutuvat integriinit - niiden toisistaan eroava säätely ja signalointi. 67 p. (125 p.) Yhteenveto 1 p. 2001.

98 AHLROTH, MERVI, The chicken avidin gene family. Organization, evolution and frequent recombination. - Kanan avidiini-geeniperhe. Organisaatio, evoluutio ja tiheä rekombinaatio. 73 p. (120 p.) Yhteenveto 2 p. 2001.

99 HYÖTYLÄINEN, TARJA, Assessment of ecotoxicological effects of creosote-contaminated lake sediment and its remediation. - Kreosootilla saastuneen järvisedimentin ekotoksikologisen riskin ja kunnostuksen arviointi. 59 p. (132 p.) Yhteenveto 2 p. 2001.

100 SULKAVA, PEKKA, Interactions between faunal community and decomposition processes in relation to microclimate and heterogeneity in boreal forest soil. - Maaperän eliöyhteisön ja hajotusprosessien väliset vuorovaiku-tukset suhteessa mikroilmastoon ja laikut-taisuuteen. 36 p. (94 p.) Yhteenveto 2 p. 2001.

101 LAITINEN, OLLI, Engineering of physicochemical properties and quaternary structure assemblies of avidin and streptavidin, and characterization of avidin related proteins. - Avidiinin ja streptavi-diinin kvaternäärirakenteen ja fysioke-miallisten ominaisuuksien muokkaus sekä avidiinin kaltaisten proteiinien karakteri-sointi. 81 p. (126 p.) Yhteenveto 2 p. 2001.

102 LYYTINEN, ANNE, Insect coloration as a defence mechanism against visually hunting predators. - Hyönteisten väritys puolustuksessa vihollisia vastaan. 44 p. (92 p.) Yhteenveto 3 p. 2001.

103 NIKKILÄ, ANNA, Effects of organic material on the bioavailability, toxicokinetics and toxicity of xenobiotics in freshwater organisms. - Orgaanisen aineksen vaikutus vierasaineiden biosaatavuuteen, toksikokinetiikkaan ja toksisuuteen vesieliöillä. 49 p. (102 p.) Yhteenveto 3 p. 2001.

104 LIIRI, MIRA, Complexity of soil faunal communities in relation to ecosystem functioning in coniferous forrest soil. A disturbance oriented study. - Maaperän hajottajaeliöstön monimuotoisuuden merkitys metsäekosysteemin toiminnassa ja häiriönsiedossa. 36 p. (121 p.) Yhteenveto 2 p. 2001.

105 KOSKELA, TANJA, Potential for coevolution in a host plant – holoparasitic plant interaction. - Isäntäkasvin ja täysloiskasvin välinen vuorovaikutus: edellytyksiä koevoluutiolle? 44 p. (122 p.) Yhteenveto 3 p. 2001.

106 LAPPIVAARA, JARMO, Modifications of acute physiological stress response in whitefish after prolonged exposures to water of anthropogenically impaired quality. - Ihmistoiminnan aiheuttaman veden laadun heikentymisen vaikutukset planktonsiian fysiologisessa stressivasteessa. 46 p. (108 p.) Yhteenveto 3 p. 2001.

107 ECCARD, JANA, Effects of competition and seasonality on life history traits of bank voles. - Kilpailun ja vuodenaikaisvaihtelun vaikutus metsämyyrän elinkiertopiirteisiin. 29 p. (115 p.) Yhteenveto 2 p. 2002.

108 NIEMINEN, JOUNI, Modelling the functioning of experimental soil food webs. - Kokeellisten maaperäravintoverkkojen toiminnan mallintaminen. 31 p. (111 p.) Yhteenveto 2 p. 2002.

109 NYKÄNEN, MARKO, Protein secretion in *Trichoderma reesei*. Expression, secretion and maturation of cellobiohydrolase I, barley cysteine proteinase and calf chymosin in Rut-C30. - Proteiinien erittyminen *Trichoderma reesei*ssä. Sellobiohydrolaasi I:n, ohran kysteiiniproteinaasin sekä vasikan kymosiinin ilmeneminen, erittyminen ja kypsyminen Rut-C30-mutanttikannassa. 107 p. (173 p.) Yhteenveto 2 p. 2002.

110 TIIROLA, MARJA, Phylogenetic analysis of bacterial diversity using ribosomal RNA gene sequences. - Ribosomaalisen RNA-geenin sekvenssien käyttö bakteeridiversiteetin fylogeneettisessä analyysissä. 75 p. (139 p.) Yhteenveto 2 p. 2002.

111 HONKAVAARA, JOHANNA, Ultraviolet cues in fruit-frugivore interactions. - Ultraviolettinäön ekologinen merkitys hedelmiä syövien eläinten ja hedelmäkasvien välisissä vuorovaikutussuhteissa. 27 p. (95 p.) Yhteenveto 2 p. 2002.

112 MARTTILA, ARI, Engineering of charge, biotin-binding and oligomerization of avidin: new tools for avidin-biotin technology. - Avidiinin varauksen, biotiininsitomisen sekä oligomerisaation muokkaus: uusia työkaluja avidiini–biotiiniteknologiaan. 68 p. (130 p.) Yhteenveto 2 p. 2002.

113 JOKELA, JARI, Landfill operation and waste management procedures in the reduction of methane and leachate pollutant emissions from municipal solid waste landfills. - Kaatopaikan operoinnin ja jätteen esikäsittelyn vaikutus yhdyskuntajätteen biohajoamiseen ja typpipäästöjen hallintaan. 62 p. (173 p.) Yhteenveto 3 p. 2002.

114 RANTALA, MARKUS J., Immunocompetence and sexual selection in insects. - Immunokompetenssi ja seksuaalivalinta hyönteisillä. 23 p. (108 p.) Yhteenveto 1 p. 2002.

115 OKSANEN, TUULA, Cost of reproduction and offspring quality in the evolution of reproductive effort. - Lisääntymisen kustannukset ja poikasten laatu lisääntymispanostuksen evoluutiossa. 33 p. (95 p.) Yhteenveto 2 p. 2002.

116 HEINO, JANI, Spatial variation of benthic macroinvertebrate biodiversity in boreal streams. Biogeographic context and conservation implications. - Pohjaeläinyhteisöjen monimuotoisuuden spatiaalinen vaihtelu pohjoisissa virtavesissä - eliömaantieteellinen yhteys sekä merkitys jokivesien suojelulle. 43 p. (169 p.) Yhteenveto 3 p. 2002.

117 SIIRA-PIETIKÄINEN, ANNE, Decomposer community in boreal coniferous forest soil after forest harvesting: mechanisms behind responses. - Pohjoisen havumetsämaan hajottajayhteisö hakkuiden jälkeen: muutoksiin johtavat mekanismit. 46 p. (142 p.) Yhteenveto 3 p. 2002.

118 KORTET, RAINE, Parasitism, reproduction and sexual selection of roach, *Rutilus rutilus* L. - Loisten ja taudinaiheuttajien merkitys kalan lisääntymisessä ja seksuaalivalinnassa. 37 p. (111 p.) Yhteenveto 2 p. 2003.

119 SUVILAMPI, JUHANI, Aerobic wastewater treatment under high and varying temperatures – thermophilic process performance and effluent quality. - Jätevesien käsittely korkeissa ja vaihtelevissa lämpötiloissa. 59 p. (156 p.) Yhteenveto 2 p. 2003.

120 PÄIVINEN, JUSSI, Distribution, abundance and species richness of butterflies and myrmecophilous beetles. - Perhosten ja muurahaispesissä elävien kovakuoriaisten levinneisyys, runsaus ja lajistollinen monimuotoisuus 44 p. (155 p.) Yhteenveto 2 p. 2003.

121 PAAVOLA, RIKU, Community structure of macroinvertebrates, bryophytes and fish in boreal streams. Patterns from local to regional scales, with conservation implications. - Selkärangattomien, vesisammalten ja kalojen yhteisörakenne pohjoisissa virtavesissä – säännönmukaisuudet paikallisesta mittakaavasta alueelliseen ja luonnonsuojelullinen merkitys. 36 p. (121 p.) Yhteenveto 3 p. 2003.

122 SUIKKANEN, SANNA, Cell biology of canine parvovirus entry. - Koiran parvovirusinfektion alkuvaiheiden solubiologia. 88 p. (135 p.) Yhteenveto 3 p. 2003.

123 AHTIAINEN, JARI JUHANI, Condition-dependence of male sexual signalling in the drumming wolf spider *Hygrolycosa rubrofasciata.* - Koiraan seksuaalisen signaloinnin kuntoriippuvuus rummuttavalla susihämähäkillä *Hygrolycosa rubrofasciata.* 31 p. (121 p.) Yhteenveto 2 p. 2003.

124 KAPARAJU, PRASAD, Enhancing methane production in a farm-scale biogas production system. - Metaanintuoton tehostaminen tilakohtaisessa biokaasuntuotantojärjestelmässä. 84 p. (224 p.) Yhteenveto 2 p. 2003.

125 HÄKKINEN, JANI, Comparative sensitivity of boreal fishes to UV-B and UV-induced phototoxicity of retene. - Kalojen varhaisvaiheiden herkkyys UV-B säteilylle ja reteenin UV-valoindusoituvalle toksisuudelle. 58 p. (134 p.) Yhteenveto 2 p. 2003.

126 NORDLUND, HENRI, Avidin engineering; modification of function, oligomerization, stability and structure topology. - Avidiinin toiminnan, oligomerisaation, kestävyyden ja rakennetopologian muokkaaminen. 64 p. (104 p.) Yhteenveto 2 p. 2003.

127 MARJOMÄKI, TIMO J., Recruitment variability in vendace, *Coregonus albula* (L.), and its consequences for vendace harvesting. - Muikun, *Coregonus albula* (L.), vuosiluokkien runsauden vaihtelu ja sen vaikutukset kalastukseen. 66 p. (155 p.) Yhteenveto 2 p. 2003.

128 KILPIMAA, JANNE, Male ornamentation and immune function in two species of passerines. - Koiraan ornamentit ja immuunipuolustus varpuslinnuilla. 34 p. (104 p.) Yhteenveto 1 p. 2004.

129 PÖNNIÖ, TIIA, Analyzing the function of nuclear receptor Nor-1 in mice. - Hiiren tumareseptori Nor-1:n toiminnan tutkiminen. 65 p. (119 p.) Yhteenveto 2 p. 2004.

130 WANG, HONG, Function and structure, subcellular localization and evolution of the encoding gene of pentachlorophenol 4-monooxygenase in sphingomonads. 56 p. (90 p.) 2004.

131 YLÖNEN, OLLI, Effects of enhancing UV-B irradiance on the behaviour, survival and metabolism of coregonid larvae. - Lisääntyvän UV-B säteilyn vaikutukset siikakalojen poikasten käyttäytymiseen, kuolleisuuteen ja metaboliaan. 42 p. (95 p.) Yhteenveto 2 p. 2004.

132 KUMPULAINEN, TOMI, The evolution and maintenance of reproductive strategies in bag worm moths (Lepidoptera: Psychidae). - Lisääntymisstrategioiden evoluutio ja säilyminen pussikehrääjillä (Lepidoptera: Psychidae). 42 p. (161 p.) Yhteenveto 3 p. 2004.

133 OJALA, KIRSI, Development and applications of baculoviral display techniques. - Bakulovirus display -tekniikoiden kehittäminen ja sovellukset. 90 p. (141 p.) Yhteenveto 3 p. 2004.

134 RANTALAINEN, MINNA-LIISA, Sensitivity of soil decomposer communities to habitat fragmentation – an experimental approach. - Metsämaaperän hajottajayhteisön vasteet elinympäristön pirstaloitumiseen. 38 p. (130 p.) Yhteenveto 2 p. 2004.

135 SAARINEN, MARI, Factors contributing to the abundance of the ergasilid copepod, *Paraergasilus rylovi*, in its freshwater molluscan host, *Anodonta piscinalis*. - *Paraergasilus rylovi* -loisäyriäisen esiintymiseen ja runsauteen vaikuttavat tekijät *Anodonta piscinalis* -pikkujärvisimpukassa. 47 p. (133 p.) Yhteenveto 4 p. 2004.

136 LILJA, JUHA, Assessment of fish migration in rivers by horizontal echo sounding: Problems concerning side-aspect target strength. - Jokeen vaeltavien kalojen laskeminen sivuttaissuuntaisella kaikuluotauksella: sivuaspektikohdevoimakkuuteen liittyviä ongelmia. 40 p. (82 p.) Yhteenveto 2 p. 2004.

137 NYKVIST, PETRI, Integrins as cellular receptors for fibril-forming and transmembrane collagens. - Integriinit reseptoreina fibrillaarisille ja transmembraanisille kollageeneille. 127 p. (161 p.) Yhteenveto 3 p. 2004.

138 KOIVULA, NIINA, Temporal perspective of humification of organic matter. - Orgaanisen aineen humuistuminen tarkasteltuna ajan funktiona. 62 p. (164 p.) Yhteenveto 2 p. 2004.

139 KARVONEN, ANSSI, Transmission of *Diplostomum spathaceum* between intermediate hosts. - *Diplostomum spathaceum* -loisen siirtyminen kotilo- ja kalaisännän välillä. 40 p. (90 p.) Yhteenveto 2 p. 2004.

140 NYKÄNEN, MARI, Habitat selection by riverine grayling, *Thymallus thymallus L.* - Harjuksen (*Thymallus thymallus L.*) habitaatinvalinta virtavesissä. 40 p. (102 p.) Yhteenveto 3 p. 2004.

141 HYNYNEN, JUHANI, Anthropogenic changes in Finnish lakes during the past 150 years inferred from benthic invertebrates and their sedimentary remains. - Ihmistoiminnan aiheuttamat kuormitusmuutokset suomalaisissa järvissä viimeksi kuluneiden 150 vuoden aikana tarkasteltuina pohjaeläinyhteisöjen avulla. 45 p. (221 p.) Yhteenveto 3 p. 2004.

142 PYLKKÖ, PÄIVI, Atypical *Aeromonas salmonicida* -infection as a threat to farming of arctic charr (*Salvelinus alpinus L.*) and european grayling (*Thymallus thymallus L.*) and putative means to prevent the infection. - Epätyyppinen *Aeromonas salmonicida* -bakteeritartunta uhkana harjukselle (*Thymallus thymallus L.*) ja nieriälle (*Salvelinus alpinus L.*) laitoskasvatuksessa ja mahdollisia keinoja tartunnan ennaltaehkäisyyn. 46 p. (107 p.) Yhteenveto 2 p. 2004.

143 PUURTINEN, MIKAEL, Evolution of hermaphroditic mating systems in animals. - Kaksineuvoisten lisääntymisstrategioiden evoluutio eläimillä. 28 p. (110 p.) Yhteenveto 3 p. 2004.

144 TOLVANEN, OUTI, Effects of waste treatment technique and quality of waste on bioaerosols in Finnish waste treatment plants. - Jätteenkäsittelytekniikan ja jätelaadun vaikutus bioaerosolipitoisuuksiin suomalaisilla jätteenkäsittelylaitoksilla. 78 p. (174 p.) Yhteenveto 4 p. 2004.

145 BOADI, KWASI OWUSU, Environment and health in the Accra metropolitan area, Ghana. - Accran (Ghana) suurkaupunkialueen ympäristö ja terveys. 33 p. (123 p.) Yhteenveto 2 p. 2004.

146 LUKKARI, TUOMAS, Earthworm responses to metal contamination: Tools for soil quality assessment. - Lierojen vasteet metallialtistukseen: käyttömahdollisuudet maaperän tilan arvioinnissa. 64 p. (150 p.) Yhteenveto 3 p. 2004.

147 MARTTINEN, SANNA, Potential of municipal sewage treatment plants to remove bis(2-ethylhexyl) phthalate. - Bis-(2-etyyliheksyyli)ftalaatin poistaminen jätevesistä yhdyskuntajätevedenpuhdistamoilla. 51 p. (100 p.) Yhteenveto 2 p. 2004.

148 KARISOLA, PIIA, Immunological characterization and engineering of the major latex allergen, hevein (Hev b 6.02). - Luonnonkumiallergian pääallergeenin, heveiinin (Hev b 6.02), immunologisten ominaisuuksien karakterisointi ja muokkaus. 91 p. (113 p.) Yhteenveto 2 p. 2004.

149 BAGGE, ANNA MARIA, Factors affecting the development and structure of monogenean communities on cyprinid fish. - Kidusloisyhteisöjen rakenteeseen ja kehitykseen vaikuttavat tekijät sisävesikaloilla. 25 p. (76 p.) Yhteenveto 1 p. 2005.

150 JÄNTTI, ARI, Effects of interspecific relationships in forested landscapes on breeding success in Eurasian treecreeper. - Lajienvälisten suhteiden vaikutus puukiipijän pesintämenestykseen metsäympäristössä. 39 p. (104 p.) Yhteenveto 2 p. 2005.

151 TYNKKYNEN, KATJA, Interspecific interactions and selection on secondary sexual characters in damselflies. - Lajienväliset vuorovaikutukset ja seksuaaliominaisuuksiin kohdistuva valinta sudenkorennoilla. 26 p. (86 p.) Yhteenveto 2 p. 2005.

175 Toivanen, Tero, Short-term effects of forest restoration on beetle diversity. - Metsien ennallistamisen merkitys kovakuoriaislajiston monimuotoisuudelle. 33 p. (112 p.) Yhteenveto 2 p. 2007.

176 Ludwig, Gilbert, Mechanisms of population declines in boreal forest grouse. - Kanalintukantojen laskuun vaikuttavat tekijät. 48 p. (138 p.) Yhteenveto 2 p. 2007.

177 Ketola, Tarmo, Genetics of condition and sexual selection. - Kunnon ja seksuaalivalinnan genetiikka. 29 p. (121 p.) Yhteenveto 2 p. 2007.

178 Seppänen, Janne-Tuomas, Interspecific social information in habitat choice. - Lajienvälinen sosiaalinen informaatio habitaatinvalinnassa. 33 p. (89 p.) Yhteenveto 2 p. 2007.

179 Bandilla, Matthias, Transmission and host and mate location in the fish louse *Argulus coregoni* and its link with bacterial disease in fish. - *Argulus coregoni* -kalatäin siirtyminen kalaisäntään, isännän ja parittelukumppanin paikallistaminen sekä loisinnan yhteys kalan bakteeritautiin. 40 p. (100 p.) Yhteenveto 3 p. Zusammenfassung 4 p. 2007.

180 Meriläinen, Päivi, Exposure assessment of animals to sediments contaminated by pulp and paper mills. - Sellu- ja paperiteollisuuden saastuttamat sedimentit altistavana tekijänä vesieläimille. 79 p. (169 p.) Yhteenveto 2 p. 2007.

181 Routtu, Jarkko, Genetic and phenotypic divergence in *Drosophila virilis* and *D. montana*. - Geneettinen ja fenotyyppinen erilaistuminen *Drosophila virilis* ja *D. montana* lajien mahlakärpäsillä. 34 p. (106 p.) Yhteenveto 1 p. 2007.

182 Benesh, Daniel P., Larval life history, transmission strategies, and the evolution of intermediate host exploitation by complex life-cycle parasites. - Väkäkärsämatotoukkien elinkierto- ja transmissiostrategiat sekä välisännän hyväksikäytön evoluutio. 33 p. (88 p.) Yhteenveto 1 p. 2007.

183 Taipale, Sami, Bacterial-mediated terrestrial carbon in the foodweb of humic lakes. - Bakteerivälitteisen terrestrisen hiilen merkitys humusjärvien ravintoketjussa. 61 p. (131 p.) Yhteenveto 5 p. 2007.

184 Kiljunen, Mikko, Accumulation of organochlorines in Baltic Sea fishes. - Organoklooriyhdisteiden kertyminen Itämeren kaloihin. 45 p. (97 p.) Yhteenveto 3 p. 2007.

185 Sormunen, Kai Markus, Characterisation of landfills for recovery of methane and control of emissions. - Kaatopaikkojen karakterisointi metaanipotentiaalin hyödyntämiseksi ja päästöjen vähentämiseksi. 83 p. (157 p.) Yhteenveto 2 p. 2008.

186 Hiltunen, Teppo, Environmental fluctuations and predation modulate community dynamics and diversity.- Ympäristön vaihtelut ja saalistus muokkaavat yhteisön dynamiikkaa ja diversiteettiä. 33 p. (100 p.) Yhteenveto 2 p. 2008.

187 Syväranta, Jari, Impacts of biomanipulation on lake ecosystem structure revealed by stable isotope analysis. - Biomanipulaation vaikutukset järviekosysteemin rakenteeseen vakaiden isotooppien avulla tarkasteltuna. 46 p. (105 p.) Yhteenveto 4 p. 2008.

188 Mattila, Niina, Ecological traits as determinants of extinction risk and distribution change in Lepidoptera. - Perhosten uhanalaisuuteen vaikuttavat ekologiset piirteet. 21 p. (67 p.) Yhteenveto 1 p. 2008.

189 Upla, Paula, Integrin-mediated entry of echovirus 1. - Echovirus 1:n integriinivälitteinen sisäänmeno soluun. 86 p. (145 p.) Yhteenveto 2 p. 2008.

190 Keskinen, Tapio, Feeding ecology and behaviour of pikeperch, Sander lucioperca (L.) in boreal lakes. - Kuhan (Sander lucioperca (L.)) ravinnonkäyttö ja käyttäytyminen boreaalisissa järvissä. 54 p. (136 p.) Yhteenveto 3 p. 2008.

191 Laakkonen, Johanna, Intracellular delivery of baculovirus and streptavidin-based vectors *in vitro* – towards novel therapeutic applications. - Bakulovirus ja streptavidiini geeninsiirtovektoreina ihmisen soluissa. 81 p. (142 p.) Yhteenveto 2 p. 2008.

192 Michel, Patrik, Production, purification and evaluation of insect cell-expressed proteins with diagnostic potential. - Diagnostisesti tärkeiden proteiinien tuotto hyönteissolussa sekä niiden puhdistus ja karakterisointi. 100 p. (119 p.) Yhteenveto 2 p. 2008.

193 Lindstedt, Carita, Maintenance of variation in warning signals under opposing selection pressures. - Vastakkaiset evolutiiviset valintapaineet ylläpitävät vaihtelua varoitussignaloinnissa. 56 p. (152 p.) Yhteenveto 2 p. 2008.

194 Boman, Sanna, Ecological and genetic factors contributing to invasion success: The northern spread of the Colorado potato beetle (*Leptinotarsa decemlineata*). - Ekologisten ja geneettisten tekijöiden vaikutus koloradonkuoriaisen (*Leptinotarsa decemlineata*) leviämismenestykseen. 50 p. (113 p.) Yhteenveto 3 p. 2008.

195 Mäkelä, Anna, Towards therapeutic gene delivery to human cancer cells. Targeting and entry of baculovirus. - Kohti terapeuttista geeninsiirtoa: bakuloviruksen kohdennus ja sisäänmeno ihmisen syöpäsoluihin. 103 p. (185 p.)Yhteenveto 2 p. 2008.

196 Lebigre, Christophe, Mating behaviour of the black grouse. Genetic characteristics and physiological consequences. - Teeren pariutumiskäyttäytyminen. Geneettiset tekijät ja fysiologiset seuraukset . 32 p. (111 p.)Yhteenveto 2 p. 2008.

197 KAKKONEN, ELINA, Regulation of raft-derived endocytic pathways – studies on echovirus 1 and baculovirus. - Echovirus 1:n ja bakuloviruksen soluun sisäänmenon reitit ja säätely. 96 p. (159 p.) Yhteenveto 2 p. 2009.

198 TENHOLA-ROININEN, TEIJA, Rye doubled haploids – production and use in mapping studies. - Rukiin kaksoishaploidit – tuotto ja käyttö kartoituksessa. 93 p. (164 p.) Yhteenveto 3 p. 2009.

199 TREBATICKÁ, LENKA, Predation risk shaping individual behaviour, life histories and species interactions in small mammals. - Petoriskin vaikutus yksilön käyttäytymiseen, elinkiertopiirteisiin ja yksilöiden välisiin suhteisiin. 29 p. (91 p.) Yhteenveto 3 p. 2009.

200 PIETIKÄINEN, ANNE, Arbuscular mycorrhiza, resource availability and belowground interactions between plants and soil microbes. - Arbuskelimykorritsa, resurssien saatavuus ja maanalaiset kasvien ja mikrobien väliset vuorovaikutukset. 38 p. (119 p.) Yhteenveto 2 p. 2009.

201 AROVIITA, JUKKA, Predictive models in assessment of macroinvertebrates in boreal rivers. - Ennustavat mallit jokien pohjaeläimistön tilan arvioinnissa. 45 p. (109 p.) Yhteenveto 3 p. 2009.

202 RASI, SAIJA, Biogas composition and upgrading to biomethane. - Biokaasun koostumus ja puhdistaminen biometaaniksi. 76 p. (135 p.) Yhteenveto 3 p. 2009.

203 PAKKANEN, KIRSI, From endosomes onwards. Membranes, lysosomes and viral capsid interactions. - Endosomeista eteenpäin. Lipidikalvoja, lysosomeja ja viruskapsidin vuorovaikutuksia. 119 p. (204 p.) Yhteenveto 2 p. 2009.

204 MARKKULA, EVELIINA, Ultraviolet B radiation induced alterations in immune function of fish, in relation to habitat preference and disease resistance. - Ultravioletti B -säteilyn vaikutus kalan taudinvastustuskykyyn ja immunologisen puolustusjärjestelmän toimintaan. 50 p. (99 p.) Yhteenveto 2 p. 2009.

205 IHALAINEN, TEEMU, Intranuclear dynamics in parvovirus infection. - Tumansisäinen dynamiikka parvovirus infektiossa. 86 p. (152 p.) Yhteenveto 3 p. 2009.

206 KUNTTU, HEIDI, Characterizing the bacterial fish pathogen *Flavobacterium columnare*, and some factors affecting its pathogenicity. - Kalapatogeeni *Flavobacterium columnare* -bakteerin ominaisuuksia ja patogeenisuuteen vaikuttavia tekijöitä. 69 p. (120 p.) Yhteenveto 3 p. 2010.

207 KOTILAINEN, TITTA, Solar UV radiation and plant responses: Assessing the methodological problems in research concerning stratospheric ozone depletion . - Auringon UV-säteily ja kasvien vasteet: otsonikatoon liittyvien tutkimusten menetelmien arviointia. 45 p. (126 p.) Yhteenveto 2 p. 2010.

208 EINOLA, JUHA, Biotic oxidation of methane in landfills in boreal climatic conditions . - Metaanin biotekninen hapettaminen kaatopaikoilla viileässä ilmastossa. 101 p. (156 p.) Yhteenveto 3 p. 2010.

209 PIIROINEN, SAIJA, Range expansion to novel environments: evolutionary physiology and genetics in *Leptinotarsa decemlineata*. - Lajien levinneisyysalueen laajeneminen: koloradonkuoriaisen evolutiivinen fysiologia ja genetiikka. 51 p. (155 p.) Yhteenveto 3 p. 2010.

210 NISKANEN, EINARI, On dynamics of parvoviral replication protein NS1. - Parvovirusten replikaationproteiini NS1:n dynamiikka. 81 p. (154 p.) Yhteenveto 3 p. 2010.

211 PEKKALA, SATU, Functional characterization of carbomoyl phosphate synthetase I deficiency and identification of the binding site for enzyme activator.- Karbamyylifosfaatti syntetaasi I:n puutteen patologian toiminnallinen karakterisaatio ja entsyymin aktivaattorin sitoutumiskohdan identifikaatio. 89 p. (127 p.) Yhteenveto 2 p. 2010.

212 HALME, PANU, Developing tools for biodiversity surveys - studies with wood-inhabiting fungi.- Työkaluja monimuotoisuustutkimuksiin - tutkimuskohteina puulla elävät sienet. 51 p. (125 p.) Yhteenveto 2 p. 2010.

213 JALASVUORI, MATTI, Viruses are ancient parasites that have influenced the evolution of contemporary and archaic forms of life. - Virukset ovat muinaisia loisia, jotka ovat vaikuttaneet nykyisten ja varhaisten elämänmuotojen kehitykseen. 94 p. (192 p.) Yhteenveto 2 p. 2010.

214 POSTILA, PEKKA, Dynamics of the ligand-binding domains of ionotropic glutamate receptors. - Ionotrooppisten glutamaattireseptoreiden ligandin-sitomisdomeenien dynamiikka. 54 p. (130 p.) Yhteenveto 3 p. 2010.

215 POIKONEN, TANJA, Frequency-dependent selection and environmental heterogeneity as selective mechanisms in wild populations. - Frekvenssistä riippuva valinta ja ympäristön heterogeenisyys luonnonvalintaa ohjaavina tekijöinä luonnonpopulaatiossa. 44 p. (115 p.) Yhteenveto 4 p. 2010.

216 KEKÄLÄINEN, JUKKA, Maintenance of genetic variation in sexual ornamentation – role of precopulatory and postcopulatory sexual selection. - Seksuaaliornamenttien geneettisen muuntelun säilyminen – parittelua edeltävän ja sen jälkeisen seksuaalivalinnan merkitys. 52 p. (123 p.) Yhteenveto 3 p. 2010.

217 SYRJÄNEN, JUKKA, Ecology, fisheries and management of wild brown trout populations in boreal inland waters. - Luontaisten taimenkantojen ekologia, kalastus ja hoito pohjoisilla sisävesillä. 43 p. (108 p.) Yhteenveto 3 p. 2010.