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Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species

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ABSTRACT

Population genetics patterns of marine fish in general and of Southern Ocean fish in particular range from virtual panmixia over ocean-wide scale to deeply fragmented populations. However the causes underlying these different patterns are not properly understood. In this paper, we tested the hypotheses that population connectivity is positively related to a combination of life history traits, namely duration of pelagic larval period and the tendency towards pelagic life style in the adulthood. To do so, we analysed the variability of six microsatellite and one mitochondrial marker (cytochrome *b*) in three Southern Ocean fish species (*Trematomus newnesi*, *Trematomus hansoni* and *Trematomus bernacchii*). They share a recent common ancestor but notably differ in their duration of pelagic larval period as well as pelagic versus benthic lifestyle. We sampled over a range of more than 5000 km for all three species and used a number of population genetics tools to investigate past and contemporary levels of connectivity. All species experienced population fluctuations, but coalescent simulations suggested that contemporary populations are in migration-drift equilibrium. Although global F_{ST} values were rather low, a significant population structure separated the High-Antarctic from the Peninsular regions in all species. The level of genetic differentiation was much lower in the pelagic *versus* benthic species. Present data suggest that past and present genetic structuring in the Southern Ocean are indeed related with the ecological traits of Antarctic fish, however the relative importance of individual factors remains unclear.

1. Introduction

Many marine species disperse primarily during the larval phase, while adults show limited movements (Bonhomme and Planes, 2000; Thorrold et al., 2007). Indeed, seventy percent of marine organisms have a planktonic stage during which larvae may disperse (Thorson, 1950). In general, fishes with longer larval duration display less genetic differentiation than those with shorter larval duration (Waples, 1987; Bay et al., 2006; Bradbury et al., 2008; Hauser and Carvalho, 2008). However, a growing body of evidence suggests the importance of other factors, such as currents and larval retention (Carreras-Carbonell et al., 2006), that may cause strong differentiation even in species with a long larval phase (Taylor and Hellberg, 2003; Planes et al., 1998). Many larvae are also capable of active vertical migration, which in combination with vertically stratified flows may allow them to avoid advection. In many cases, particularly in later stages of their pelagic developmental phase, larvae may be able to swim even faster than ambient currents (Leis, 2006).

Direct measurements of larval/individual dispersal of small organisms are difficult to obtain in any marine environment (Bay et al., 2006; Hedgecock et al., 2007; and references therein) but population genetics studies, although not without limitations, provide some of the most valuable tools for estimating such parameters (Hedgecock et al., 2007; Selkoe et al., 2008; Skillings et al., 2011). Populations evolve through changes in allele frequencies, which in turn change through

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evolutionary forces such as mutation, random genetic drift, gene flow and selection. In the absence of environmental change and over prolonged periods of time, a population will reach a genetic equilibrium determined by the opposing evolutionary forces of drift and migration. Historical factors such as climate associated extinctions and recolonisations or changes in the direction of currents also play an important role in shaping the population structure of marine species (Maggs et al., 2008). Genetic methods for estimating effective dispersal and connectivity are becoming increasingly effective. However publications often focus on low latitude reef species and general conclusions concerning the processes driving differentiation within species remain hard to draw (Bohonak, 1999; Bay et al., 2006).

The Southern Ocean, in spite of obvious challenges for sampling, offers a unique environment to study the effect of biological and environmental forces on patterns of dispersal and evolution in marine organisms. Characterised by two major currents that promote dispersal on a circumpolar scale, the majority of the surface flow moves in a clockwise (eastward) motion around Antarctica forming the Antarctic Circumpolar Current (ACC). Along the margins of the continent, however, there is a westward current, the Antarctic Slope Current (ASC) (Orsi et al., 1995). Application of genetic tools has revealed that patterns of population differentiation in Antarctic/Southern Ocean organisms range from virtual panmixia on a circum-Antarctic scale to isolated populations or even crypic species (for an overview see Rogers, 2007, 2012; Volckaert et al., 2012). The causes for such differences are not fully clear but the ultimate outcome likely depends on the interplay of several factors. Some studies emphasize the dominant roles of major climatic and geological events affecting southern polar organisms more or less simultaneously (Thatje et al., 2008). Other studies suggest that conditions for survival and dispersal differ across Antarctic biogeographic regions and hence the genetic structure of given species depends on its distribution range and the local conditions, especially temperature (McGaughran et al., 2010; Patarnello et al., 2011).

Yet other studies highlight the dominant role of intrinsic factors such as habitat preference in shaping the genetic structure of Antarctic species. Comparative analysis of four trematomids, *Trematomus bernacchi*, *Trematomus pennelli*, *Pagothenia borchgrevinki* and *Trematomus newnesi* showed a qualitatively different response of benthic and pelagic species to the Pleistocene glaciations (Janko et al., 2007). Benthic species in particular are expected to express a more pronounced genetic structure than pelagic ones (Rogers, 2007, 2012), which may either reflect lower dispersal at larval and adult stages or a higher complexity of the benthic environment, promoting habitat specialization. Such a situation may become extreme when populations have been historically separated during cycles of glaciation, suggesting that life style may decisively affect the evolution and adaptability of the species. Although Matschiner et al. (2009) recently showed that passive larval dispersal within Antarctic currents may be sufficient to homogenise populations of benthic fish species at a scale of hundreds of kilometres, one may expect that the length of pelagic larval phase in combination with specialisation to a benthic or pelagic life history in adulthood may have a prominent effect on the genetic build-up of a species' genepool (Purcell et al., 2006; Bradbury et al., 2008; Papetti et al., 2012).

The strength of a comparative approach lies in the possibility to disentangle the major determinants of population structure of Southern Ocean organisms and to broaden our scope of understanding of marine ecological speciation in general. Here we focus on the Trematominae, an endemic tribe of notothenioid teleosts from the Southern Ocean widely used as a model of cold adaptation (Patarnello et al., 2011). The group forms a monophyletic species flock strikingly diverse in morphology and demonstrates significant evolutionary versatility with a variety of ecological forms including species with benthic, pelagic or cryopelagic ecology (Janko et al., 2011; Rutschmann et al., 2011).

This study aims at comparing genetic diversity, genetic differentiation and environmental and life-history (traits) between three fish species with distinct characteristics living over the same environmental gradient. For this the nuclear and mitochondrial genetic structure and demographic history of *T. bernacchii*, *T. hansoni* and *T. newnesi* was compared. The former two are bottom-feeding fish that lay demersal eggs (with *T. bernacchii* even laying eggs in sponges and displaying nest guarding behaviour) (DeWitt et al., 1990; Kock et al., 2006; La Mesa et al., 2006). In contrast, *T. newnesi* prefers a semi-pelagic or even cryopelagic habitat. The larval phase of all three species is pelagic but differs in duration; *T. hansoni* has a long larval phase spanning over the winter, while the pelagic larval phase of *T. newnesi* is considerably shorter and accomplished during the first fall (Duhamel et al., 1993; detailed information on larval ecology of *T. bernacchii* remains undetermined). Adult individuals may differ substantially in dispersal ecology, since *T. newnesi* populations are known to largely vary in local abundance, indicative of seasonal migrations, while *T. bernacchii* is a rather sedentary fish (as an extreme example of this behaviour, we noted the re-capture of a specimen at the Ross Sea region, which had been tagged two years ago in the very same spot (J. MacDonald pers. Comm..). We hypothesize that life history traits such as larval duration and adult ecology affect genetic structure and diversity.

2. Material and methods

2.1. Sampling, DNA purification, DNA amplification and species validation

Samples of *T. newnesi* Boulenger, 1902, *T. hansoni* Boulenger, 1902 and *T. bernacchii* Boulenger, 1902 of the family Nototheniidae were collected using land-based sampling in Adelie Land and the Ross Sea and by bottom trawls during various scientific expeditions (Fig. 1, Table 1). For *T. newnesi*, two temporal samples were available from the same site at Adelie Land. Specimens were morphologically identified on site and muscle or fin tissue was preserved in 100% ethanol.

Total genomic DNA was extracted using the Nucleospin Extraction kit (Macherey-Nagel GmBH) following the manufacturer's specifications. Amplification of a set of six microsatellites followed the methods described in Van Houdt et al. (2006). PCR products were visualised on an ABI 3130 Genetic Analyser (Applied Biosystems). Allele size was determined by means of an internal Genescan 500-LIZ size standard and genotypes were obtained using GENEMAPPER v.3.7 (Applied Biosystems). Mitochondrial sequences of the cytochrome *b* gene (*cyt b*) for a subset of individuals were obtained according to the protocol of Janko et al. (2007) using the primers: CytBU15786-Tremato (5′TGAGGkGGrTTTTCGGTAGATA-3′) and CytB-L16317-Tremato (5′-GATrTAnGGrTCCTCAaCGGG-3′). Haplotypes were compared to those of Janko et al. (2007). Novel sequences were submitted to genbank JX138967-JX138996. For an overview of the accession number see Table A4–6. Sequences were aligned and analyses were performed in BioEdit v.7.0.9. Morphological identification was validated genetically either using the method described in Van de Putte et al. (2009) or by blasting the *cyt b* sequences. The former method assigns specimens to an *a priori* defined baseline group of eight trematomid species based on samples for which there was a congruent morphological and *cyt b* identification. Analyses were performed in STRUCTURE v.2.3 (Pritchard et al., 2000) using the non-admixture model in combination with the non-correlated allele frequency model. A burn in period of 10^5 steps and 10^6 Markov Chain Monte Carlo simulations was used. A total of 5 runs were carried out to check the repeatability of the results. Assignments scores (q) were plotted and the proportion of correctly assigned individuals ($q > 0.8$) was calculated.

2.2. Microsatellites

2.2.1. Genetic diversity

Genotype and allele frequencies of the microsatellite loci were used to obtain standard estimates of genetic diversity within and between sample sites. Tests for null alleles and other potential technical artefacts, such as stuttering and large allele dropout, were performed