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Population divergences despite long pelagic larval stages: lessons from crocodile icefishes (Channichthyidae)

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Abstract

Dispersal via pelagic larval stages plays a key role in population connectivity of many marine species. The degree of connectivity is often correlated with the time that larvae spend in the water column. The Antarctic notothenioid fishes develop through an unusually long pelagic larval phase often exceeding 1 year. Notothenioids thus represent a prime model system for studying the influence of prolonged larval phases on population structure in otherwise demersal species. Here, we compare the population genetic structure and demographic history of two sub-Antarctic crocodile icefish species (*Chaenocephalus aceratus* and *Champsocephalus gunnari*) from the Scotia Arc and Bouvet Island in the Atlantic sector of the Southern Ocean to delineate the relative importance of species-specific, oceanographic and paleoclimatic factors to gene flow. Based on 7 (*C. aceratus*) and 8 (*C. gunnari*) microsatellites, as well as two mitochondrial DNA markers (cytochrome *b*, D-loop), we detect pronounced population genetic structure in both species (AMOVA FSTs range from 0.04 to 0.53). High genetic similarities were found concordantly in the populations sampled at the Southern Scotia Arc between Elephant Island and South Orkney Islands, whereas the populations from Bouvet Island, which is located far to the east of the Scotia Arc, are substantially differentiated from those of the Scotia Arc region. Nonetheless, haplotype genealogies and Bayesian cluster analyses suggest occasional gene flow over thousands of kilometres. Higher divergences between populations of *C. gunnari* as compared to *C. aceratus* are probably caused by lower dispersal capabilities and demographic effects. Bayesian skyline plots reveal population size reductions during past glacial events in both species with an estimated onset of population expansions about 25 000 years ago.

Keywords: adaptive radiation, generation time, larval dispersal, notothenioids, population genetics

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Introduction

Larval dispersal plays a key role in population connectivity of marine organisms and is central to our understanding of marine ecology (Cowen & Sponaugle 2009). Drivers influencing dispersal can either be physical or biological, and their combination results in a diverse range of connectivity patterns between species (Cowen & Sponaugle 2009; Hellberg 2009). The extent of connectivity, and hence gene flow, between populations has great implications for evolution and the ability of species to cope with changing environmental conditions or impacts from human exploitation (Palumbi 1994; Botsford *et al.* 2001).

Gene flow through larval dispersal is especially important for benthic and demersal species, which are strictly confined to suitable habitats, such as shelf areas,

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during their adult stage. For these sedentary species, the degree of population connectivity may be greatly enhanced through a pelagic larval phase in the water column, where they are subject to passive drift by ocean currents. Therefore, the pelagic larval duration (PD) is often thought to be a main factor determining the structure of marine populations (Shanks 2009), and the time spent adrift during larval stages has been repeatedly found to be positively related to dispersal capability (e.g. Riginos & Victor 2001; Shanks *et al.* 2003). However, a growing number of studies indicate that populations can be highly structured even in species with long PDs (e.g. Levin 2006; Marko *et al.* 2007; White *et al.* 2010).

Antarctic fishes of the perciform suborder Notothenioidei are ideal model organisms to study the influence of long PDs on the connectivity of geographically distant populations. More than 100 notothenioid species from six families inhabit the shelves and slopes of the Antarctic continent and sub-Antarctic islands, where they are the dominant taxa in terms of diversity, abundance and biomass (Eastman 2005). Besides their ability to express antifreeze glycoproteins that prevent their body fluids from freezing in the cold Antarctic waters, they are also characterized by lacking a swim bladder (Eastman 1993). Although predominantly demersal, some notothenioids gained buoyancy by morphological adaptations, such as increased lipid deposition and reduced skeletal mineralization, leading to benthic, epibenthic, semi-pelagic and pelagic life-history strategies (Eastman 1993). Despite differing in adult life-history strategies, the larval development of notothenioids is always pelagic and unusually long. The early developmental stages, from hatching of larvae until juveniles return to a demersal life, may take about 9 months, as, for example, in the benthic *Gobionotothen gibberifrons*, or last about 1 year, as in the benthopelagic *Champsocephalus gunnari* and benthic *Chaenocephalus aceratus* (Kellermann 1989; Kock & Kellermann 1991; North 2001). During this time, larvae may be dispersed with the currents and gene flow by larval dispersal may counteract population divergence even between distantly located islands. The presumably great potential for dispersal of notothenioid larvae is further enhanced through the world's largest current system, the Antarctic Circumpolar Current (ACC). The ACC flows in easterly direction without interruption around Antarctica and is relatively well studied owing to its importance in driving the global ocean circulation (Orsi *et al.* 1995; Schmitz 1995; Rintoul 2000). The high velocity and net volume of the ACC are supposed to have a homogenizing effect on populations around Antarctica (Eastman 1993), and larvae dispersed with the ACC can significantly enhance the connectivity of populations, as indicated by similar species assemblages on islands connected by the ACC (e.g. Permitin 1973). Nonetheless, Antarctic species with high dispersal potential may show significant population genetic structure and genetic studies increasingly reveal cases of cryptic speciation (e.g. Rogers 2007; Allcock *et al.* 2011), challenging the overall homogenizing effect of the ACC.

Previous population genetic studies revealed a diverse pattern of connectivity between notothenioid populations. In the Atlantic sector of the Southern Ocean (Fig. 1), genetic homogeneity was found in populations of the benthic nototheniid *G. gibberifrons* along the Scotia Arc islands (Matschiner *et al.* 2009). A similar pattern arose previously from allozymes in the benthopelagic *C. gunnari* (Carvalho & Warren 1991 in Kock & Jones 2005) and from enzyme polymorphism data in the benthic *Lepidonotothen squamifrons* (Schneppenheim *et al.* 1994), but later studies based on SNPs and mtDNA revealed differences between northern [South Georgia (SG), Shag Rocks] and southern (South Shetlands, SSh) populations in both cases (Kuhn & Gaffney 2006; Jones *et al.* 2008). Significant population differentiation between SG and SSh was also inferred for the

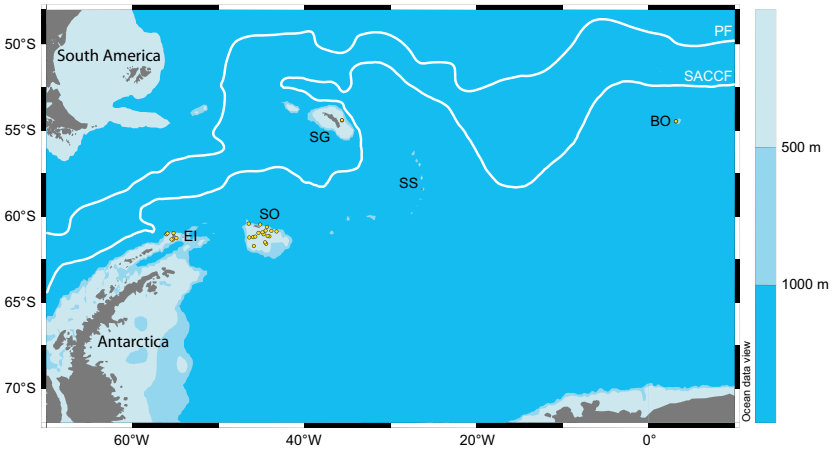

Fig. 1 Map of the study area including sampling locations (yellow dots) and fronts (white lines). EI, Elephant Island; SO, South Orkney Islands; SG, South Georgia; SS, South Sandwich Islands; BO, Bouvet Island; PF, Polar Front; SACCF, Southern Antarctic Circumpolar Current Front.

notothenioid *Trematomus hansoni* (Van de Putte *et al.* 2012). More strikingly, preliminary studies found no significant differentiation between populations of the northern Scotia Arc islands and Bouvet Island (BO), which are separated by ~2300 km of deep ocean, in five notothenioid species, including *C. gunnari* and *L. squamifrons* (Kuhn & Gaffney 2006; Jones *et al.* 2008). However, a significant population differentiation between these areas in the large *Dissostichus eleginoides* suggests that genetic homogeneity is not a general rule (Rogers *et al.* 2006). On a more regional scale, genetic homogeneity is the predominant pattern found in eight notothenioid species in the southern Scotia Arc area [SSh including Elephant Island (EI), northern Antarctic Peninsula (AP) and South Orkney Islands (SO)] (Papetti *et al.* 2007, 2012; Damerau *et al.* 2012; Van de Putte *et al.* 2012). Notable exceptions are the two icefishes *C. aceratus* and *C. gunnari*, which showed a slight, but significant differentiation between EI and SO populations based on microsatellites (Papetti *et al.* 2009, 2012; Damerau *et al.* 2012).

In this study, we investigate the role of pelagic larval dispersal in two sub-Antarctic species of crocodile icefishes (Channichthyidae) in a comparative approach based on multiple markers to assess the effect of long PDs and strong currents on the genetic structures of populations, which are geographically separated by large distances of deep ocean. We sampled Blackfin icefish (*C. aceratus*) and Mackerel icefish (*C. gunnari*) at four locations in the Southern Ocean (SG, SO, EI and BO) to encompass areas connected by the ACC in a west–east direction (Fig. 1). Although connected by the ACC, SG is separated from the other islands by the Southern ACC Front (SACCF), which runs south of the island and might act as oceanographic barrier for gene flow.

Both study species inhabit the shelves and slopes down to about 700–770 m depth (Iwami & Kock 1990; Kock & Stransky 2000). Adult movement between islands is constrained by ocean basins of 3000 m depth or more. *C. aceratus* is a sedentary benthic species with a generation time of 6–8 years, while *C. gunnari* is an active benthopelagic species with a generation time of only 3 years (Kock & Kellermann 1991). Both species spawn inshore between austral autumn and winter (Kock 1989, 1992), and the pelagic development until juveniles return to a demersal life lasts throughout the winter of the following year (North 2001). Hence, the early development of both species is similar and takes more than 1 year. Nonetheless, the early developments differ in the following traits: *C. aceratus* is a nest-guarding species and larvae hatch after about 3–4 months of incubation, whereas *C. gunnari* spawns nonguarded demersal eggs and hatching occurs after about 5–6 months (Kock & Kellermann 1991; Everson *et al.* 2001; Detrich *et al.* 2005 and references therein). Consequently, eggs of *C. gunnari* are more likely to be dispersed with the currents, resulting in a higher potential for gene flow. Data on the exact larval duration are scarce, but the occurrence of transforming larvae and juveniles in summer and late summer suggests that the larval phase of both species is completed within 2–6 months, depending on location (e.g. Kellermann & Ślósarczyk 1984; North 1990, 2001). However, regional differences of spawning times and rates of development leave the aforementioned times as approximations (Everson 1984; North 2005). For example, latitudinal shifts in spawning times were reported for both species with a 1–2 months spawning delay in the southerly populations of SO and SSh as compared to SG (Kock & Kellermann 1991). Regardless of regional differences, the PDs are unusually long in both species.

We used population genetic tools to examine the connectivity between islands based on the hypothesis of genetically similar populations when larvae are dispersed successfully along the prevailing current of the ACC. Alternatively, larval behaviour and oceanographic retention mechanisms or barriers may substantially limit gene flow between localities (Loeb *et al.* 1993; Young *et al.* 2012). Given the equally long PDs of both species, observed differences between population genetic structures are probably caused by species-specific traits including demographic effects. Because glacial cycles had major impacts on the Antarctic marine fauna in the past and ice-sheet expansions limited the available shelf habitats during glacial maxima (Anderson *et al.* 2002; Convey *et al.* 2009), we additionally assessed the demographic histories of both species to identify how species responded to major glacial cycles with Bayesian skyline plots (BSPs).

Material and methods

Sample collection

Samples of *Chaenocephalus aceratus* and *Champsocephalus gunnari* were collected by bottom trawls between 131 and 398 m depth at four island shelves in the Atlantic sector of the Southern Ocean: 1. Elephant Island (EI), 2. South Orkney Islands (SO), 3. South Georgia (SG) and 4. Bouvet Island (BO; Fig. 1). Muscle tissues for DNA extraction were collected from adult specimens during three expeditions: from December 2005 to January 2006, during ANT-XXIII/8 aboard *RV Polarstern*, in February–March 2009, during the United States Antarctic Marine Living Resources (US AMLR) finfish survey aboard *RV Yuzhmorgeologiya* and from February to April 2011 during ANT-XXVII/3 again aboard *RV Polarstern*. All tissue

samples were stored in 95% ethanol prior to DNA extraction.

Mitochondrial DNA sequencing

DNA extraction, amplification of partial cytochrome *b* (cyt *b*) and D-loop mitochondrial DNA (mtDNA) as well as sequencing followed previously established protocols (Damerau *et al.* 2012). D-loop sequences of samples from EI and SO generated as part of a former study (Accession nos JN241690–JN241705) were complemented with new data from all four study locations. Cyt *b* sequences were newly amplified for all individuals. The additional sequencing for this study was carried out on a 3500 Genetic Analyzer (Applied Biosystems) following the manufacturer's protocol. All sequences were automatically aligned with CODONCODE ALIGNER (CodonCode Corp.), and alignments were manually optimized.

Microsatellite genotyping

Nine microsatellite markers were genotyped in both species using previously published primers: Ca26 was originally isolated from *C. aceratus* (Susana *et al.* 2007), Cr15, Cr38, Cr127, Cr236, Cr259 from *Chionodraco rastrospinosus* (Papetti *et al.* 2006) and Trne20, Trne53, Trne66 from *Trematomus newnesi* (Van Houdt *et al.* 2006). Microsatellites Cr38 and Cr236 failed to amplify in *C. aceratus*, whereas Ca26 was omitted in *C. gunnari*, resulting in 7 and 8 markers in the final data set, respectively. PCR set-ups were used as in Damerau *et al.* (2012), and genotypes were subsequently assessed on a 3500 Genetic Analyzer (Applied Biosystems). We used GENEMAPPER v4.0 (Applied Biosystems) to score allele sizes before binning them automatically with TANDEM (Matschiner & Salzburger 2009).

mtDNA data analyses

DNA polymorphism was examined as haplotype (H_D) and nucleotide diversities (π) for every species and sampling site using DNASP v5.10 (Librado & Rozas 2009). Haplotype genealogies were constructed from phylogenetic trees as suggested by Salzburger *et al.* (2011). We selected the model of sequence evolution according to the Bayesian information criterion (BIC) in JMODELTEST (Posada 2008) and applied it to the inferred maximum-likelihood (ML) tree, as implemented in PAUP* v4.0a112 (Swofford 2003).

Genetic differentiation within and among populations were measured by analysis of molecular variance (AMOVA) based on traditional *F*-statistics with 16 000 permutations in ARLEQUIN v3.5 (Excoffier & Lischer 2010). The statistical power of the mtDNA sequences to detect significant genetic differentiation between populations was examined with POWSIM v4.0 (Ryman & Palm 2006) as described in Damerau *et al.* (2012) for F_{ST} values between 0.01 and 0.8.

The demographic histories were examined with Tajima's D and Fu's F neutrality tests (Tajima 1989; Fu 1997) using 1000 simulation steps as implemented in ARLEQUIN v3.5. In these tests, negative significant deviations from neutrality might indicate either balancing selection or population expansion. In addition, population size changes were reconstructed from Bayesian skyline plots (BSPs) using BEAST v1.74 (Drummond *et al.* 2005, 2012) and visualized in TRACER v1.5 (Rambaut & Drummond 2007). Substitution models of sequence evolution and their parameters were chosen as described above. Substitution rates for each species and marker were inferred by constructing phylogenetic trees with publicly available sequences from GenBank (for Accession nos, see Table S1, Supporting information) that were time-calibrated as described in Matschiner *et al.* (2011), using their channichthyid and bathydraconid divergence date estimates as temporal constraints (Data S1–S6, Supporting information). The derived branch rates per site and million years (myr) for cyt *b* and D-loop were 0.0108 and 0.0149 in *C. aceratus* and 0.0107 and 0.0184 in *C. gunnari*, respectively. Strict substitution rates were applied as molecular clock model and estimated from a normal distribution with means based on the derived branching rates, variation of 0.01 and upper boundary of 0.08. To account for uncertainties with the exact substitution rate, we further explored substitution rates of 0.02, 0.04 and 0.08 substitutions/site/myr. The time to the most recent common ancestor (tMRCA) was directly inferred from the tree prior. Each analysis was first run at species level (including sequences of all specimens of a species) for 50 million Markov chain Monte Carlo (MCMC) generations with a burn-in of 10%. Parameters were checked for convergence and effective sampling sizes (ESS) of at least 100 in TRACER v1.5. We then applied the same analysis at population level (including sequences of all specimens of a population) and ran two replicate analyses to validate the consistency of the results. If parameters did not converge or ESS were <100, we extended the MCMC until these criteria were fulfilled.

Microsatellite data analyses

Microsatellite loci were tested for the presence of null alleles with MICROCHECKER v2.2.3 (Van Oosterhout *et al.* 2004). The number of alleles per locus and sample site was calculated with FSTAT (Goudet 2001) using the implemented rarefaction method to allow for

differences in sample sizes. Allele size ranges, genotypic linkage disequilibrium between loci and Hardy–Weinberg-Equilibrium (HWE) per species and population were analysed with GENEPOP v4.0.10 (Raymond & Rousset 1995).

The genetic population differentiation was assessed with AMOVA in ARLEQUIN v3.5 analogous to the mtDNA data, but including within individual variation. Where applicable, we repeated the AMOVA excluding null-allele loci (ENA). Moreover, we calculated Jost's D as alternative measure of population differentiation with GENEALEX 6.5 (Peakall & Smouse 2012 and references therein). In contrast to F_{ST} measures, which are based on the variation in allele frequencies, Jost's D measures the proportion of allele variation among populations and may deliver more accurate results of population differentiation when heterozygosities are high (Jost 2008). In addition, we used a Bayesian approach to identify clusters of individuals with the software STRUCTURE v2.3.1 (Pritchard *et al.* 2000). For each species, 20 runs with 10^3 steps of burn-in and 10^5 MCMC replications were conducted for up to 6 admixing clusters (K). In a second pass, we incorporated information of individual sampling locations to help STRUCTURE with clustering.

Drifter trajectories

Trajectories of satellite-tracked drifting buoys (drifters) of the Global Drifter Program (Lumpkin & Pazos 2007) as means to compare the directionality of ocean currents with gene flow/larval dispersal were plotted following the protocols of Matschiner *et al.* (2009). For all drifters passing the regions of BO, EI, SG and SO between 15 February 1979 and 31 December 2009, interpolated data were downloaded from http://www.aoml.noaa.gov/phod/dac/index.php. Four polygons were defined to encompass the respective shelf areas at 500 m depth. Polygon vertices were 54.100°S 2.563°E, 54.100°S 4.025°E, 54.750°S 4.025°E, 54.750°S 2.563°E for BO, 61.668°S 54.642°W, 61.668°S 56.511°W, 60.890°S 56.511°W, 60.890°S 54.642°W for EI, 54.517°S 39.440°W, 53.735°S 39.440°W, 53.415°S 37.584°W, 53.735°S 35.779°W, 55.065°S 34.355°W, 55.513°S 35.779°W for SG and 62.249°S 44.839°W, 61.451°S 43.951°W, 61.207°S 42.374°W, 60.767°S 42.848°W, 60.308°S 46.621°W, 60.466°S 47.308°W, 61.634°S 46.861°W for SO. The trajectories of drifters leaving these polygons were plotted for 105 and 400 days – the span of potential pelagic larval dispersal in our study species (Fig. 2).

Results

mtDNA data

Overall, 247 *Chaenocephalus aceratus* and 192 *Champsocephalus gunnari* specimens were analysed for this study. Sequence alignments of cyt *b* generated from 241 *C. aceratus* individuals covered 697 base pairs (bp) whereas D-loop sequences from 179 individuals covered 333 bp. Fewer individuals could be sequenced of *C. gunnari*, resulting in alignments of 634 bp from 114 specimens for cyt *b* and 337 bp from 148 specimens for D-loop (GenBank Accession nos KC907761–KC907839). The genetic diversity for cyt *b* was about twice as high as in D-loop in both species (Table 1, Supporting information). Overall, *C. aceratus* showed slightly higher genetic diversity

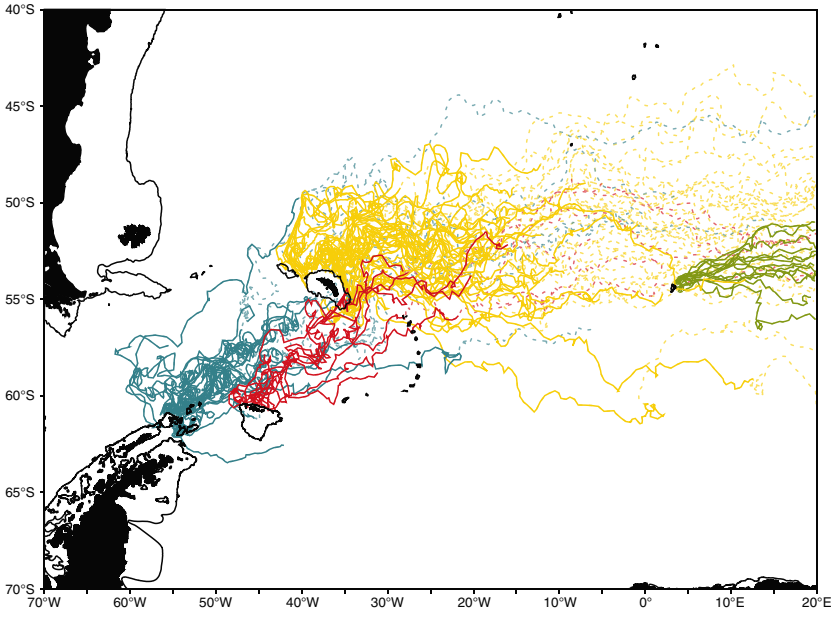

Fig. 2 Trajectories of drifters leaving the 500-m isobath of the study areas Elephant Island (blue), South Orkney Islands (red), South Georgia (yellow) and Bouvet Island (green) for the duration of 105 (solid lines) and 400 (dashed lines) days.

Table 1 Genetic diversities of cyt *b* and D-loop mtDNA sequences per species, population and locus

	Sequences	Haplotypes	Private haplotypes	H_D	π
Chaenocephalus aceratus cyt *b* (697 bp)	241	41	—	0.771	0.005
BO	38	5	2	0.704	0.005
EI	36	9	5	0.703	0.004
SG	65	26	18	0.882	0.005
SO	102	15	7	0.533	0.005
C. aceratus D-loop (333 bp)	179	11	—	0.477	0.002
BO	28	5	1	0.471	0.002
EI	36	3	1	0.398	0.001
SG	40	5	3	0.665	0.003
SO	75	5	2	0.376	0.001
Champsocephalus gunnari cyt *b* (634 bp)	114	16	—	0.756	0.003
BO	23	1	0	0	0
EI	22	6	4	0.411	0.001
SG	36	9	5	0.680	0.002
SO	33	7	3	0.382	0.001
C. gunnari D-loop (337 bp)	148	11	—	0.391	0.001
BO	33	2	0	0.170	0.001
EI	63	8	5	0.412	0.002
SG	24	4	2	0.601	0.002
SO	28	3	1	0.204	0.001

bp, base pairs; H_D, haplotype diversity; π, nucleotide diversity; BO, Bouvet Island; EI, Elephant Island; SG, South Georgia; SO, South Orkney Islands.

indices than *C. gunnari*. Populations at SG were genetically more diverse than at BO, EI and SO. These trends are also well reflected in the constructed haplotype genealogies (Fig. 3). For cyt *b,* no genetic variation was found at BO in *C. gunnari*. In general, POWSIM suggested higher power of cyt *b* than D-loop sequences to detect population differentiation, and this difference is more pronounced in *C. aceratus* (Fig. S1, Supporting information).

Microsatellite data

Microsatellites could be genotyped for 230 and 177 specimens of *C. aceratus* and *C. gunnari*, respectively (Table S2, Supporting information). All loci were polymorphic and showed no linkage disequilibrium. Null alleles were present at single loci within individual populations, but these were not excluded from further analyses, as they did not change the results qualitatively. Two loci showed null alleles in all populations of *C. gunnari* (Cr38, Trne20) probably caused by stuttering, and additional analyses were performed excluding these loci from the data set. At BO, *C. gunnari* showed homozygote excess at most loci (Cr38, Cr127, Cr236, Trne20, Trne53), but these were not caused by scoring errors. In general, genetic diversities were highest at SG and lowest at BO (Table S3, Supporting information). In accordance with mtDNA data, allelic diversity in *C. aceratus* was higher than in *C. gunnari*. The power of microsatellites to distinguish between slightly differentiated populations was higher than for mtDNA sequences as inferred from POWSIM, and the proportion of significances reached 1 even for F_{ST} values as small as 0.005 (Fig. S1, Supporting information).

Genetic population structure

The genetic population differentiation estimated with F_{ST} were not congruent between markers, but showed some striking parallels between species. Global F_{ST}s for *C. aceratus* and *C. gunnari* were highly significant, with lowest F_{ST} values for microsatellites (0.021 and 0.022) and highest for cyt *b* (0.192 and 0.443, respectively, Table 2). Microsatellite data show significant pairwise population differentiation with the highest degree of divergence for the populations at BO (Table 3). Excluding null-allele loci from the analysis of *C. gunnari* did not alter the results. A comparison between values of Jost's D and F_{ST} suggests that the actual differentiation between populations is higher than indicated by F_{ST}.

Mitochondrial DNA data show significant differentiation between all populations but EI-SO for cyt *b*, whereas D-loop data suggest significant differences only for the populations at SG in both species. In general, the degree of differentiation between populations is approximately 2.5-fold higher in the benthopelagic *C. gunnari* compared with the benthic *C. aceratus*. The

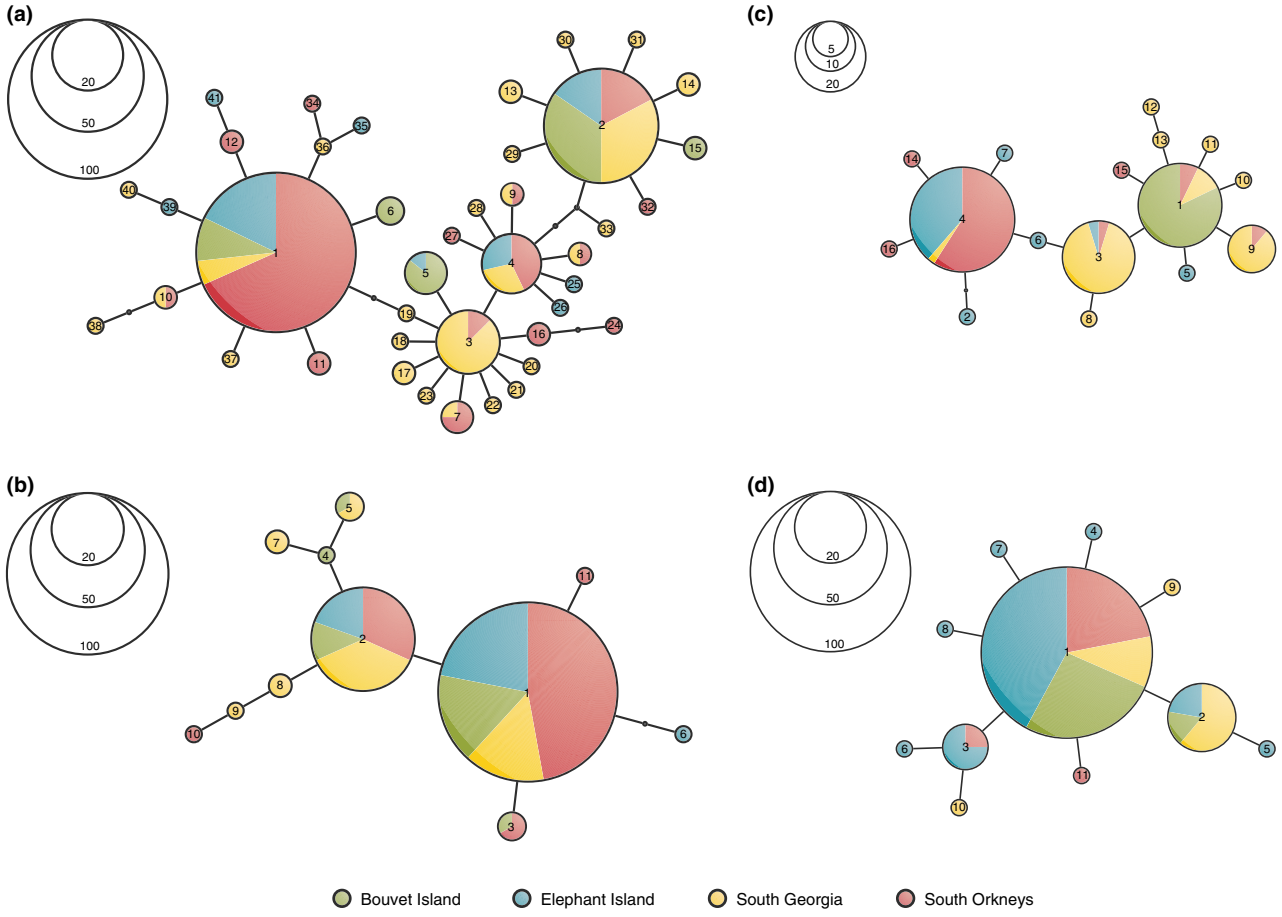

Fig. 3 Haplotype genealogies for *Chaenocephalus aceratus* (a, b) and *Chaenocephalus gunnari* (c, d). Upper row cyt *b*, lower row D-loop.

least population differentiation as measured by all markers was found at the southern Scotia Arc between EI and SO.

Although the haplotype genealogies revealed no strict separation of genetically distinct populations in any species, star-like genealogies with unique haplotypes in all populations except for *C. gunnari* at BO are indicative of local population expansions and self-recruitment. In general, the D-loop haplotype genealogies displayed only one or two central haplotypes of ancestral polymorphisms, depending on the species. Bayesian cluster analyses with STRUCTURE identified 2–3 clusters within each species, depending on the information given about the sampling localities (Table S4, Supporting information). Without sampling site information, STRUCTURE suggested the existence of two clusters (BO and a cluster composed of EI, SO, SG) for each species (Fig. 4). The incorporation of sampling site information resulted in three clusters each, which correspond to BO, SG and the group of EI and SO. Excluding loci with null alleles did not change the results in *C. gunnari*. Overall, the combined STRUCTURE analyses confirmed the pattern revealed by F_{ST} and Jost's D of genetic similarities in the southern Scotia Arc, but high divergence of BO populations.

Demographic histories

Tests of neutrality delivered varying results between genes, species and populations (Table S5, Supporting information). Nonetheless, growing population sizes was evident for both species. Fu's F's showed significant negative deviations from the neutral expectation on species levels, indicating an overall increase in population sizes, as supported by the star-like haplotype genealogies and increasing skyline plots (Fig. 5). The BSPs mostly re-covered the pattern of demographic expansions but show subtle differences between species and populations. While overall population sizes clearly increased since the beginning of the last warming period, this trend is less pronounced for D-loop data in *C. aceratus*. Remarkably, the populations at BO of both species may either be exceptionally small or may even have declined in population size, as suggested by cyt *b* in *C. aceratus* and the impossibility to reconstruct

Table 2 Results of analysis of molecular variance (AMOVA) for the two study species *Chaenocephalus aceratus* and *Champsocephalus gunnari* from four locations, (Bouvet Island, Elephant Island, South Georgia, South Orkneys) measured by microsatellites, cyt *b* and D-loop mtDNA

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	F_{ST}	*P*
C. aceratus						
Microsatellites						
Among pops	3	48.101	0.121	4.15	0.042	0
Within pops	226	660.271	0.116	3.97		
Within individuals	230	618.500	2.689	91.88		
Total	459	1326.872	2.926	100		
Cyt *b*						
Among pops	3	53.004	0.290	17.67	0.177	0
Within pops	237	320.168	1.351	82.33		
Total	240	373.212	1.641	100		
D-loop						
Among pops	3	2.247	0.012	5.07	0.051	0.008
Within pops	175	40.211	0.230	94.93		
Total	178	42.458	0.242	100		
C. gunnari						
Microsatellites ENA						
Among pops	3	61.433	0.207	8.18	0.082	0
Within pops	173	414.222	0.070	2.77		
Within individuals	177	399.000	2.254	89.06		
Total	353	874.655	2.531	100		
Cyt *b*						
Among pops	3	20.400	0.235	53.66	0.537	0
Within pops	110	22.328	0.203	46.34		
Total	113	42.728	0.438	100		
D-loop						
Among pops	3	3.601	0.029	14.41	0.144	0
Within pops	144	25.156	0.175	85.59		
Total	147	28.757	0.204	100		

ENA, excluding loci with null alleles.

reliable BSPs in *C. gunnari* due to the lack of genetic diversity. Information gained from BSPs about population size changes ceases with time and is not informative for the time prior to the LGM.

Drifter trajectories

Drifters leaving the shelf areas of the four study locations floated consistently in northeasterly or easterly direction with the ACC (Fig. 2). Every drifter leaving one of the shelf areas has the potential to reach any island located downstream of its place of origin within 400 days. Once entrained in the ACC, the majority of drifters remained within a core area of the current, although individual trajectories were highly variable. Drifters at the northern and southern edge of the ACC showed an undulating pathway probably caused by local eddy systems. Retention of drifters in lee gyres behind islands was evident from the tip of the Antarctic Peninsula, SG, and to a lesser extent SO. Under the assumption of passive dispersal, larvae should have the potential to establish gene flow between all islands in the investigated area.

Discussion

Population genetic structures

Despite long PDs and oceanographic connectivity between islands through the ACC, the data presented here indicate substantial genetic population structure in both *Chaenocephalus aceratus* and *Champsocephalus gunnari* in the study area. The highest level of population differentiation is revealed between the Scotia Arc and BO, whereas the highest similarities were found in concordance with previous population genetic studies in the southern Scotia Arc area (Papetti *et al.* 2009, 2012; Damerau *et al.* 2012; Van de Putte *et al.* 2012). Accordingly, the populations at SG and BO each represent discrete populations.

Table 3 Pairwise population differentiations. (a) Genetic differentiation (F_{ST} | Jost's D) of *Chaenocephalus aceratus* populations based on microsatellites. (b) Genetic differentiation (F_{ST}) of *C. aceratus* populations based on mtDNA. Below diagonal cytochrome *b*, above diagonal D-loop. (c) Genetic differentiation (F_{ST} | Jost's D) of *Champsocephalus gunnari* populations based on microsatellites; below diagonal F_{ST} | D_{est} values including loci with null alleles (INA), above diagonal excluding loci with null alleles (ENA). (d) Genetic differentiation (F_{ST}) of *C. gunnari* populations based on mtDNA. Below diagonal cytochrome *b*, above diagonal D-loop

	EI	SO	SG	BO
a				
EI	—			
SO	0.03* | 0.12*	—		
SG	0.04* | 0.17*	0.01* | 0.06*	—	
BO	0.08* | 0.28*	0.07* | 0.25*	0.07* | 0.29*	—
b				
EI	—	−0.01	0.08*	−0.02
SO	0.03	—	0.13*	−0.02
SG	0.12*	0.28*	—	0.06*
BO	0.09*	0.26*	0.05*	—
c				
EI	—	0.02* | 0.10*	0.03* | 0.15*	0.17* | 0.50*
SO	0.03* | 0.08*	—	0.02* | 0.08*	0.13* | 0.31*
SG	0.04* | 0.12*	0.04* | 0.10*	—	0.16* | 0.44*
BO	0.18* | 0.37*	0.15* | 0.27*	0.14* | 0.27*	—
d				
EI	—	0.01	0.20*	0.03
SO	−0.02	—	0.33*	0.01
SG	0.41*	0.44*	—	0.31*
BO	0.80*	0.77*	0.58*	—

BO, Bouvet Island; EI, Elephant Island; SG, South Georgia; SO, South Orkneys.
*Significant differentiation at 5% level.

The identified population divergence between the northern (SG) and the southern (EI, SO) Scotia Arc populations conforms to previous nongenetic assessments based on meristic and morphological characters, such as dorsal fin ray counts, vertebra counts and proportional length measurements (Gubsch & Hoffmann 1981; Kock 1981; Gubsch 1982; Sosiński 1985) for either one or both study species. In *C. aceratus*, the finding of a population boundary between AP and SG was furthermore supported based on otolith chemistry (Ashford *et al.* 2010). However, a preliminary genetic study based on mitochondrial ND2 gene sequences and small sample sizes did not reveal signs of divergence in *C. aceratus* (Jones *et al.* 2008).

A larger number of molecular studies were aimed at delineating the population structure of *C. gunnari*, a species of commercial interest, but the obtained results were inconsistent. An early allozyme-based study suggested high similarities between SG in the north and SO in the south (Carvalho & Warren 1991 in Kock & Jones 2005), whereas a combination of mitochondrial and nuclear SNPs showed a clear separation (Kuhn & Gaffney 2006). Our measures of population differentiation now give further support for the subdivision of *C. gunnari* into northern and southern Scotia Arc populations.

Bouvet Island is the geographically most isolated island in our study, and, in contrast to previous investigations, we found that the populations of both species showed the highest genetic differentiation towards any other island in the study area (Kuhn & Gaffney 2006; Jones *et al.* 2008). Although significant differences between SSh and BO were found for *C. gunnari* based on SNPs, this difference was attributed to the population of SSh, as this was also significantly differentiated from Shag Rocks, SG and Heard Island (Kuhn & Gaffney 2006). However, the number of samples from BO used in both these studies was rather low (four and three specimens, respectively), which might have resulted in unrepresentative estimates. Indeed, a study on *Dissostichus eleginoides* with 10 individuals from the area around BO showed significant differences towards SG with microsatellites (Rogers *et al.* 2006). The differences between the presented and previous studies can therefore be explained by varying degrees of marker polymorphisms and sample sizes.

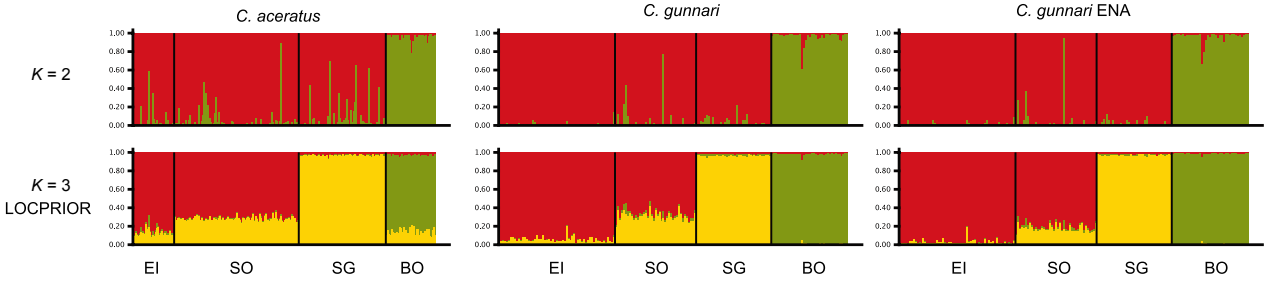

Fig. 4 Individual probabilities of cluster assignments from the software STRUCTURE. All individuals are grouped according to their sampling locality (EI, Elephant Island; BO, Bouvet Island; SG, South Georgia; SO, South Orkneys). Each vertical line represents the probabilities for a single individual to belong to one of the clusters (K) used in the analysis. LOCPRIOR, analysis including data of sampling localities; ENA, excluding loci with null alleles.

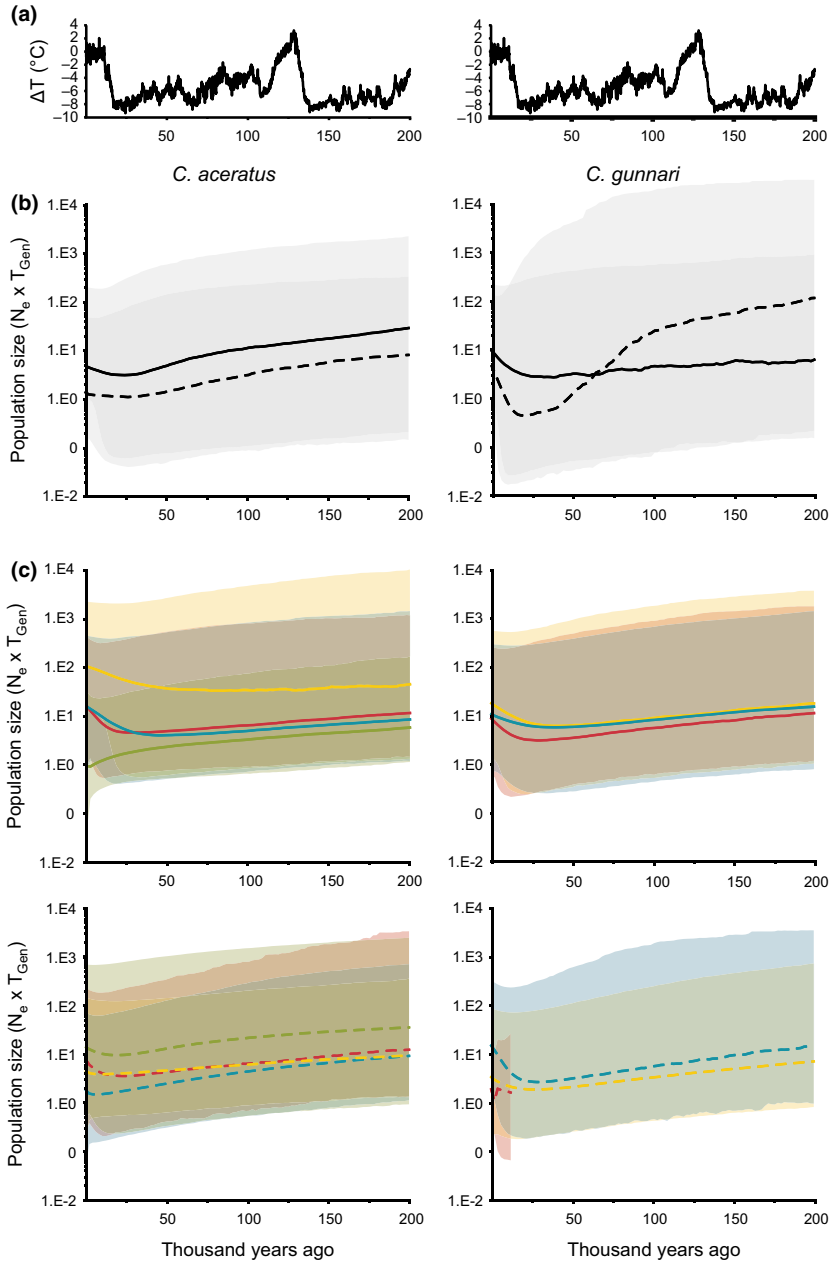

Fig. 5 Bayesian skyline plots showing population size changes per species, population and genetic marker for the last 200 000 years. (a) Plots of relative temperature change ΔT (°C) from Petit *et al.* (1999), (b) BSPs for each species (including all individuals of a species), (c) BSPs for each population (including all individuals of a population) and marker (cyt *b*, solid lines; D-loop, dashed lines). Population size changes are plotted as the product of effective population size (N_e) and generation time (T_{Gen}). Colour codes as in Figs. 2–3.

Gene flow by larval dispersal

The drifter trajectories show potential oceanographic connectivity among populations along the ACC, and the high similarity of teleost species assemblages at SG and BO (Permitin 1973) suggests that larvae are the primary means for gene flow in demersal notothenioids. Regarding the vertical distribution of larvae of both study species, which are restricted to the upper 250 m of the water column, with high abundances in the surface layer around dusk (North 1991; Loeb *et al.* 1993), it can be assumed that passively dispersing larvae would approximately follow the direction of the ACC similar to the surface drifters. Moreover, the pelagic early developmental stages last long enough to passively reach islands downstream. Yet, our finding of pronounced genetic population structures shows that pelagic phases of more than 1 year do not necessarily imply high levels of gene flow. Therefore, effective larval retention mechanisms or low rates of larval survival in oceanic waters must be responsible for reduced gene flow among populations. Also, the chance of reaching downstream islands by passive dispersal seems rather low, given that most drifters passed downstream shelves outside the 500-m isobaths contours. Unfortunately, information about larval survival in oceanic waters is scarce, but it has been argued that the long PDs of notothenioids are generally at odds with a

successful survival strategy (White 1998). Interestingly, it has repeatedly been shown that notothenioid larvae are primarily distributed in neritic waters, with decreasing abundances towards the outer shelf, whereas larvae of mesopelagic fishes, such as myctophids, are more abundant beyond the slope in oceanic waters (e.g. Efremenko 1983; Loeb *et al.* 1993). Retention mechanisms in the form of lee gyres at islands, in which the larvae can become entrapped, are known from the Scotia Sea (Efremenko 1983) and are also visible beyond the slope from the drifter trajectories near AP, SG and SO. Moreover, slow exchange of water masses across the shelf break may further limit offshore dispersal, as indicated by the zooplankton distribution at SG (Atkinson & Peck 1990).

Additional factors favouring larval retention are adult spawning and larval vertical migration behaviours. In a recent study, Young *et al.* (2012) compared the larval retention of *C. gunnari* and the notothenid *Notothenia rossii* at SG with a numerical modelling approach and found mean retentions of 31.3% and 5.3%, respectively. The supposed main reasons for this difference are the longer PD of *N. rossii* eggs and larvae as well as the location of spawning sites (inshore–offshore). Further, diel vertical migrations enhanced retention of larvae in both species, whereas the time of spawning had no effect. As both our study species are known to migrate inshore for spawning (Kock & Kellermann 1991) and larvae carry out diel vertical migrations (North 1988), these behaviours help to explain low levels of gene flow despite long PDs. Indeed, high levels of self-recruitment and growing population sizes are evident from the star-like haplotype genealogies with private haplotypes in most populations, possibly also reflecting local adaptations.

For both species, the SACCF is not a strict barrier to gene flow. Although SG is separated from any other study location oceanographically by the SACCF and the local populations were significantly differentiated to any other island with all markers, the distribution of haplotypes exclusively shared with SG populations indicates at least temporally high levels of gene flow. For example, in *C. aceratus*, the populations of SO and SG share five exclusive haplotypes, which is the highest number of exclusively shared haplotypes in our study. In addition, the degree of differentiation between these two populations is comparatively low. Therefore, the SACCF does not constitute a strong barrier to gene flow for the studied channichthyids, unlike the Polar Front (PF), which has been found to be a strong barrier to gene flow in *D. eleginoides* populations from the South American shelves and the area of Shag Rocks and SG (Rogers *et al.* 2006). In contrast to the PF, which is marked by sharp temperature and salinity gradients at the sea surface, the SACCF is characterized by a temperature maximum at 500 m depth (Orsi *et al.* 1995), which probably has little influence on larvae in the upper water layers.

Despite strong evidence for gene flow by larval dispersal with the ACC in the study area (Matschiner *et al.* 2009), the distribution of genetic diversity, which is highest at SG and lower at the southerly populations of EI and SO, might be indicative of gene-flow events against the current. One possible scenario is that the southerly populations have been completely eradicated during past glacial maxima (e.g. Thatje *et al.* 2005) and subsequently recolonized from SG, possibly by larvae entrained in detached eddies during heavy storms in the area. However, the reduced genetic diversities at EI and SO in comparison with SG might also reflect a lower survival rate during glacial maxima near the Antarctic continental shelf.

Based on our results, we conclude that the PD itself is not a reliable estimator for the level of gene flow. Unusually long PDs do not predict genetic homogeneity among populations, even though they are located within the trajectory of one of the world's strongest currents known. Instead, local oceanography as well as spawning and larval behaviours might regulate larval retention considerably.

Species-specific gene-flow patterns

Although our results indicate the existence of three different populations in the study area for both studied icefishes, the species-specific degrees of differentiation and haplotype distributions suggest lower dispersal capabilities for *C. gunnari*. The cyt *b* haplotype genealogy of *C. gunnari* resembles a stepping-stone pattern in parallel with the direction of the ACC. Under the assumption that dispersal results in shared haplotypes, which would also be evident from colonization events, connections exist between all populations except EI and BO. Even when larvae from EI were dispersed with the ACC and transported past SG, the capabilities of young *C. gunnari* to survive such long distance dispersal appear low. This observation is further corroborated by the STRUCTURE analyses, when no sampling site information was provided for clustering (Fig. 4). High population assignment probabilities of individual *C. gunnari* from the Scotia Arc to belong to BO, which indicate genetic similarities and, hence, potential origins of former migrants, vary between locations and are negligible for samples collected at EI, low for SG and highest for SO.

By contrast, the cyt *b* haplotype genealogy of *C. aceratus* is less indicative of a stepping-stone pattern, and haplotypes are shared between all populations. The existence of a haplotype exclusively shared between EI and BO might indicate direct gene flow between these two distant populations. Nonetheless, additional sampling

from SO, SG and preferably South Sandwich Islands could prove that this haplotype is also found in between. In addition, STRUCTURE assigns high probabilities for single *C. aceratus* to belong to BO in every cluster from the Scotia Arc. These findings and the lower genetic differentiation of *C. aceratus* populations as compared to *C. gunnari* indicate higher dispersal capabilities of *C. aceratus*. However, the high degrees of genetic differentiation suggest that these distances are realized only rarely.

Since our study species' resemble each other in regard to adult inshore spawning, diel vertical larval migrations and the total time larvae spend in the water column, the underlying mechanisms causing the differences in population divergences are probably demographic.

Demographic factors influencing population divergence

In addition to the varying degrees of connectivity between the populations of *C. aceratus* and *C. gunnari* that can be explained by differences in successful larval dispersal, it is likely that the disparity in the extent of genetic differentiation is also caused by generation-time effects and differing effective population sizes (Ne).

The generation-time hypothesis predicts faster nucleotide substitution rates per unit time in species with shorter generation times, as DNA replication errors (probably during stem cell division) accumulate more often per absolute time (e.g. Laird *et al.* 1969; Li *et al.* 1996). Without gene flow, populations of species with short generation times will therefore diverge more rapidly. Our results are in congruence with this hypothesis and show elevated levels of divergence in populations of *C. gunnari*, whose generation time of 3 years is about half the generation time of *C. aceratus* in which individuals spawn for the first time after 6–8 years. However, the unknown times of colonization events do not allow a final conclusion, and this point requires further examination.

The effective population sizes also influence population divergences, as genetic drift acts faster in small populations (Kimura & Ohta 1969). Estimates of census population sizes and the respective spawning proportions in the study area are available from biomass surveys, but regional and temporal differences in biomass as well as differing sizes of specimen between species prohibit reliable estimates of population sizes within the scope of this study. It must be noted that biomass estimates for both species fluctuate heavily between years and are associated with large confidence intervals. For example, at EI, the estimated biomass of *C. aceratus* from 1981 until 1996 varied from 768 to 7619 t, while the biomass of *C. gunnari* varied from 502 to 2059 t (Kock 1998). Estimates on total and spawning stock biomass at EI from the year 1998 indicate that a larger proportion of *C. aceratus* (40.3%) spawned as compared to *C. gunnari* (Jones & Kock 2005; Convey *et al.* 2009), but little is known about the temporal stability of these ratios. Unfortunately, Ne estimates based on our genetic data did not result in unambiguous estimates and depended upon methods (data not shown), leaving the relative importance of Ne on population divergences an open question to be resolved.

Our findings are important as they show that simple reductions in the underlying causes for genetic divergence among populations are neither single parametric (such as the PD) nor are they restricted to certain life-history stages (such as the larval stage). Comparative studies among species are highly valuable for disentangling the complexity of factors shaping the genetic structure of populations, but the results will always be limited by our ecological knowledge of the investigated species.

Demographic responses to the LGM

Antarctic species encountered major challenges for survival during repeated glacial periods over the past million years (Convey *et al.* 2009). Data from ice cores suggest cyclical temperature oscillations over a range of 10 °C since about 400 000 years ago (Petit *et al.* 1999) with the last glacial maximum (LGM) at about 20–14 kya at the Antarctic Peninsula (Anderson *et al.* 2002). During glacial maxima, the Antarctic ice sheets expanded far over the continental shelf (Hambrey & Barrett 1993), and the maximum expansion of sea ice was displaced about 10° latitude to the north in the Atlantic Ocean sector as compared to its current state (Gersonde *et al.* 2005). Habitat loss and changes in population size were the consequences for many Antarctic species, and genetic imprints for such events are found in a diverse range of taxa including kelp (Fraser *et al.* 2009), shrimps (Dambach *et al.* 2012), krill (Zane *et al.* 1998), octopus (Strugnell *et al.* 2012) as well as notothenioid fishes (Patarnello *et al.* 2011).

For notothenioids, there is evidence from genetic data that pelagic species were less affected by population bottlenecks caused by recent glaciation events than benthic species (Janko *et al.* 2007). For example, population expansion in the cryopelagic *Pagothenia borchgrevinki* could have started as early as 70 kya (Janko *et al.* 2007) and even earlier in the truly pelagic *Pleuragramma antarctica* at about 111–126 kya (Zane *et al.* 2006), but in the benthic notothenioids *Trematomus bernacchii*, *T. pennellii* and *Gobionotothen gibberifrons*, the onsets of possible population expansions were estimated to be more recently at about 50, 25 and 33 kya, respectively (Janko *et al.* 2007; Matschiner *et al.* 2009). Also, population expansions in the benthopelagic *C. gunnari* occurred at an intermediate time of 37 kya or later (Kuhn & Gaffney

2006). Although these results are tempting to conclude a succession of population size changes from pelagic to benthic species, the large confidence intervals associated with them that range from thousands to even millions of years do not allow an unambiguous conclusion. The difficulty in dating population size changes is the use of a correctly calibrated molecular clock, because substitution rates vary between taxa, lineages, loci and timescale (see Box 2 in Allcock & Strugnell 2012). The assumed substitution rates used for dating such events are hence only approximations to the 'real' molecular clock, and it has recently been suggested that expansion events in notothenioids were more recent than stated above (Patarnello *et al.* 2011).

In contrast to previous studies, in which mismatch distributions were applied, our coalescent approach resulted in population expansion times starting 25 kya or later for both channichthyids. Although this corresponds to the lower CI bounds of previous estimations, our results suffer from large HPDs, too, and prevent a clear dating for the expansions. However, BSPs based on phylogenetically derived molecular clocks often overestimate the timing of historical, postglacial population size changes (Ho *et al.* 2005; Grant *et al.* 2012). We examined the effect of substitution rate on the BSPs by conducting further analyses using rates of 0.02, 0.04 and 0.08 substitutions/site/myr, which were all higher than our phylogenetically inferred rate of 0.01. Although the plots remained highly identical in shape (data not shown), the slopes of the population expansions steepened with increasing rates and population growths started between 18 and 12 kya. Our inferred estimate of the population expansion onset of 25 kya might therefore indeed be rather conservative.

Conclusions

The two channichthyids *Chaenocephalus aceratus* and *Champsocephalus gunnari* show a clear population genetic structure despite prolonged pelagic development of more than 1 year and connectivity through one of the world's largest current systems. Nonetheless, occasional gene flow is evident even over thousands of kilometres possibly through larval dispersal with the ACC. A greater divergence between populations of *C. gunnari* as compared to *C. aceratus* may indicate both a reduced capability for successful larval dispersal as well as influences from demographic effects, but additional reasons such as local adaptations should be explored in future studies. The low levels of gene flow between sub-Antarctic island populations as well as the reduction in population sizes during glacial maxima indicate that the notothenioid diversity is possibly driven by allopatric speciation.

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M.D., W.S. and R.H. conceived and designed the study. M.D. performed population genetic analyses. M.M. contributed the drifter map, haplotype genealogies and inferred the substitution rates from the phylogenetic tree. M.D. wrote the manuscript with helpful comments from M.M., W.S. and R.H.

Data accessibility

All DNA sequences from this study are available on GenBank, Accession nos KC907761–KC907839. Sequence alignments for the provisional phylogenetic trees (nexus format), the constructed trees (tree format) and their respective BEAUTi input files are available in the Data S1–S6 (Supporting information). Fragment lengths of microsatellites can be accessed in the form of CONVERT formatted txt files from the Data S7, S8 (Supporting information).

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Tests of statistical power for microsatellite and mtDNA data sets as inferred with POWSIM 4.0.

Table S1 Sequence accession numbers used for provisional phylogenetic trees based on cyt *b* and D-loop data.

Table S2 Species-specific microsatellite properties.

Table S3 Population specific microsatellite properties and inbreeding coefficient (F_{IS}).

Table S4 Mean Ln P(D) values (SD) of 20 runs for K=1–6 from the software Structure per species.

Table S5 Neutrality tests (Tajima's D and Fu's F) per species and population analysed for cyt *b* and D-loop.

Data S1 Cytochrome *b* sequence alignment file for the phylogenetic tree construction (nexus format).

Data S2 Cytochrome *b* phylogenetic tree (tree format).

Data S3 Cytochrome *b* input file for the software BEAST (xml format).

Data S4 D-loop sequence alignment file for the phylogenetic tree construction (nexus format).

Data S5 D-loop phylogenetic tree (tree format).

Data S6 D-loop input file for the software BEAST (xml format).

Data S7 Microsatellite data file for *C. aceratus* (Convert format).

Data S8 Microsatellite data file for *C. gunnari* (Convert format).