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High genetic diversity and connectivity in a common mesopelagic fish of the Southern Ocean: The myctophid *Electrona antarctica*

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ABSTRACT

Many marine pelagic fish species are characterized by subtle but complex genetic structures and dynamics, depending on the balance between current-mediated larval dispersal and adult active homing behavior. The circumantarctic continuous hydrodynamics of the Southern Ocean is a prime example of a system with a potentially great homogenizing effect among distant populations. We tested this hypothesis by analyzing the contemporary genetic relatedness among populations of a common and endemic mesopelagic fish of the Southern Ocean, *Electrona antarctica*. Seven newly developed species-specific microsatellite markers were used to investigate patterns of neutral genetic variation in 11 geographically widespread samples ($n=400$) collected between 2006 and 2007. We detected a very high level of genetic diversity, but a striking lack of genetic differentiation on a circumantarctic scale, indicating large effective population sizes complemented with high levels of admixture. These findings underscore the large scale homogenizing effect of the Southern Coastal Current, leading to a high level of connectivity of our model species in the Southern Ocean, which is congruent with its huge biomass and central role in marine food webs. As an important Antarctic marine living resource this species may as such be managed on a circumantarctic level, although the demographic stability of this stock should be estimated urgently.

1. Introduction

Patterns of genetic structure in marine organisms range from closed to open populations (Hellberg et al., 2002). The latter pattern is more common because many marine species show a suite of characteristics, which promote gene flow in an environment that often lacks definite physical boundaries. Factors promoting gene flow include a large number of spawning propagules, great dispersal capabilities and large population sizes (Maes et al., 2006; Palumbi, 2003). However other traits related to organismal ecology, life-history (e.g., high mortality) and behavior (e.g., phylopatry and larval retention) may counteract these homogenizing factors. Additionally, bathymetry and hydrodynamics (especially ocean currents and fronts) can influence connectivity and dispersal (Hedgecock et al., 2007). The interaction among often contradictory processes results in the frequently – although not exclusively – observed patterns of low-structure-high-gene flow in marine fishes, ranging from a subtle structure (Hemmer-Hansen et al., 2007; Waples, 1998; Ward et al., 1994) to a complete lack of genetic differentiation (Klanten et al., 2007; Muss et al., 2001; Waples et al., 2008).

In the Southern Ocean, the Antarctic Circumpolar Current (ACC), which circles in an eastward direction around the continent, is the major factor influencing connectivity among pelagic fishes. Patterns of low-structure-high-gene flow are likely to dominate in this largely open hydrodynamic system. This has been confirmed by various studies examining the genetic structure of pelagic taxa such as Antarctic silverfish *Pleuragramma antarcticum* (Zane et al., 2006), Antarctic toothfish *Dissostichus mawsoni* (Kuhn and Gaffney, 2008; Parker et al., 2002), Antarctic krill *Euphasia superba* (Zane et al., 1998; Zane and Patarnello, 2000) and ice krill *E. crystallorophias* (Jarman et al., 2002; Jarman and Nicol, 2002), as well as the demersal humped rockcod *Gobionotothen gibberifrons* (Matschiner et al., 2009). All of these studies found either weak or no genetic structure, concluding that the absence of genetic breaks among populations was due to the tremendous homogenizing forces of the ACC on dispersing pelagic larvae and the lack of homing at the adult stage.

The mesopelagic *Electrona antarctica* is endemic to the waters of the Southern Ocean, south of the Antarctic Polar Front (APF) (Hulley, 1990). It is the most abundant mesopelagic species in the Southern Ocean and represents an important energetic link in

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the ecosystem, both as a consumer of meso-zooplankton (Pakhomov et al., 1996; Pusch et al., 2004) and as a prey for birds and mammals (Casaux et al., 2003; Quillfeldt, 2002). Between the years 1979/1980 and 1991/1992, an estimated 200,000 metric tonnes of Southern Ocean myctophids were caught for fish meal (Anon, 1990a, b). *E. antarctica* has a life cycle of about three years and is believed to attain sexual maturity in the second year, where it reproduces as batch spawner, with a peak in the austral autumn–winter (Lubimova et al., 1983). The pelagic larvae metamorphose at about 20 mm following a larval phase of an estimated 2–4 months (Efremenko, 1986). From this stage onwards they migrate diurnally with peak abundances between 0 and 300 m at night and 650 and 950 m during daytime. Males reach a length of 82 mm and females 103 mm (Greely et al., 1999; Hulley, 1990).

Our aim was to study for the first time the genetic pattern of a mesopelagic fish in the Southern Ocean, to assess whether the homogenizing force of the ACC influences this species as strongly as commonly observed in pelagic species. The first objective was to develop a set of high quality microsatellite markers for *E. antarctica* to evaluate the level of genetic diversity in this species within the Southern Ocean. Secondly, we investigated the spatial variation among populations to unveil potential genetic barriers to gene flow. Finally, we define research priorities for further studies in a eco-evolutionary context.

2. Material and methods

E. antarctica (Myctophidae; Teleostei) individuals were caught by the research vessels *Polarstern*, *Aurora Australis* and *James Clark Ross* across the Southern Ocean in 2006 and 2007 (Table 1). Where the number of sampled individuals was small, samples were clustered regionally into oceanographically congruous zones (e.g., Lazarev Sea, Cosmonaut Sea and Cooperation Sea) (Fig. 1).

Muscle tissue or fin clips were collected from each freshly caught fish and stored in 96% ethanol for subsequent DNA extraction and microsatellite genotyping. Microsatellite markers were developed from a fin clip of a single *E. antarctica*, captured in the oceanic zone of the Lazarev Sea. Genomic DNA was extracted using the Nucleospin Extraction kit (Machery-Nagel GmbH). Using the "Fast Isolation by AFLP of Sequences Containing repeats" (FIASCO) protocol (Zane et al., 2002) with some modifications, a microsatellite enriched library was constructed. We performed a selective hybridization with di-[$(CA)_{15}$], tetra-[$(CTTT)_5$, $(ACGC)_5$, $(GTCT)_5$] and penta-[$(TAACC)_4$] repeat probes. Fifty picomoles of each 5′ biotinylated oligonucleotide probe were hybridized with 1.0 μg of adaptor-ligated DNA. Hybridized probe-DNA was captured using magnetic beads (Streptavidin MagneSpher Paramagnetic Particles, Promega). After washing of the beads, the eluted fragments were cloned into the TA cloning vector (Invitrogen). To extract plasmid DNA, each bacterial recombinant clone was put into a 96-well plate in 100 μl of water and incubated at 96 °C for 5 min. Ten microlitres of the plasmid extract was added to a 50 μl reaction volume containing 20 mM Tris–HCl, pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U of Taq DNA polymerase (Invitrogen) and 20 pmol each of standard M13 forward and reverse primers. The polymerase chain reaction (PCR) was carried out on a GenAmp PCR system 2700 thermocycler (Applied Biosystems, Foster City, CA, USA) with 30 cycles of 30 s at 96 °C, 30 s at 60 °C and 60 s at 72 °C.

After purification with the 'GFX PCR DNA and Gel Band Purification kit' (GE Healthcare), 64 PCR products were sequenced in both directions using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems). PCR primers were designed using the Fastpcr program (v. 3.6.60, Institute of Biotechnology) for 14 clones containing tandem repeats, and each microsatellite locus was tested on four individuals. Loci were amplified in a 10-μl volume containing 100 ng of *E. antarctica* genomic DNA, 2 pmol of each locus-specific primer, 0.6 U of Taq DNA polymerase (Invitrogen) and other reagents as mentioned above. The PCR was carried out with 30 cycles of 30 s at 96 °C, 30 s at 57 °C and 30 s at 72 °C. Only loci showing consistent amplification and those that were revealed to be polymorphic in our test panel were retained for further genotyping.

Genotyping with the newly developed microsatellite markers included in total 400 individuals. DNA was extracted from the field samples as described above. The seven loci (Table 2) were amplified in two multiplex PCR protocols with five and two loci, using the QIAGEN Multiplex PCR kit (QIAGEN) following the manufacturer's recommendations. PCR products were visualized on an ABI3130-Avant Genetic Analyzer (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).

Tests for null alleles and other potential technical artefacts, such as stuttering and large allele dropout, were performed using the software package MICRO-CHECKER v. 2.2.1 (Van Oosterhout et al., 2004). Genetic diversity of the microsatellites was quantified per locus and per sampling site from genotype and allele frequencies. Observed (H_O) and unbiased expected heterozygosities (H_E) were calculated with the software package GENETIX v. 4.05.2 (Belkhir et al., 2002). The number of alleles (N), Allelic Richness (R_A) and Private Allelic Richness (R_{AP}) were determined using HP-RARE (Kalinowski, 2005). Deviations from Hardy–Weinberg equilibrium (HWE) were tested using the inbreeding coefficient F_{IS} (Weir and Cockerham, 1984) calculated in GENETIX and significance assessed with permutation tests (1000 replicates). Linkage disequilibrium for all loci and sampling sites was assessed using GENEPOP v. 4 (Rousset, 2008). Significance was tested with a Markov Chain Monte Carlo (MCMC) method of 1000 batches of 2000 iterations with the first 500 iterations discarded before sampling.

Table 1
Sample collection sites with location code, total sample size (n) and sampling period. * indicates clusters of samples. Sampling coordinates (decimal latitude and longitude) indicate average sampling location.

Area	Location code	Latitude	Longitude	n	Sampling period
Bellinghausen Sea	BS	−67.28	−82.99	12	Jan. 2007
South Shetlands*	KGI	−63.10	−59.83	15	Jan.–Feb. 2007
Elephant Island	EI	−61.41	−54.95	18	Feb. 2007
Scotia Arc 1	SA1	−60.55	−48.93	38	Oct.–Nov. 2006
Scotia Arc 2, South Orkneys	SA2	−60.43	−44.51	50	Oct.–Nov. 2006
Scotia Arc 3	SA3	−59.56	−44.27	79	Oct.–Nov. 2006
Scotia Arc 4, South Georgia	SA4	−52.96	−40.33	47	Oct.–Nov. 2006
Lazarev Sea, North*	LS061	−61.46	2.21	23	Jun.–Aug. 2006
Lazarev Sea, South*	LS062	−67.43	0.80	33	Jun.–Aug. 2006
Cosmonaut Sea*	BW2	−64.00	42.22	44	Feb. 2006
Cooperation Sea*	BW3	−63.59	68.08	41	Mar. 2006

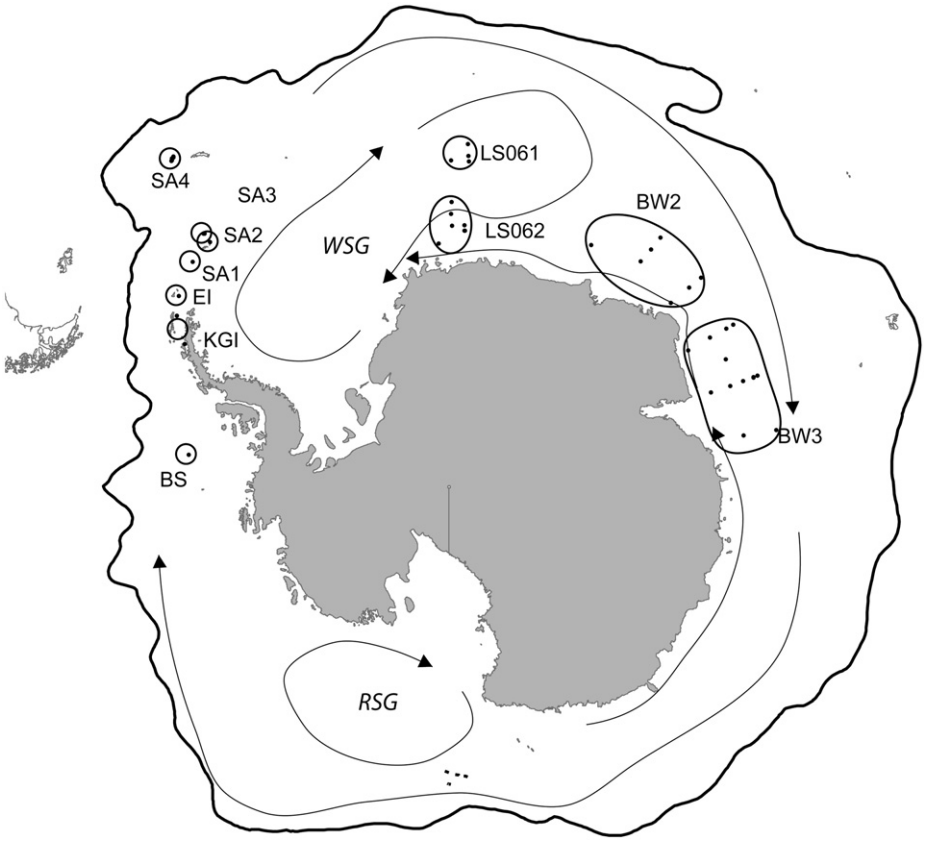

Fig. 1. Sampling locations and major features of the Southern Ocean with the Antarctic Polar Front as a northern boundary. Arrows within the Antarctic Polar Front indicate major current patterns. WSG: Weddell Sea Gyre, RSG: Ross Sea Gyre. Details on sampling areas are listed in Table 1.

Table 2
Properties of the microsatellites newly isolated from *Electrona antarctica* ($n=48$; Lazarev Sea). *indicate primers used for the second multiplex.

Locus	Repeat	Primer sequence	Size (bp)	T (°C)	Concentration (μM)
Elan001	$(AC)_{22}$	ACTGCACAACACATTCATGCAGGT AGTTTGGTGCTCGCTCACTGG	278–424	52	0.2
Elan009	$(AC)_{14}$	CCCTCCCACTTCCAGACAGC GGGTTGAAACGGATTGTAACGTGTC	139–193	52	0.2
Elan013	$(GT)_4$ GC$(GT)_3$ GC$(AC)_2$ GT $(CG)_2$ GTGC$(GT)_{15}$	TGCGTGCATGTGTCAATAAA CCACCGCCACAAGTATGAG	202–250	52	0.2
Elan025	(AC)17 AAACT (AT)5	AGCGTTTGGTTGGTTTTCAC CACAATCAAAACGCACATCC	215–291	52	0.2
Elan035*	(AC)26	TGGGCCTGATTTCACCGCAGG TTTGAGGCCAGTAGTACCAGCCA	168–234	52	0.2
Elan043	(AC)10(GCAC)7(AC)4	TGCACAACACTTTGGCTCAACA ACAAGGTTTCCTGCCACGACAGC	140–214	52	0.2
Elan114*	(CA)2 CG (CA)16	AAAGGCGGAGAACACTGAAA CTGCCGAAGTTCGAGTCCTA	151–337	52	0.2

Genetic differentiation was characterized using hierarchical *F*-statistics, (θ) (Weir and Cockerham, 1984) and significance of multi-locus F_{ST} was assessed with permutation tests (1000) in GENETIX. Isolation by distance was tested by comparing linearized genetic differentiation values ($F_{ST}/1-F_{ST}$) with Log geographic distance using a Mantel test (Mantel, 1967). A model-based clustering algorithm, implemented in the software package STRUCTURE v.2.3.2 (Hubisz et al., 2009; Pritchard et al., 2000), was used to estimate the potential number of populations, including the geographical location as prior (LocPrior). STRUCTURE uses a Bayesian Markov chain Monte Carlo approach that organizes individuals into a predefined number of clusters (K). These analyses were performed 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 performed to check the repeatability of the results, followed by the *K*-estimation procedure of Evanno et al. (2005). The analysis was performed without correlating sampling location to population membership.

Point-estimates of effective population size (Ne) were calculated with NEESTIMATOR v. 1.3 (Peel et al., 2004) using the linkage disequilibrium method (Bartley et al., 1992). The linkage disequilibrium method estimates effective population size (Ne) by assessing linkage disequilibrium (D) and correlations among alleles (r) at different loci. In finite populations both D and r will

depart from 0 due to genetic drift, migration, selection and physical linkage (Hill, 1981; Bartley et al., 1992). In an ideal population both D and r will be 0 resulting in a negative estimate of Ne that is reported as infinite (Hill, 1981).

To assess the statistical power of our microsatellite markers compared to the observed levels of differentiation (F_{ST}), we used a modified version of the program POWSIM (Ryman and Palm, 2006) tailored to incorporate a higher numbers of alleles. This program allows estimating the probability of type I and type II errors for a given set of loci. It estimates the chance of a false positive (α-error or type I error) and the power, which is equal to 1, minus the probability of a false negative (type II error).

3. Results

3.1. Microsatellite development

Seven loci that produced consistent banding patterns were retained for the genotyping (GenBank accession numbers FJ800828.1–FJ800834.1). Genetic variation and Hardy–Weinberg equilibrium tests for the seven loci were initially calculated for a sample of *E. antarctica* from the Scotia Arc near South Georgia (SA4, Table 1). After Bonferroni correction none of the individual markers in this population showed a significant homozygote excess, however there was overall homozygote excess based on the combined 5 or 7 loci (Appendix A).

Table 3
Summary statistics for seven microsatellite loci: total number of alleles per locus (N_A), allelic richness (R_A) and allelic richness of private alleles (R_{AP}) (standardized to the smallest population $n=12$), mean expected (H_E), observed (H_O) heterozygosities and mean F_{IS}. The percentage of null alleles (% Null) was estimated using the Brookfield 1 method (Brookfield, 1996). Bold F_{IS} values are significant estimates after Bonferroni correction.

Locus name	N_A	R_A	R_{AP}	H_E	H_O	F_{IS}	% Null
Elan001	79	17.99	1.793	0.948	0.913	**0.056***	1.80
Elan009	35	13.65	0.663	0.928	0.878	**0.073***	2.59
Elan013	29	12.04	0.317	0.911	0.889	0.043	1.14
Elan025	35	13.85	0.484	0.922	0.818	**0.131***	5.41
Elan035	37	14.42	0.630	0.934	0.917	0.037	0.83
Elan043	47	15.62	0.808	0.942	0.946	0.014	0
Elan114	66	12.61	1.763	0.882	0.778	**0.137***	5.53

3.2. Genetic diversity in natural populations

The level of microsatellite polymorphism was very high for all areas at all seven loci with 29–79 alleles per locus (mean $N_A=46.86\pm18.70$). This was also reflected in the high observed and expected mean heterozygosities ($H_O=0.877\pm0.06$ and $H_E=0.924\pm0.02$) (Table 3).

Genetic variability was homogenously distributed across geographical areas as suggested by the low standard deviations of the mean allelic richness after rarefaction (14.31 ± 0.27), and the mean observed (0.88 ± 0.02) and expected (0.92 ± 0.01) heterozygosities (Fig. 2, Table 4). The overall proportion of private alleles (after rarefaction) was moderate (6%). Tests for linkage disequilibrium across all populations were non-significant after Bonferroni correction. The inbreeding coefficient (F_{IS}) varied between 0.014 (*Elan043*) and 0.137 (*Elan025*) with a mean F_{IS} of 0.070 (±0.05); three loci (*Elan013*, *Elan035* and *Elan043*) were in HWE for all samples. Sample groups from eight sampling areas showed a significant departure from HWE based on using all 7 loci, indicating a homozygote excess. Testing for large allele dropout and stuttering did not reveal significant effects, however, a test for null alleles suggested possible null alleles at some loci. Two loci, *Elan025* and *Elan114*, showed significant null allele frequencies in more than five samples. Therefore, calculations on the original data were replicated, either using corrected allele frequencies according to Brookfield (Brookfield, 1996) or leaving out the most problematic loci (e.g., *Elan025* and *Elan114*). Replicates using the corrected allele frequencies did not change the results and were not retained for further analyses. Removing the two problematic loci reduced the number of significant mean F_{IS} values to four sample groups.

3.3. Genetic differentiation among natural populations and statistical power

No significant overall genetic differentiation could be found among populations on a global level (Weir and Cockerham, 1984) based on all seven loci ($F_{ST}=-0.00006$; 95% CI: -0.00097–0.00088). All pair-wise F_{ST} estimates were non-significant after

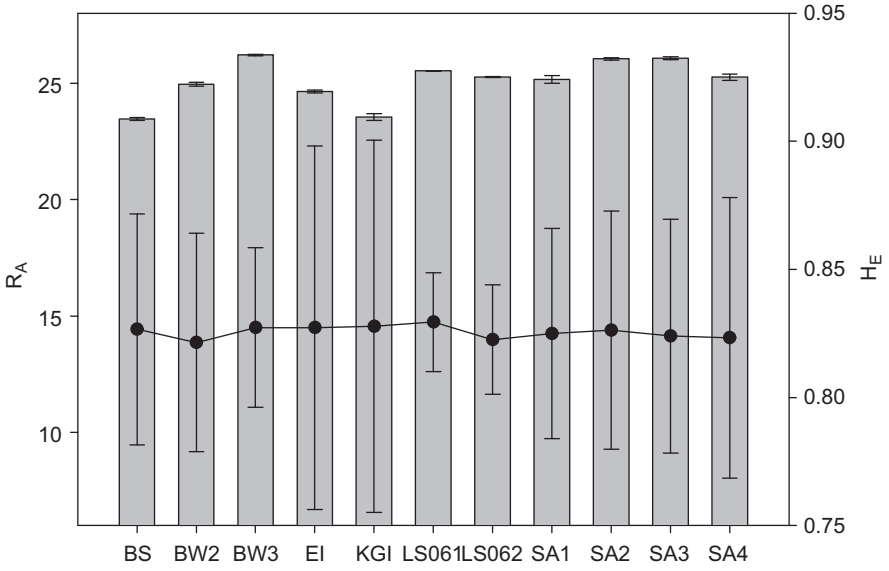

Fig. 2. Genetic diversity: Allelic richness (R_A) (standardized to the smallest population size of $n=12$), and expected heterozygosity (H_E) for all sampled locations. For location abbreviations see Table 1.

Table 4
Summary statistics for each region sample based on 7 and 5 loci. Population size (N), mean, number of alleles (N_A), allelic richness (R_A), allelic richness of private alleles (R_{AP}) (standardized to the smallest population size of $n=12$), mean expected (H_E), observed (H_O) heterozygosities and mean F_{IS}. Bold F_{IS} values are significant estimates after Bonferroni correction. For location abbreviations see Table 1.

Location	N	N_A	R_A	R_{AP}	H_E	H_O	F_{IS}
7 loci							
BS	12	14.43	14.43	1.258	0.909	0.869	**0.087***
KGI	15	16.43	14.56	1.078	0.910	0.914	0.030
EI	18	17.71	14.50	1.153	0.919	0.870	**0.083***
SA1	38	23.57	14.25	0.641	0.924	0.875	**0.066***
SA2	50	27.43	14.39	0.890	0.932	0.877	**0.069***
SA3	79	30.29	14.13	0.791	0.932	0.873	**0.070***
SA4	47	25.43	14.07	0.891	0.925	0.877	**0.062***
LS061	23	20.43	14.74	0.746	0.928	0.893	0.060
LS062	33	21.71	13.99	0.829	0.925	0.858	**0.089***
BW2	44	26.00	13.86	0.736	0.922	0.883	0.054
BW3	41	25.86	14.50	1.142	0.934	0.857	**0.094***
5 loci							
BS	12	15.20	15.20	0.817	0.918	0.883	0.081
KGI	15	17.40	15.35	1.158	0.922	0.932	0.023
EI	18	19.20	15.55	1.220	0.931	0.933	0.026
SA1	38	23.80	14.70	0.676	0.939	0.900	**0.054***
SA2	50	28.20	14.83	0.837	0.938	0.924	0.025
SA3	79	30.80	14.40	0.634	0.938	0.905	**0.042***
SA4	47	26.40	14.69	0.950	0.938	0.902	**0.050***
LS061	23	20.80	14.98	0.617	0.931	0.903	0.053
LS062	33	22.20	14.13	0.663	0.928	0.904	0.042
BW2	44	27.80	14.62	0.799	0.935	0.914	0.035
BW3	41	25.80	14.80	0.894	0.937	0.893	**0.060***

Bonferroni correction (Appendix B). The same pattern was observed after exclusion of the loci with possible null alleles (Global $F_{ST}=-0.00070$; 95% CI: -0.00157–0.00001).

We analyzed the statistical power for an observed F_{ST} of 5×10^{-4} ($Ne=10^3$, $t=10$) with the software package POWSIM. Estimates of α-error were low (7% on average) and increased slightly with the sample size. The current sampling design has an α-error of 7% and is capable of detecting significant F_{ST} values of 0.0020 or more, which is below the level of differentiation commonly observed between populations of marine taxa (DeWoody and Avise, 2000). In order to obtain a power superior of 90% for the F_{ST} observed in this study, sample sizes of at least 100 individuals are needed.

There was no significant correlation between geographical distance (Ln (km)) and genetic distance $[F_{ST}/(1-F_{ST})]$ based on all loci (Mantel test, $r=0.031$, $P=0.37$; regression, $y=-0.0009+0.00005\cdot x$; $P=0.8868$; $r^2=0.0004$). Similar patterns were observed when only five high quality loci were incorporated in the analysis (Mantel test, $r=0.030$ $P=0.40$; regression, $y=-0.0026+0.0001\cdot x$; $P=0.7469$; $r^2=0.0020$).

3.4. Model based clustering analysis

The absence of structure as measured with classical F-statistics and genetic distances leaves open the possibility for population admixture, resulting in the observed non-significant F_{ST} values. We therefore applied a model based clustering algorithm to search for potential sub-populations using all sampled myctophids. For the non-admixture model, the Bayesian MCMC cluster analysis using seven microsatellite markers showed a decrease in likelihood peaking at $K=1$. Calculation of ΔK corroborated the inability to detect population structure (Fig. 3) (Evanno et al., 2005). Using only five microsatellite markers yielded a similar result (data not shown). Therefore there was no indication that groups were admixed; they represent a single population.

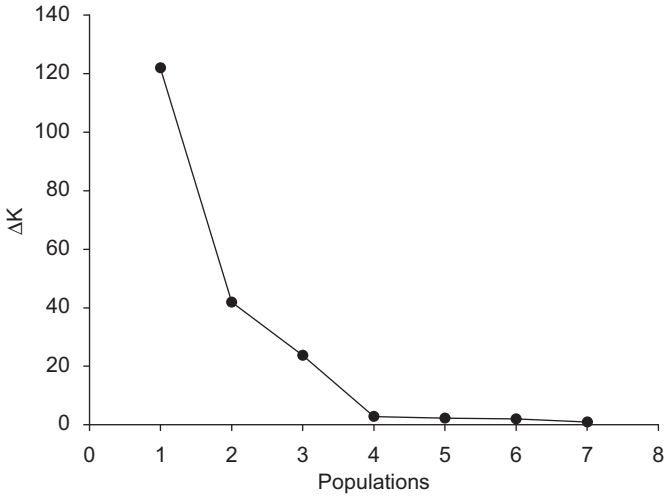

Fig. 3. Structure: ΔK values calculated following Evanno et al. (2005) for K groups (from $K=1$ to 8) using seven microsatellite markers.

Table 5
Linkage disequilibrium estimates of the effective population size (Ne) for sampling regions, sample clusters and all samples combined (∞ = infinite).

Location	n	7 loci			5 loci		
		Ne	95% C.I.		Ne	95% C.I.	
BS	12	**44.8**	29.3	88.8	**43.7**	25.4	128.5
KGI	15	**62.4**	40.9	123	**55.6**	33.6	141.4
EI	18	**74.4**	50.1	138	**66.7**	41.9	148.1
SA1	38	**361.4**	203	1460	**529**	200.8	∞
SA2	50	**959.1**	411	∞	**844.5**	315.0	∞
SA3	79	**1402.8**	660	∞	**580.9**	342.5	1740.0
SA4	47	**1939.6**	473	∞	**1500**	341.6	∞
LS061	23	**90.5**	64.2	149	**161.7**	81.6	2013.4
LS062	33	**553.7**	208	∞	∞	278.6	∞
BW2	44	**1025.9**	381	∞	**2598.9**	381.1	∞
BW3	41	**501.6**	264	3884	**319.8**	172.1	1727.0
[BS]	12	**44.8**	29.3	88.8	**43.7**	25.4	128.5
[KGI, EI]	33	**181.3**	123	333	**274.0**	142.8	2154.5
[SA1, SA2, SA3, SA4]	214	∞	∞	∞	∞	5811.5	∞
[LS061, LS062]	56	**6257.4**	1904	inf	∞	778.9	∞
[BW2, BW3]	85	**2303.1**	894	inf	**1108.6**	529.4	∞
All	400	∞	∞	∞	**2009.8**	1515.0	2942.7

Since no significant differentiation could be observed between populations, population size was estimated for population samples as well as for the hierarchical structure used in the population analysis (Table 5). Ne estimates ranged from 44 to 1940 for single area's samples. Clustering samples increased Ne estimates. Overall Ne estimates were in the order of thousands when all populations were clustered.

4. Discussion

Knowledge of the genetic connectivity and population size of marine Antarctic fish is important for understanding the processes of population divergence and maintenance in a relatively closed polar ecosystem. Here we present a first study on the genetic variability, using microsatellite markers, of a common mesopelagic fish in the Southern Ocean. Using a novel set of seven species-specific microsatellite loci we detected high levels of genetic diversity. Considering that *E. antarctica* is an abundant pelagic species in an environment that promotes gene flow these finding are congruent with previous studies on genetic diversity

(DeWoody and Avise, 2000; McCusker and Bentzen, 2010). As this is a pilot study for this species, we provide a critical analysis of our method, before further discussing the causes of this high genetic diversity and limited genetic structure.

The interpretation of a weak population structure hinges on dense genome sampling and the quality of the analyses. The golden standard of assessing subtle structure in marine organisms requires a combination of many neutral and selection sensitive markers (Nielsen et al., 2009). As this was the first study for this species, there was no a priori information on the amount of required markers or the sampling size. This necessitates a critical analysis of the data quality and statistical power. Significant deviations from HWE due to homozygote excess were observed in three out of seven loci of *E. antarctica*. Homozygote excess has been attributed to a number of factors: population substructuring or admixture, inbreeding, selection at or near a microsatellite locus, null alleles (Dakin and Avise, 2004) and small sample size. Null alleles are a common artefact at microsatellite loci across taxa (Ardren et al., 1999; Callen et al., 1993; Girard and Angers, 2007), especially in large marine fish populations where the genetic diversity is high (O'Reilly et al., 2004). Our multi-locus analysis indicated that homozygote excess was not concordant across loci but rather locus-specific. Two loci displayed homozygote excess in a large number of populations indicative of locus-specific problems that could be related to selection at these loci or null alleles. Removing these loci improved overall estimates of heterozygote excess considerably. We recommend not using the loci Elan025 and Elan11 until the causes of homozygote excess have been clarified.

Removal of the two suspect loci did not eliminate a tendency for overall homozygote excess (but not for individual markers). This might be indicative of either inbreeding (non random mating) or population substructuring (Wahlund effect). Effective population sizes of marine fish are three to four orders of magnitude lower than the census size (Hauser et al., 2002; Palstra et al., 2009). This has been attributed to the large variance in family size from sweepstakes recruitment (Hedgecock, 1994; Li and Hedgecock, 1998). Another important cause for heterozygote deficiencies may be attributed to the Wahlund effect, defined as the inclusion of individuals in a sample that has originated from genetically differentiated populations (Hartl and Clark, 2007). Three out of four significant deviations from HWE were observed in single location samples from the Scotia Arc; this could mean that the Scotia Sea acts as a funnel where different high and low latitude populations are admixed. Such an admixture process could also explain the overall indication of homozygote excess.

The various models tested using STRUCTURE did not reveal any genetic structure for *E. antarctica*. The current genotyping and associated analysis failed to detect significant genetic structure among *E. antarctica* area samples. No signs of isolation by distance were observed and assignment analysis revealed just one population. The POWSIM analysis reveals that under the current sampling regime and this marker set we could only have detected significant F_{ST} values of 0.0020 or more. At this level this would mean that even for a significant F_{ST} value at this level only a fraction (0.20%) of the variance would be explained by differences among populations. Indicating that even with a significant differentiation, it would have limited ecological relevance.

As such it might be tempting to conclude that *E. antarctica* lives in panmictic populations in the Southern Ocean. However caution is advised before reaching this conclusion (Hedgecock et al., 2007): Regardless of statistical power, there is no means of knowing whether the populations of *E. antarctica* have reached equilibrium. The lack of variance may reflect insufficient time since separation to establish a drift-migration equilibrium (Hedgecock et al., 2007). When F_{ST} values are small, as often observed in marine populations (<0.05), small errors in measuring F_{ST} will have a relative large effect, making it impossible to distinguish moderate gene flow from random mating (Palumbi, 2003; Waples, 1998). Furthermore under the current conditions statistical power does constrain the detection of population differentiation. Nevertheless our analysis shows that the set of microsatellite markers used should provide a significant result if the F_{ST} value would be in the order of 0.002, in which instance a 'low-structure-high-gene flow' scenario would be indicated. Finally Bayesian assignment failed to detect any genetic partitioning. Our results clearly show that genetic divergence is indeed low, which is indicative of high levels of gene flow between populations.

Estimates of effective population size (Ne) based on methods without temporal replicate samples, such as the linkage disequilibrium method, are often strongly biased. While the influence of the number of loci used for the Ne estimate is minor, there is a large bias due to sampling size (England et al., 2006). In order to obtain reasonable estimates, sample size has to be equal or larger than Ne. In large marine species the estimate of Ne based on linkage disequilibrium is often biased due to the effect of large populations and migration. Due to these biases Ne should be considered merely indicative (Ovenden et al., 2007). Estimates of Ne were relatively high. For the larger area samples ($n>40$) estimates of Ne were in the order of magnitude 1000. Clustering area samples into regional clusters increased the Ne estimate considerably, indicating a higher effective population size for the whole population.

The myctophid *E. antarctica* joins a growing group of planktonic and pelagic organisms of the Southern Ocean, in which the ACC seems to preclude the development of genetic structure on a large scale (Jarman and Nicol, 2002; Parker et al., 2002; Shaw et al., 2004; Zane et al., 2006, 1998). Historically the Southern Ocean has been isolated from northern waters by the APF for the full period of the Pleistocene, the time window relevant for population structuring. Below the APF the clockwise flow of the ACC should propagate connectivity among populations. For a long time Southern Ocean marine populations were considered demographically open (Ward et al., 1994). However this view is challenged by an increasing literature revealing genetic structure both at a geographic and a temporal scale (Patarnello et al., 2007).

Despite the growing list of fish displaying insignificant variation across thousands of kilometers and even across the entire Southern Ocean, some studies have found significant geographic differentiation, mostly in species with distinct demersal life stages (Matschiner et al., 2009). Primarily, deep water often acts as a barrier to migration for the demersal species (e.g., *D. elengoides*) (Rogers et al., 2006), unlike similar species with a mesopelagic life style (*D. mawsoni*) (Smith and Gaffney, 2005). Also, gene flow may be constrained at local scales. For example, the benthic black-fin icefish *Chaenocephalus aceratus*, which has a pelagic larval stage, showed genetic differentiation between samples from the South Shetlands-Elephant Island and the South Orkneys (Papetti et al., 2009). Antarctic krill, *E. superba,* shows little differentiation on a circumantarctic scale, but they do show significant differentiation between South Georgia and the Eastern Weddell Sea (Zane and Patarnello, 2000), which are separated by the Scotia-Weddell confluence. For the krill *E. crystallorophias*, (Jarman and Nicol, 2002) found no significant genetic differentiation between samples collected 5000 km apart, while samples as close as 16 km showed significant genetic differentiation. Krill forms dense aggregation, which difference often displays considerable differences in weight, sex-ratio and length measurements. Such aggregation behavior would promote genetic variance both at a small geographic scale as well as on a temporal scale.

There is increasing evidence that Antarctic fish similarly displays considerable temporal genetic variability. For the pelagic *P. antarcticum* it was not possible to distinguish between

geographic population structure and weak temporal differentiation (Zane et al., 2006). The benthic *C. aceratus* showed both temporal and spatial genetic variances on a limited geographic scale (Papetti et al., 2007, 2009). Temporal genetic variability, with significant differences in the genetic composition of recruits over time, may be attributed to a sweepstakes effect where only a small proportion of the available gene pool successfully contributes to the replenishment of the population (Hedgecock, 1994), often due to stochastic larval mortality.

Such sweepstake effects are often further enhanced by a match-mismatch effect where asynchrony between reproductive activity and suitable environmental conditions may lead to reduced larval survival due to variation of food availability (Cushing, 1990). Reproductive failure by a large portion of the adult population leads to recruitment variation and changes in cohort size, with concomitant temporal genetic variation (Maes et al., 2006) and a Wahlund effect, caused by the mixing of genetically distinct populations, resulting in a reduction of observed heterozygosity (Hartl and Clark, 2007; Papetti et al., 2009). Pelagic species in the Southern Ocean such as *E. antarctica* and *E. superba*, might be highly susceptible to such a scenario.

The ACC is likely to be an important factor in promoting larval dispersal and hence connectivity in a clockwise direction around the Antarctic continent (Matschiner et al., 2009; Papetti et al., 2009). Also, postlarval dispersal should be affected in mesopelagic organisms; their small size and limited control of movement is likely to lead to low levels of population discreteness. Thus the ACC may be responsible for the mixing of populations and cohorts over time with a constant influx of individuals from westerly regions. Such a system would have a homogenizing effect on the genetic structure. Additionally, the Drake Passage forms a funnel for the ACC and could induce local mixing of populations traveling through the Drake Passage and the adjacent Scotia Sea.

5. Conclusions

Our findings support the hypothesis that the ecological and life-history traits of *E. antarctica* and the oceanographic properties of the Southern Ocean lead to limited geographical differentiation. Under such circumstances it would be more surprising if geographical differentiation were discrete. But there is room for improved knowledge. In order to better understand these patterns, more focussed sampling is needed both in time and space. In order to improve the statistical power of these results, sampling density ($n > 100$ per sample) should be increased substantially and replicated for different years. This is a real challenge given the extreme conditions in the Southern Ocean. A second strategy, less dependent on the local conditions, would be to improve genome sampling using a combination of neutral and adaptive markers. Subtle patterns in adaptive traits are often evolving from the selection in the standing genetic variation. Current technological progress is such that implementation of this approach has become feasible (Hemmer-Hansen et al., 2007; Nielsen et al., 2009).

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Appendix A

(See Table A1).

Appendix B

(See Table B2).

Table A1
Electrona antarctica: location codes, number of alleles (N_A), allelic richness (A_R), expected heterozygosity (H_E), observed heterozygosity (H_O) and F_{IS} according to Weir and Cockerham (1984) for each microsatellite locus. * indicates significant F_{IS} values after Bonferroni corrections. For location abbreviations see Table 1.

	Elan001	Elan009	Elan013	Elan025	Elan035	Elan043	Elan114	Five	All
BS									
N_A	14	15	14	15	16	17	10	15.20	14.43
A_R	14.00	15.00	14.00	15.00	16.00	17.00	10.00	15.20	14.43
H_O	0.750	0.917	0.833	0.833	1.000	0.917	0.833	0.883	0.869
H_E	0.913	0.913	0.910	0.913	0.931	0.924	0.858	0.918	0.909
F_{IS}	0.221	0.040	0.127	0.130	−0.031	0.051	0.072	0.081	**0.087**
KGI									
N_A	19	18	11	15	20	19	13	17.40	16.43
A_R	16.97	15.98	10.15	13.64	17.09	16.59	11.49	15.35	14.56
H_O	0.929	1.000	0.933	0.867	0.933	0.867	0.867	0.932	0.914
H_E	0.929	0.933	0.880	0.918	0.933	0.933	0.840	0.922	0.910
F_{IS}	0.037	−0.037	−0.026	0.090	0.035	0.106	0.003	0.023	0.030
EI									
N_A	24	19	14	15	18	21	13	19.20	17.71
A_R	18.93	15.42	12.22	12.71	14.21	16.97	11.06	15.55	14.50
H_O	1.000	0.889	0.944	0.833	0.833	1.000	0.588	0.933	0.870
H_E	0.952	0.934	0.909	0.898	0.915	0.944	0.884	0.930	0.919
F_{IS}	−0.021	0.076	−0.011	0.101	0.118	−0.030	**0.361***	0.026	**0.083***
SA1									
N_A	34	18	18	23	20	29	23	23.80	23.57
A_R	17.51	13.01	12.35	14.39	14.03	16.61	11.86	14.7	14.25

Table A1 (*continued*)

	Elan001	Elan009	Elan013	Elan025	Elan035	Elan043	Elan114	Five	All
H_O	0.895	0.868	0.842	0.842	0.921	0.974	0.784	0.900	0.875
H_E	0.956	0.928	0.919	0.935	0.936	0.953	0.841	0.939	0.924
F_{IS}	0.077	0.077	0.097	0.112	0.030	−0.008	0.082	**0.054***	**0.066***
SA2									
N_A	43	20	21	23	28	29	28	28.20	27.43
A_R	18.73	12.60	12.35	13.72	14.99	15.50	12.83	14.83	14.39
H_O	0.980	0.860	0.920	0.800	0.960	0.900	0.720	0.924	0.877
H_E	0.967	0.920	0.913	0.932	0.942	0.947	0.904	0.938	0.932
F_{IS}	−0.003	0.075	0.003	**0.152***	−0.009	0.060	**0.214***	0.025	**0.069***
SA3									
N_A	55	23	20	29	26	30	29	30.80	30.29
A_R	18.35	12.77	11.81	14.78	14.10	14.99	12.15	14.40	14.13
H_O	0.885	0.870	0.873	0.798	0.962	0.937	0.785	0.905	0.873
H_E	0.965	0.925	0.913	0.944	0.941	0.947	0.890	0.939	0.932
F_{IS}	**0.090***	0.066	0.050	**0.162***	−0.016	0.018	**0.124**	**0.042***	**0.070***
SA4									
N_A	45	22	19	22	21	25	24	26.40	25.43
A_R	18.98	13.87	12.03	13.69	13.94	14.61	11.35	14.69	14.07
H_O	0.891	0.872	0.872	0.872	0.936	0.936	0.761	0.902	0.877
H_E	0.966	0.932	0.913	0.934	0.937	0.942	0.852	0.938	0.925
F_{IS}	0.088	0.075	0.055	0.076	0.011	0.017	0.118	**0.049***	**0.062***
LS061									
N_A	27	20	17	18	19	21	21	20.80	20.43
A_R	17.10	14.74	13.00	12.86	14.91	15.15	15.41	14.98	14.74
H_O	1.000	0.826	0.826	0.783	0.864	1.000	0.950	0.903	0.893
H_E	0.941	0.930	0.917	0.908	0.934	0.935	0.928	0.931	0.928
F_{IS}	−0.040	0.134	0.121	0.160	0.098	−0.048	0.001	0.053	0.060
LS062									
N_A	28.00	19.00	16.00	20.00	24.00	24.00	21.00	22.20	21.71
A_R	16.23	13.55	11.30	13.64	15.03	14.52	13.64	14.13	13.99
H_O	0.903	0.813	0.970	0.818	0.867	0.969	0.667	0.904	0.858
H_E	0.942	0.922	0.908	0.928	0.937	0.930	0.909	0.928	0.925
F_{IS}	0.058	0.135	−0.053	**0.133***	0.092	−0.026	**0.284***	0.042	**0.089***
BW2									
N_A	43	25	20	22	22	29	21	27.80	26.00
A_R	17.63	14.54	12.46	12.64	12.97	15.50	11.30	14.62	13.86
H_O	0.932	0.886	0.932	0.818	0.864	0.955	0.796	0.914	0.883
H_E	0.954	0.939	0.920	0.908	0.918	0.945	0.872	0.935	0.922
F_{IS}	0.035	0.067	−0.001	0.111	0.071	0.002	0.099	0.035	**0.054***
BW3									
N_A	34	23	17	20	22	33	32	25.80	25.86
A_R	16.03	13.79	12.03	12.68	14.79	17.35	14.85	14.78	14.50
H_O	0.878	0.854	0.829	0.732	0.951	0.951	0.805	0.893	0.857
H_E	0.941	0.929	0.916	0.922	0.943	0.958	0.929	0.937	0.934
F_{IS}	0.079	0.094	0.106	**0.218***	0.004	0.019	**0.145***	**0.060***	**0.094***
Total									
N_A	79	35	29	35	37	47	66	23.42	46.86
A_R	17.99	13.65	12.04	13.85	14.42	15.62	12.61	14.84	14.31
H_O	0.913	0.878	0.889	0.818	0.917	0.946	0.778	0.909	0.877
H_E	0.948	0.928	0.911	0.922	0.933	0.942	0.882	0.937	0.924
F_{IS}	0.056	0.073	0.043	0.131	0.037	0.015	0.137	0.045	0.070

Table B2
Pair-wise multi-locus F_{ST} estimates based on all seven loci (above diagonal) excluding Elan025 and Elan114 (below diagonal) between sample region pairs of *E. antarctica*. Note that no measure was significant after the Bonferroni correction had been applied.

F_{ST}	BS	KGI	EI	SA1	SA2	SA3	SA4	LS061	LS062	BW2	BW3
BS		−0.004	0.002	−0.003	0.000	−0.003	0.001	−0.001	0.002	0.003	−0.005
KGI	−0.005		0.001	−0.008	−0.004	−0.005	−0.002	−0.004	−0.002	−0.002	−0.004
EI	−0.004	−0.005		0.001	0.002	0.002	0.004	−0.004	0.004	0.003	−0.002
SA1	−0.005	−0.008	−0.004		−0.001	−0.001	−0.001	−0.002	0.001	0.000	0.001
SA2	0.001	−0.002	−0.001	−0.002		0.001	0.000	−0.003	0.001	0.002	−0.001
SA3	−0.005	−0.005	−0.002	−0.002	0.002		−0.001	0.000	0.001	0.001	0.001
SA4	−0.003	−0.002	−0.001	−0.001	0.000	−0.001		−0.002	0.001	0.000	0.001
LS061	−0.003	−0.008	−0.002	−0.004	−0.003	−0.001	−0.005		−0.002	0.000	−0.003
LS062	0.004	−0.002	0.003	0.001	0.004	0.003	0.000	0.000		0.001	0.001
BW2	−0.001	−0.005	−0.001	−0.001	0.002	0.001	−0.002	−0.002	0.003		0.002
BW3	−0.007	−0.005	−0.003	−0.002	0.000	0.000	−0.002	−0.002	0.004	0.000	

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