Marine Biology (2006) 149: 915–924
DOI 10.1007/s00227-006-0256-x

RESEARCH ARTICLE

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Genetic structure of Patagonian toothfish (*Dissostichus eleginoides*) populations on the Patagonian Shelf and Atlantic and western Indian Ocean Sectors of the Southern Ocean

Received: 9 April 2005 / Accepted: 10 January 2006 / Published online: 4 February 2006

Abstract The genetic structure of Patagonian toothfish populations in the Atlantic and western Indian Ocean Sectors of the Southern Ocean (SO) were analysed using partial sequences of the mitochondrial 12S rRNA gene and seven microsatellite loci. Both haplotype frequency data ($F_{ST} > 0.906$, $P < 0.01$) and microsatellite genotype frequency data ($F_{ST} = 0.0141$–0.0338, $P < 0.05$) indicated that populations of toothfish from around the Falkland Islands were genetically distinct from those at South Georgia (eastern Atlantic Sector SO), around Bouvet Island (western Atlantic Sector SO) and the Ob Seamount (western Indian Ocean Sector of the SO). Genetic differentiation between these populations is thought to result from hydrographic isolation, as the sites are separated by two, full-depth, ocean-fronts and topographic isolation, as samples are separated by deep water. The South Georgia, Bouvet and Ob Seamount samples were characterised by an identical haplotype. However, microsatellite genotype frequencies showed genetic differentiation between South Georgia samples and those obtained from around Bouvet Island and nearby seamounts ($F_{ST} = 0.0037$, $P < 0.05$). These areas are separated by large geographic distance and water in excess of 3,000 m deep, below the distributional range of toothfish (< 2,200 m). No significant genetic differentiation was detected between samples around Bouvet Island and the Ob Seamount although comparisons may have been influenced by low sample size. These localities are linked by topographic features, including both ridges and seamounts, that may act as oceanic "stepping stones" for migration between these populations. As for other species of deep-sea fish, Patagonian toothfish populations are genetically structured at the regional and sub-regional scales.

Communicated by J.P. Thorpe, Port Erin

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Introduction

Benthopelagic fish species, from bathyal depths, often have very wide geographic distributions. Populations occur on continental slopes, the slopes of oceanic islands and on axial and non-axial seamounts that maybe separated by thousands of kilometres of deep ocean. These species are often strong swimmers as adults, are highly fecund and have long-duration pelagic eggs, larvae and/or juvenile stages. As a null hypothesis it is assumed that such life-histories confer a high capacity for dispersal across large distances. Given the lack of obvious physical barriers to dispersal in the oceans, this should lead to high levels of migration between distant populations and genetic homogeneity on regional or even oceanic scales.

In some cases, there is evidence for gene-flow between populations of deep-water benthopelagic fish over large geographic distances. Examples include: slender armourhead (*Pseudopentaceros wheeleri* Hardy 1983), wreckfish (*Polyprion americanus* Bloch and Schneider 1801) and alfonsino (*Beryx splendens* Lowe 1834) (Martin et al. 1992; Sedberry et al. 1996; Hoarau and Borsa 2000). However, in many cases, genetic differentiation has been detected between populations on interoceanic, intraoceanic and even intraregional scales (reviewed in Creasey and Rogers 1999; Rogers 2003; see also Stockley et al. 2005; Aboim et al. 2005).

The Patagonian toothfish, *Dissostichus eleginoides*, is a benthopelagic species that grows to more than 2 m in length and reaches 95 kg in weight (North 2002). It is an active predator and scavenger that preys on fish, crustaceans (especially prawns), cephalopods and other invertebrates, although the relative importance of these groups in the diet depends on geographic locality and fish size (Pilling et al. 2001; Goldsworthy et al. 2002; Xavier et al. 2002; Arkhipkin et al. 2003). Patagonian toothfish are found at depths of between 200 and 2,500 m around islands and seamounts of the Sub-Antarctic, North of 55°N, and on the shelf and continental slopes of southern Chile and Argentina (Gon and Heemstra 1990).

Patagonian toothfish are thought to spawn at about 1,000–1,500 m depth, and female fish produce between 50,000 and 500,000 large pelagic eggs (Kock et al. 1985; Chikov and Melnikov 1990; Agnew et al. 1999). Eggs and larvae occur in the upper 500 m of the ocean (Evseenko et al. 1995). The duration of the pelagic phase from eggs, through larvae, to juveniles probably lasts for around 1 year (Des Clers et al. 1996). Juvenile fish eventually become associated with the seabed, and age-class 1 between 14 and 25 cm long occur in bottom trawls in waters around 140 m depth (North 2002; British Antarctic Survey unpublished data). Growth of Patagonian toothfish is fairly rapid for the first 10 years and males and females mature at 7 and 12 years, respectively (Horn 2002). Toothfish live for more than 50 years and tend to migrate down-slope, as they grow larger (Horn 2002; Collins et al. 2003). Data on movements of adult Patagonian toothfish are limited and somewhat contradictory. Tagging studies suggest that sub-adult (immature) fish (<85 cm total length) have a tendency to remain within a relatively small area (15 nm; Williams et al. 2002; Tuck et al. 2003; Marlow et al. 2003). However, these studies also indicate that low numbers of sub-adult fish may migrate between locations within regions (see Williams et al. 2002 for Heard/McDonald islands, Kerguelen, Crozet and Marion/Prince Edward Islands). Recent tagging studies around South Georgia, in the Atlantic Sector of the Southern Ocean (SO), have demonstrated that very occasionally toothfish tagged at South Georgia have been recaptured on the Patagonian Shelf (Marlow personal communication Renewable Resources Assessment Group, Imperial College, London). Long-distance, transoceanic dispersal of individual fish has also been reported on one occassion (Møller et al. 2003). It must be noted that because toothfish migrate into increasingly deep waters, as they grow larger (Williams et al. 2002), there is little data on the behaviour of large mature fish.

Several studies on the genetics of Patagonian toothfish have already been completed. These have included the identification of molecular markers for species identification (Smith et al. 2001) but the majority have focused on resolving the genetic population structure of Patagonian toothfish, for the purposes of stock identification (Smith and McVeagh 2000; Appleyard et al. 2002, 2004; Shaw et al. 2004). These studies have demonstrated marked genetic differentiation between populations of Patagonian toothfish located in different geographic regions, namely Falklands, South Georgia, Heard/McDonald Islands and Macquarie Islands. Within regions, a different picture emerges depending on locality. Populations within the area of Prince Edward/Marion Islands, Crozet Islands and Heard/McDonald Islands are genetically homogenous (Appleyard et al. 2004). Comparisons between the Patagonian Shelf and South Georgia/Shag Rocks have revealed marked genetic differentiation (Shaw et al. 2004). These localities are separated by a deep-water channel (more than 2,000 m depth) and by two oceanic fronts, the Polar Front (PF), and the Subantarctic Front (SAF).

Previous studies clearly show that Patagonian toothfish demonstrate marked genetic differentiation on regional and even sub-regional scales. However, a complete picture for the entire distribution of the species is not yet completed on the regional scale. In this study, genetic analysis of populations of Patagonian toothfish from the Patagonian Shelf and Atlantic and western Indian Ocean Sectors of the SO is presented. This includes the first genetically analysed samples of individuals from the southeast Atlantic Sector of the SO around Bouvet*ø*ya (Bouvet Island), a locality lying almost exactly between previously analysed localities in South Georgia, and Crozet, Prince Edward/Marion Islands. Bouvet is separated from South Georgia by deep water of abyssal depths. However, Bouvet Island and the islands and seamounts to the east are potentially linked by ridges and seamounts that may act as stepping-stones for dispersal of toothfish either in the adult or larval and juvenile stages. This study therefore provides a test that toothfish may disperse along topographic features such as seamounts and ridges but that deep water presents a barrier to dispersal for this species.

Materials and methods

Samples

Toothfish samples were collected by long line from commercial fishing vessels over a period of several years but mainly in 1997 and 2003. Samples were collected from several geographic areas including from around the Falkland Islands, Shag Rocks, South Georgia, Bouvet Island, Speiss Seamount, Ob Seamount and Meteor Seamount (Fig. 1). Table 1 presents the location of each sample along with the numbers of toothfish sampled for the present study.

Samples were dissected immediately following capture and a small sample of muscle, liver or gill tissue preserved in 99% ethanol. Samples were transported back to British Antarctic Survey, Cambridge, United Kingdom for genetic analysis.

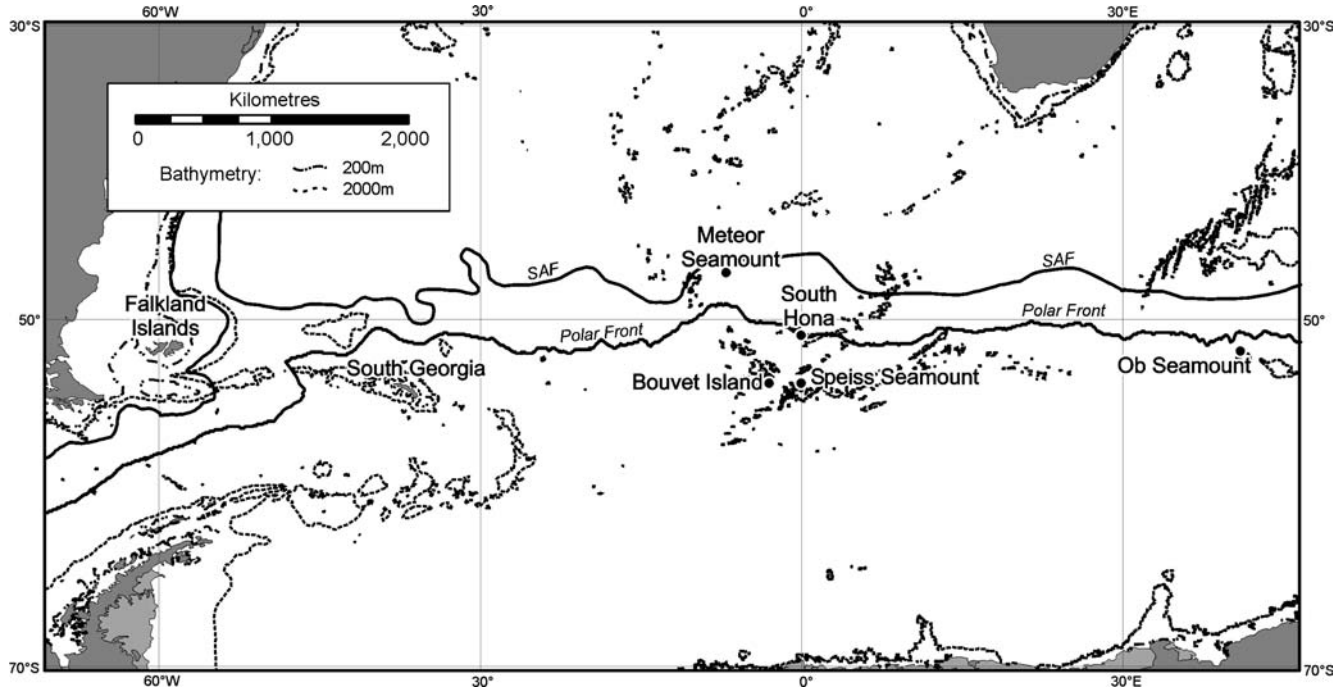

Fig. 1 Dissostichus *eleginoides*: map showing the region in which sampling was undertaken for this study. *SAF* Sub-Antarctic Front

Table 1 *Dissostichus eleginoides*: sampling localities (latitudes and longitudes) on the Patagonian Shelf and in the East Atlantic, West Atlantic and East Indian Ocean Sectors of the SO

Sample area	Latitude and longitude	Date
Bouvet, South Hona	51°S 0°	November 1997
Bouvet, Meteor Seamount	47°S 7°W	November 1997
Bouvet, Speiss Seamount	54°S 0°	November 1997
E. Indian Ocean, Ob Seamount	52°S 41°E	November 1997
Bouvet Island	54°26S 3°24W	November 1997
South Georgia, Shag Rocks	53°30S 42°W	July 2002
South Georgia, Shag Rocks	53°30S 42°W	July 2002
South Georgia	53°49S 36°09W	January 2003
South Georgia	53°49S 35°50W	January 2003
South Georgia	53°53S 35°47W	January 2003
South Georgia	53°24S 37°01W	January 2003
South Georgia	53°32S 37°02W	January 2003
South Georgia	53°34S 37°18W	January 2003
South Georgia	53°47S 39°18W	January 2003
South Georgia	54°54S 38°17W	January 2003
South Georgia	55°22S 36°12W	January 2003
South Georgia	54°44S 35°09W	January 2003
South Georgia	54°53S 34°21W	January 2003
South Georgia	54°57S 34°16W	January 2003
South Georgia	53°48S 35°51W	January 2003
Falklands	48°46S 56°19W	July 2002
Falklands	48°30S 56°49W	July 2002
Falklands	48°39S 57°13W	July 2002
Falklands	48°38S 57°16W	August 2003
Falklands	52°17S 55°46W	July 2002
Falklands	48°26S 57°35W	August 2003

DNA extraction

In the laboratory, excess ethanol was blotted from the tissue and total genomic DNA was then extracted from small (1–3 mg) sections of tissue using DNeasy kits (Qiagen, Crawley, West Sussex, UK), following the manufacturers instructions for animal tissue. The extracted DNA was resuspended in elution buffer and then checked on a Syngene Gene Genius documentation system (Syngene, Cambridge, UK), with Gene Snap Version 2.60.0.14 documentation software, by comparison with a known standard (1 Kb DNA ladder, Gibco BRL, Rockville, MD, USA) on a 1% agarose gel, following electrophoresis. DNA was also quantified using an Eppendorf Biophotometer (UV spectrophotometer, Eppendorf, Hamburg, Germany) by measuring absorbance at 260 nm. DNA was subsequently stored at -20°C until used for analysis.

Mitochondrial DNA amplification

The genetic variation was assessed between individuals from the different sampling localities for the 16S rDNA and the 12S rDNA mitochondrial partial sequences. Amplification of the 16S rDNA region was carried out using the primers 16AR (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16SBR (5′-CCG GTC TGA ACT CAG ATC ACG-3′) Palumbi et al. (1991). The reaction components for 16S amplification were as follows: 100 μl reaction; 10 μl 10× PCR buffer (Tris–HCl, KCl pH 7.8, contains 15 mM $MgCl_2$), 20 μl Q-solution, 2 μl dNTPs (10 mM of each dNTP), 1 μl (10 pM) of each

primer, 0.5 μl Taq polymerase (2.5u), 64.5 μl dH_2O, 1 μl sample DNA (2 ng); all reagents from Qiagen. Reaction conditions were: 94°C for 4 min, 30 cycles of 94°C 30 s, 50°C 1 min, 72°C 1 min, then 72°C for 10 min. All reactions were carried out on an MJ Research Peltier Thermal Cycler 225 DNA Engine Tetrad-Gradient.

Amplification of the partial 12S rDNA region was carried out using the newly developed primers 12STF1 (5′-TCC CTA ACC ACT CTT TAC GC-3′) and 12STR1(5′-CTC CAA CCT CCT TGT TTC G-3′). The reaction components for 12S amplification were as follows: 50 μl reaction; 5 μl 10× PCR buffer, 1 μl dNTPs, 1 μl $MgCl_2$ (25 mM), 0.5 μl each primer, 0.25 μl Taq-polymerase, 41.25 μl dH_2O, 0.5 μl sample (1 ng); all reagents Qiagen. Reaction conditions were as for 16S rDNA.

DNA sequencing

Cycle sequencing reactions were carried out using DYEnamic ET terminator reagent premix according to the manufacturer's instructions (Amersham Biosciences Ltd, Little Chalfont, Buckinghamshire, UK). Samples were purified, following cycle sequencing, using DyeEx dye terminator removal kits (Qiagen) according to manufacturer's instructions. Products of sequencing reactions were subsequently visualised on a Megabace 500 automated capillary DNA sequencing machine running Sequence Analyzer Version 2.4 software (Amersham Biosciences). Sequence fluorograms were checked by eye, especially where insertion/deletions (indels) were detected by alignment (see below). In all cases, indels were found to result from failure of the software to record nucleotide peaks following multiple "A" or "T" sequences or because of low peaks for "G".

Microsatellites

Seven microsatellite loci were PCR amplified using the primers developed by Reilly and Ward (1999) and Smith and McVeagh (2000): To2 (forward 5′-CTC TGA AGA TGA ATT GGT GGA TGC-3′ reverse 5′-CAT CAT GTC ACC CTG TCT TTA ACG-3′), To5 (forward 5′-CAC AGA CCA GCA CTA CAA CCC AAG G-3′ reverse 5′-AAG TGT AGT AAT CCA AAT GCA CGC-3′), cmrDe2, cmrDe4, cmrDe9, cmrDe13, cmrDe30. Reverse primers were labelled with a fluorescent dye (To2R—NED, Applied Biosystems, Warrington, Cheshire, UK; To5R—FAM, Transgenomic, Glasgow, UK; De2R—HEX, Transgenomic; De4R—-FAM, Transgenomic; De9R—HEX, Transgenomic; De13R—FAM; De30R—FAM, Transgenomic). PCR amplifications were carried out as 10 μl reactions with the following components: 10× PCR buffer 1 μl, Q-solution 2 μl, $MgCl_2$ 0.4 μl, dNTPs 0.2 μl, 0.5 μl of each primer, 0.5 μl 1:5 Taq polymerase (0.5u), dH_2O 3.9 μl, 1 μl sample DNA (2 ng). Reactions conditions were as follows: 93°C 10 min then 40 cycles of 93°C 30 s, 54°C 1 min, 72°C 2 min, then 72°C 10 min followed by a slow cooling of 60, 50, 40, 30, 20°C for 1 h each. The size of PCR products was estimated using Genotyper Version 1.1 on a Megabace 500 automated capillary DNA sequencing machine.

Data analysis

12S rRNA

Sequence data was aligned using the programme Clustal X (Thompson et al. 1997) using default parameters. Sequences were then trimmed from both ends to allow comparison of good sequence for the maximum number of individuals. Base frequencies were calculated and intrapopulation diversity and diversity across all samples was analysed by estimating gene diversity (h), the probability that two randomly choosen haplotypes are different (Nei 1987), and nucleotide diversity (π), the probability that randomly chosen homologous nucleotides are different (Tajima 1983; Nei 1987).

Overall genetic differentiation between samples from the Falkland Islands, Bouvet Island, Meteor Seamount, Speiss Seamount and Ob Seamount were estimated using pairwise F-statistics (F_{ST}; Wright 1951). Differentiation was also estimated using Exact testing (Raymond and Rousset 1995; Goudet et al. 1996). All sequence analyses were carried out using Arlequin Version 2.0 (Schneider et al. 2000).

Microsatellites

Estimates of observed and expected heterozygosities and Exact tests of conformity to Hardy–Weinberg expectations (Guo and Thompson 1992) were carried out using Arlequin Version 2.0 (Schneider et al. 2000). Tests for linkage disequilibrium between genotypes at each pair of loci was tested using FSTAT 2.9.3.2 (Goudet 1995). This software estimates the significance of association between genotypes at pairs of loci in each sample by using the log-likelihood ratio G-statistic.

Genetic differentiation was analysed using Exact tests of genetic differentiation between each site across all loci using Genepop Version 3.3. Pairwise F_{ST}s were estimated using Weir and Cockerham's (1984) implementation of Wright's (1978) F-statistics, with significance being estimated using permutation tests in FSTAT. A matrix of F_{ST} values were compared to a matrix of geographic distances in kilometres between the sample areas, again using Genepop. These approaches do not allow the specific testing of hypotheses that relate geographic proximity of populations to genetic population structure. Analysis of molecular variance (AMOVA) was therefore also used to analyse population structure at three levels of hierarchy; (1) within populations, (2) between each population (3) between three groups of

populations (Falklands, South Georgia, eastern Atlantic/Indian Ocean Sectors). This analysis was implemented using Arlequin Version 2.0.

Results

Sequences

No variation was found for the 16S rRNA partial sequences (Accession Number: AM180545) so this gene was abandoned for purposes of population genetic analysis. Partial sequences of 249 base pairs (bp) of the 12S rRNA were obtained for 151 individuals across all populations. Overall nucleotide composition was: C = 22.58%, T = 29.23%, A = 22.97% and G = 25.22%. A total of three haplotypes (Accession Nos.: AM180546, AM180547, AM180548) were detected in this short region with all mutations being substitutions (two transitions and one transversion). The samples from around the Falklands region showed a gene diversity (h) of 0.1193 ($\pm$0.0756) and a nucleotide diversity (π) of 0.000730 ($\pm$0.001079). All other populations were fixed for a single haplotype that occurred in one individual in the Falklands samples (see Table 2). For the Falklands samples 32 out of 33 specimens exhibited a different haplotype to all other populations. F_{ST}s were highly significant in all pairwise comparisons between the Falklands and all other samples. Pairwise comparisons between populations other than the Falkland Islands showed no differentiation (Table 3).

Microsatellites

A total of 274 individuals were genotyped for seven microsatellite loci. Of these loci, one, To5 was monomorphic in all but one population, Speiss Seamount. Heterozygosity of the other loci was high, ranging from 0.41463, in De13, to 1.0000 in To2 and De2. Numbers of alleles varied from 4 (De30) to 34 (De9). Pairwise genotypic disequilibria were not detected and significant deviations from Hardy–Weinberg expectations were only found in two populations, both for the same locus, De9 (Table 4). It should be noted that this locus also showed the most deviations from Hardy–Weinberg expectations in Appleyard et al. (2002) and significant deviation in a single sample in Shaw et al. (2004). This may reflect difficulties in scoring this locus or occasional occurrence of null alleles although results across the three studies are inconsistent and not thought to be of sufficient magnitude to adversely influence overall conclusions.

Analysis of molecular variance detected significant genetic differentiation between populations but after sequential Bonferroni correction significant differentiation was not detected between groups of populations (Table 5). Exact tests of pairwise genetic differentiation and pairwise F_{ST}s (excluding the Bouvet samples because of insufficient sample size) showed significant results for all comparisons between the Falkland Islands and all other populations after Bonferroni correction (Table 6). Comparisons between South Georgia and Speiss seamount also showed significant genetic differentiation although for Exact tests this result was insignificant following Bonferroni correction (Table 6). Comparisons between Meteor, Speiss and Ob seamounts were not significant under any tests prior to or following Bonferroni correction (Table 6). A Mantel test of F_{ST} values versus geographic distance between samples was not significant.

Following these analyses, populations around Bouvet Island (Meteor, Speiss) were pooled and then subsequently pooled with the Ob Seamount sample. F_{ST} analysis and Exact tests for comparisons between the three regional samples (Falklands, South Georgia, West Atlantic/East Indian Ocean Sectors of the SO) were carried out (Table 7). All comparisons between the Falkland Island samples and other populations were significant (F_{ST}s $P < 0.05$; Exact tests $P < 0.01$). Comparisons between South Georgia and the pooled samples of Speiss and Meteor Seamounts were significant at the $P < 0.05$ level.

Discussion

This study confirms the findings of Shaw et al. (2004) in demonstrating clear genetic differentiation between populations located on the Patagonian Shelf (Falkland Islands) and South Georgia. This differentiation is apparent in both mitochondrial haplotype data for partial sequences of the 12S rRNA and in analysis of genotype frequencies for microsatellites. As with Shaw et al. (2004), this differentiation is marked in the haplotype data, where the Falkland Islands populations are

Table 2 *Dissostichus eleginoides*: 12S rRNA partial sequence haplotype distributions for all populations sampled

	Falklands (N = 33)	South G (N = 45)	Bouvet (N = 10)	Meteor (N = 16)	Speiss (N = 17)	Ob (N = 30)
Haplotype						
1	31	0	0	0	0	0
2	1	0	0	0	0	0
3	1	45	10	16	17	30

N number of sequences sampled for each geographic locality

Table 3 *Dissostichus eleginoides*: pairwise F_{ST}s estimated from haplotype frequency data

	Falklands	South G	Bouvet	Meteor	Speiss	Ob
Falklands	–					
South G	0.94771*	–				
Bouvet	0.90677*	0.00000	–			
Meteor	0.91788*	0.00000	0.00000	–		
Speiss	0.91948*	0.00000	0.00000	0.00000	–	
Ob	0.93563*	0.00000	0.00000	0.00000	0.00000	–

*significant at $P < 0.000001$ level

Table 4 *Dissostichus eleginoides*: observed (H_O) and expected (H_E) Heterozygosities for microsatellite loci for each population sampled

Sample	Falklands ($N = 87$)	South G ($N = 59$)	Bouvet ($N = 11$)	Meteor ($N = 27$)	Speiss ($N = 43$)	Ob ($N = 47$)
Locus						
To2						
N_a	25	17	9	12	13	14
H_O	0.91765	0.89831	1.00000	0.88462	0.78571	0.82609
H_E	0.92503	0.88932	0.85714	0.86802	0.85829	0.85475
To5						
N_a	1	1	1	1	2	1
H_O					0.02326	
H_E					0.04624	
De2						
N_a	29	24	12	20	22	24
H_O	0.83750	0.86667	0.81818	1.00000	0.92683	0.93478
H_E	0.94583	0.94272	0.95238	0.95248	0.93797	0.94625
De4						
N_a	18	15	8	13	12	14
H_O	0.78481	0.83636	0.90909	0.80769	0.90476	0.82609
H_E	0.89349	0.83720	0.89177	0.86576	0.86776	0.82537
De9						
N_a	34	29	12	23	28	30
H_O	0.92593	0.96226	0.80000	0.76923*	0.80488*	0.95652
H_E	0.96365	0.95957	0.93684	0.94495	0.95574	0.95939
De13						
N_a	8	9	6	7	6	7
H_O	0.68293	0.56140	0.45455	0.55556	0.41463	0.48780
H_E	0.76410	0.64881	0.63636	0.57722	0.42608	0.51340
De30						
N_a	16	14	4	7	14	10
H_O	0.64368	0.59322	0.72727	0.44444	0.57500	0.53333
H_E	0.71131	0.67695	0.70996	0.62753	0.60253	0.56305

N number of individual genotyped
N_a number of alleles detected for each locus in each population

dominated by individuals with a different haplotype to all other populations in the present study. A single individual out of 33 from the Falkland Islands area showed a "Southern Ocean" haplotype. This may have arisen through a sampling error, arising either on a fishing vessel or in subsequent labelling of collected samples. However, the migration of an individual fish from South Georgia to the Patagonian Shelf is possible given recent data from tagging studies in the Atlantic Sector of the SO (Marlow personal communication).

The question arises as to what mechanisms may prevent dispersal and gene-flow between populations of Patagonian toothfish around the Falkland Islands and South Georgia/Shag Rocks. In terms of larval dispersal it is notable that these two regions are divided by the PF and, further to the north and west, the SAF, the two

Table 5 *Dissostichus eleginoides*: hierarchical AMOVA

Source of variation	Total variance	Fixation indices	*P*-value
Among groups	0.01	0.00006	0.04888
Among populations within groups	-0.01	-0.00007	0.00782*
Within populations	100.01	-0.00013	0.60606

*significant at the $P < 0.05$ level

Table 6 *Dissostichus eleginoides*: pairwise F_{ST}s below diagonal, probabilities associated with Exact tests of population differentiation above diagonal

	Falklands	South G.	Meteor	Speiss	Ob
Falklands		< 0.00001**	0.00057**	< 0.00001**	< 0.00001**
South G.	0.0141**		0.05665	0.01884	0.55568
Meteor	0.0226*	0.0018		0.44232	0.11476
Speiss	0.0338**	0.0038*	-0.0024		0.15040
Ob	0.0283**	0.0013	0.0005	-0.0014	

*significant at $P < 0.05$ level after sequential Bonferroni correction
**significant at $P < 0.01$ level

main current cores of the Antarctic Circumpolar Current (ACC). These are fast flowing bottom-reaching cores, associated with thermohaline fronts and with slower-moving water between. They are constrained to lie within deep passages of the North Scotia Ridge, with the PF lying in the 3,300 m deep Shag Rocks Passage. To the North of these features, around the Falkland Islands and slopes of the Patagonian Shelf, residual current flows are generally towards the north (Glorioso 2000). Therefore it is most unlikely that eggs and larvae produced on the Patagonian Shelf will be advected towards South Georgia.

The oceanography around South Georgia and Shag Rocks is also highly complex. Currents in this region are dominated by the southern sectors of the ACC, most notably the Southern ACC Front (Meredith et al. 2003a), This approaches South Georgia from the southwest, before looping anticyclonically around the island, topography having a very strong influence on direction of flow locally (Thorpe et al. 2005). In addition, an anticyclonic circulation has been observed by deployment of surface drifters and other oceanographic methods on the Northwest Georgia Rise, north of South Georgia (Meredith et al. 2003b). This feature is associated with the formation of a Taylor Column with resultant upwelling and localised increases in productivity. Such features have been associated with larval retention on seamounts and banks, although in this case, this feature in itself would not explain a lack of gene flow between the Falklands and South Georgia (Mullineaux and Mills 1997; Fock and Zidowitz 2004). Instead it is likely that both current–topography interactions and the overall northeastward ACC flow in this region together, mean that it is highly unlikely that larvae in the vicinity of South Georgia are transported towards the Patagonian Shelf. Records of eggs and larvae of Patagonian toothfish are sparse despite extensive sampling in the Atlantic sector of the SO but several eggs and larvae have been captured close to the continental shelf of South Georgia (Evseenko et al. 1995). Furthermore, net-sampling of larvae and juveniles around South Georgia/Shag Rocks suggests that early juvenile fish probably migrate onshore in this region, again retaining the growing fish around the only shallow topography in the area, South Georgia and Shag Rocks (North 2002). This would leave adult dispersal as the only likely means of migration between these populations.

Previous tagging studies have suggested that toothfish migrate in to deeper water as they grow and that small number of sub-adult fish may also undertake migrations. In addition low numbers of toothfish, tagged in South Georgia have been recaptured on the Patagonian Shelf, indicating that at least some fish undertake long-distance migration across oceanic fronts. Microsatellite data from adult fish previously sampled on the North Scotia Ridge, between the Falklands and South Georgia areas, may be intermediate between these two genetically distinct populations (Shaw et al. 2004). This is consistent with adult migration across this zone but levels must be small to prevent genetic homogenisation between the respective shelves in this zone. An alternative possibility is that there is a barrier to reproduction between these populations although there is no evidence to suggest this at the present time. Note that Shaw et al. (2004) also pointed to marked differences between mitochondrial and nuclear microsatellite data. One explanation of this is that male fish are exhibiting different migratory behaviour than females.

For other populations, genetic structure is less resolved. Haplotypes for the partial 12S rRNA sequence for populations at South Georgia, around Bouvet and at the Ob Seamount were identical. Analysis of the separate samples collected from these regions only revealed some differentiation between South Georgia and samples from around Bouvet and the Ob Seamount. Most of

Table 7 Pairwise comparisons with pooled samples for Meteor & Speiss Seamounts and Meteor and Speiss and Ob Seamounts for F_{ST}s and Exact tests of population differentiation

Pairwise comparison	F_{ST}	Exact test *P*-values
Falklands versus South G	0.0141*	< 0.00000**
Falklands versus Meteor/Speiss	0.0307*	< 0.00000**
Falklands versus Ob	0.0283*	< 0.00000**
Falklands versus Meteor/Speiss/Ob	0.0307*	< 0.00000**
South G versus Meteor/Speiss	0.0037*	0.00567*
South G versus Ob	0.0013	0.56408
South G versus Meteor/Speiss/Ob	0.0030*	0.05571
Ob versus Meteor/Speiss	-0.0003	0.09565

*$P < 0.05$ after Bonferroni correction
**$P < 0.01$

these results were insignificant following Bonferroni corrections. Given that no differentiation was detected between the Meteor and Speiss populations, pooling of these samples was carried out. This did show significant genetic differentiation between each of the three regions (Falklands, South Georgia, West Atlantic Sector) using both F_{ST} analysis and Exact testing (note that AMOVA did not suggest significant differentiation attributable to nominal groups of populations). Comparisons between South Georgia and the Ob Seamount by itself were not significant. This contrasts to previous studies that have compared Patagonian toothfish populations in the Indian Ocean Sector of the SO with South Georgia/Shag Rocks (Appleyard et al. 2002). However, as noted by Shaw et al. (2004) the levels of genetic differentiation with microsatellite loci were generally low in most cases. As such, sample size in the Ob Seamount Sample may have influenced the results in the present study. In addition, the times during which samples were collected from these areas (South Georgia versus Bouvet region and Ob Seamount) differed by 5–6 years. Temporal genetic variation in the sampled populations may have influenced comparisons of genotype frequencies. Toothfish are extremely long-lived fish and it is likely that temporal genetic variation in populations will be limited over the timescales represented by this study. No evidence of genetic heterogeneity within populations has been detected in previous studies of toothfish, although these have only spanned 2–3 years (Appleyard et al. 2002, 2004). Temporal genetic variation in fish has been shown to be insignificant or relatively small compared to that related to spatial genetic structure in other species with shorter life-cycles (e.g. Ruzzante et al. 1997; Hansen et al. 2002). Comparisons between the Ob Seamount and pooled Meteor and Speiss Seamount samples also did not show significant genetic differentiation.

That the South Georgia/Shag Rocks populations are genetically distinct from those around Bouvet (junction of the Mid-Atlantic Ridge and South-west Indian Ridge) is unsurprising given previous evidence for regional differentiation in Patagonian toothfish populations (Smith and McVeagh 2000; Appleyard et al. 2002; Shaw et al. 2004). These two areas are separated by a large geographic distance of open-ocean of depths in excess of 3,000 m and no intervening seamounts or islands that allow for step-wise dispersal. Although the evidence for differentiation between the Ob Seamount and South Georgia was not present, previous studies have shown genetic differentiation between Heard and Macdonald Islands, on the Kerguelen Plateau, and South Georgia/Shag Rocks (Appleyard et al. 2002). No consistent significant genetic differentiation has been detected between Marion Prince Edward Islands, Crozet and Kerguelen suggesting that adult migration and/or larval dispersal is sufficient within this region to give a homogenous genetic structure. The present study did not show significant genetic differences between the Speiss/Meteor Seamounts and the Ob Seamount. These sites are potentially linked by oceanic ridge systems and seamounts that may act as oceanic "stepping stones" across this region. Overall this and previous studies support the existence of genetically distinct populations in the Falklands Island area, South Georgia/Shag Rocks and the Bouvet/Crozet/Heard and Macdonald Islands. It should be noted, however, that pooling of the Speiss and Meteor Seamount samples moved comparisons with Ob Seamount samples towards significance. Finer Scale comparisons between the Bouvet area and Marion/Prince Edward Islands, Crozet and Kerguelen would be useful in resolving the question of differentiation between Bouvet and sites to the east.

Patagonian toothfish therefore share the feature of many other species of commercially valuable deep-sea fish in that they demonstrate genetic differentiation at regional and, in the case of the East-Atlantic Sector of the SO, intraregional genetic differentiation. Insignificant correlation between F_{ST}s and geographic distance between samples suggest that this differentiation is not simply related to geographic distance between populations and that fronts may play a role in differentiation in the western Atlantic sector of the SO. Fronts have been implicated as barriers to gene flow in other species. For example the subtropical convergence to the south of New Zealand is thought to act as a barrier to gene-flow in populations of orange roughly, *Hoplostethus atlanticus* (Smith et al. 1996) and pink ling, *Genypterus blacodes* (Smith and Francis 1982; Smith and Paulin 2003). In other cases, distance has been implicated in the isolation of populations of fish and squid separated by ocean basins. Genetic differentiation has been detected in populations located around the Azores and those on the European continental shelf in black-spot seabream, *Pagellus bogaraveo* (Stockley et al. 2005) and veined squid, *Loligo forbesi* (Shaw et al. 1999). The Tasman Sea has been found to be a barrier to gene flow in hoki, *Macruronus novaezelandiae* (Milton and Shaklee 1987; Baker et al. 1995), pink ling (Smith and Paulin 2003) and black oreo, *Allocyttus niger* (Ward et al. 1998). Historical patterns of dispersal and colonisation may also strongly influence the population structure of deep-sea fish populations, as indicated in recent studies of the bluemouth, *Helicolenus dactylopterus* in the North Atlantic (Aboim et al. 2005). In toothfish wide areas of deep water isolate populations inhabiting topographic islands such as ridges, seamounts and the slopes of continental shelves and islands. On intraregional scales, such as the East Atlantic, oceanographic factors are important. The role of history in structuring toothfish populations is unclear from present studies but may also have played a role at intraregional and interregional scales.

Acknowledgements We thank Dr Eduardo Balguerías for sampling and provision of toothfish samples from Speiss, Meteor and Ob Seamounts and Bouvet Island. Dr Paul Brickle provided toothfish samples from the Falklands Islands whilst samples from the Shag Rocks/South Georgia regions were provided by scientific observers working on commercial longline vessels and from a survey conducted by the FRV "Dorada". Simon Morley was funded by the

Government of South Georgia and South Sandwich Islands (GSGSSI), through the South Georgia Project. Kester Jarvis received an Undergraduate Research Bursary (Ref: URB/01449/G) from the Nuffield Foundation for a summer internship at British Antarctic Survey, Cambridge. We also acknowledge Dr Michael Meredith for comments on the oceanographic discussion in the paper, Dr Tony North and Dr Martin Collins for comments on toothfish biology, and Prof. Paul Rodhouse and Prof Christopher Rapley for provision of facilities at British Antarctic Survey, Cambridge.

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