POPULATION GENETIC STRUCTURE OF PATAGONIAN TOOTHFISH IN THE WEST INDIAN OCEAN SECTOR OF THE SOUTHERN OCEAN

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Abstract

Molecular markers were used to investigate the stock structure of Patagonian toothfish (*Dissostichus eleginoides*) in the West Indian Ocean sector of the Southern Ocean. Four collections of *D. eleginoides* from Kerguelen (n = 1), Crozet (n = 1) and Prince Edward and Marion (n = 2) Islands were typed using two fragments of mitochondrial DNA (mtDNA) and seven nuclear microsatellite loci. MtDNA haplotype diversity ranged from 0.331 to 0.351. Observed heterozygosities per locus per collection ranged from 0.000 to 0.900 and allele counts from 1 to 21. There was no evidence of significant mtDNA heterogeneity among the four collections and only weak and inconsistent heterogeneity (based on minor allele frequency differences) at three microsatellite loci among the four collections. Neither mtDNA nor microsatellite F_{ST} values indicated population sub-structuring among collections. Genetic variance estimates of both mtDNA (F_{ST} = –0.016) and microsatellites (F_{ST} = –0.005) indicated all variation was seen within collections. Comparisons with previously collected genetic data from Heard and McDonald Island collections (n = 4) were not significant among the geographic groups (mtDNA ϕ_{CT} = –0.003; microsatellite ϕ_{CT} = 0.004), indicating a lack of genetic differentiation among these West Indian Ocean sector fishing locations.

Résumé

Des marqueurs moléculaires ont servi à étudier la structure du stock de légine australe (*Dissostichus eleginoides*) dans le secteur ouest indien de l'océan Austral. Quatre prélèvements de *D. eleginoides* de Kerguelen (n = 1) et des îles du prince Edouard et Marion (n = 2) ont fait l'objet d'une classification effectuée au moyen de deux fragments d'ADN mitochondrial (ADNmt) et de sept loci microsatellites nucléaires. La diversité de l'haplotype d'ADNmt variait entre 0,331 et 0,351. Les niveaux observés d'hétérozygotie par locus variaient entre 0,000 et 0,900 et le nombre d'allèles de 1 à 21. Aucune hétérogénéité d'ADNmt d'importance n'était évidente sur les quatre prélèvements et seule une hétérogénéité faible et changeante (reposant sur des différences mineures de fréquence des allèles) était apparente à trois loci microsatellite sur les quatre prélèvements. Ni l'ADNmt ni les valeurs microsatellites F_{ST} n'indiquaient une sous-structure de la population parmi les prélèvements. Les estimations de la variance génétique de l'ADNmt (F_{ST} = –0,016) et des microsatellites (F_{ST} = –0,005) indiquaient que toute la variation était observée dans les prélèvements. Les comparaisons avec les données génétiques qui avaient été collectées précédemment aux îles Heard et McDonald (n = 4) n'étaient pas significatives parmi les groupes géographiques (ADNmt ϕ_{CT} = –0,003; microsatellite ϕ_{CT} = 0,004), ce qui met en évidence l'absence de différentiation génétique entre les lieux de pêche du secteur ouest de l'océan Indien.

Резюме

Для исследования структуры запаса патагонского клыкача (*Dissostichus eleginoides*) в западной части индоокеанского сектора Южного океана использовались молекулярные маркеры. Был определен тип для четырех коллекций *D. eleginoides*

из районов о-вов Кергелен (n = 1), Крозе (n = 1), а также Принс-Эдуард и Марион (n = 2) с использованием двух фрагментов митохондриальной ДНК (мтДНК) и семи ядерных микросателлитных локусов. Разнообразие гаплотипов мтДНК лежало в диапазоне от 0.331 до 0.351. Наблюдаемые гетерозиготности по локусу на коллекцию варьировали от 0.000 до 0.900, а число аллелей – от 1 до 21. Свидетельств значительной гетерогенности мтДНК между этими четырьмя коллекциями не имелось и наблюдалась лишь слабая и неустойчивая гетерогенность (исходя из незначительных различий в частоте аллелей) в трех микросателлитных локусах для этих коллекций. Ни мтДНК, ни значения микросателлитных F_{ST} не указывают на наличие популяционной подструктуры в коллекциях. Оценки генетической изменчивости мтДНК (F_{ST} = –0.016) и микросателлитов (F_{ST} = –0.005) указывают на изменчивость в пределах отдельных коллекций. Отличия по сравнению с ранее собранными генетическими данными по коллекциям с о-вов Херд и Макдональд (n = 4) были незначительными между этими географическими группами (мтДНК ϕ_{CT} = –0.003; микросателлитная ϕ_{CT} = 0.004), что свидетельствует об отсутствии генетической дифференцировки между этими промысловыми участками в западной части Индоокеанского сектора.

Resumen

Se utilizaron marcadores moleculares para estudiar la estructura del stock de austromerluza negra (*Dissostichus eleginoides*) en el sector occidental del Océano Índico, Océano Austral. Se caracterizaron cuatro muestras de *D. eleginoides* recolectadas en Kerguelén (n = 1), Crozet (n = 1) y las islas Príncipe Eduardo y Marion (n = 2) utilizando dos fragmentos de ADN mitocondrial (mtADN) y siete loci microsatélite del ADN nuclear. La diversidad de haplotipos del mtADN fue de entre 0,331 y 0,351. Las heterocigozidades observadas por locus de cada muestra variaron entre 0,000 y 0,900, y el número de alelos de 1 a 21. No hubo indicios de una heterogeneidad significativa del mtADN entre las cuatro muestras, y solamente se observó una heterogeneidad débil e inconstante (sobre la base de pequeñas diferencias entre las frecuencias de alelos) en tres loci microsatélite en las cuatro muestras. No se demostró la existencia de subestructuras en la población representada por las muestras, ni con mtADN ni con los valores F_{ST} de microsatélites. Las estimaciones de la variabilidad genética del mtADN (F_{ST} = –0,016) y los microsatélites (F_{ST} = –0,005) indicaron que la variabilidad se dio dentro de cada muestra. Las comparaciones con los datos genéticos de muestras recolectadas anteriormente en las islas Heard y McDonald (n = 4) no fueron significativas para los grupos geográficos (mtADN ϕ_{CT} = –0,003; microsatélite ϕ_{CT} = 0,004), indicando que no hay diferenciación genética entre los peces de los caladeros situados en el sector occidental del Océano Índico.

Keywords: *Dissostichus eleginoides*, stock structure, mtDNA, microsatellites, gene flow, CCAMLR

Introduction

The Southern Ocean toothfish fishery has increased in economic value since the start of commercial exploitation, approximately 17 years ago, within the area of application of CCAMLR. Little is known about the stock structure or degree of stock separation of Patagonian toothfish (*Dissostichus eleginoides*, Smitt 1898) within each sector of the Southern Ocean. The amount of linkage via possible pelagic larval drift or by adult migration (Williams et al., 2002) is also unknown. This general lack of knowledge regarding stock structure of *D. eleginoides* adds to the difficulty of effectively managing this resource. The minimal information available on *D. eleginoides* population structure globally suggests the presence of at least three discrete *D. eleginoides* stocks across the Southern Ocean (Smith and McVeagh, 2000; Appleyard et al., 2002 and references within).

In a recent genetic study by the authors, no significant differentiation between Macquarie Island collections (sampled both temporally and spatially) was observed, nor was differentiation between temporally or spatially separated Heard Island and McDonald Islands (HIMI) collections observed (Appleyard et al., 2002). However, mitochondrial DNA (mtDNA) markers strongly supported *D. eleginoides* population sub-structuring within the greater Southern Ocean (i.e. among the various oceanic sectors) (F_{ST} = 0.445, P < 0.001) (Appleyard et al., 2002). The genetic heterogeneity observed between the sectors indicated restricted gene flow, with fish at each of the fishing locations comprising independent units (Appleyard et al., 2002). In the same study, significant overall microsatellite differentiation among the sectors was not apparent (F_{ST} = –0.009, P = 0.785), although some individual loci showed small but significant differentiation (Appleyard et al., 2002). In each

Table 1: Sample sizes and average lengths of *Dissostichus eleginoides* from the West Indian Ocean sector.

Locality	Abbreviation	Date of Sampling	Sample Size	Average Length (mm) (s.e)
Crozet Islands	Crozet	2 December 2001	60	732.20 (18.61)
Kerguelen Islands	Kerguelen	9 December 2001	30	675.67 (10.71)
Prince Edward and Marion1	PEMI1	Presumed 2001	36	unknown
Prince Edward and Marion2	PEMI2	25 March 2003	30	848.00 (43.51)

case, the genetic differentiation was attributable to differences among, rather than within, fishing locations. However, an earlier microsatellite analysis in collections of *D. eleginoides* from the Atlantic, Indian and Pacific Ocean sectors of the Southern Ocean by Smith and McVeagh (2000) revealed significant allelic heterogeneity among populations (F_{ST} = 0.028, P < 0.001), although this was not apparent from allozyme analysis (F_{ST} = 0.019, P = 0.080).

Movements of *D. eleginoides* on a more local level and, hence, finer-scale population structure are less defined (Williams et al., 2002). Tagging data suggest that generally *D. eleginoides* do not move more than 15 n miles from their tagging point and do not usually move between grounds within fishing localities (Williams et al., 2002). It has been proposed that deep-water basins might prevent *D. eleginoides* mixing (Appleyard et al., 2002). More recently, from over 7 000 tagged fish at HIMI and 1 200 recaptures, four tagged fish were caught at the Crozet Islands and seven tagged fish were recorded at the Kerguelen Islands (Williams et al., 2002 and unpublished data). These fish had been at liberty for 0.7 to 3.8 years, demonstrating that under some circumstances *D. eleginoides* (albeit rarely) are capable of wide movements across deep waters from one fishery to another (Williams et al., 2002).

Thus, there is an increased interest in investigating the more fine-scale population structure of toothfish within ocean basins and, in particular, in the West Indian Ocean sector of the Southern Ocean. A number of countries fish in both CCAMLR and national fishing grounds (i.e. Australia – HIMI, South Africa – Prince Edward and Marion Islands (PEMI) and France – Kerguelen Islands and Crozet Archipelago) within this sector (Williams et al., 2002). The HIMI and Kerguelen zones are contiguous with water no deeper than 600 m separating the two areas (Williams et al., 2002). In contrast, water deeper than 2 000 m separates the Crozet and PEMI zones (Williams et al., 2002).

It is largely unknown if stocks of *D. eleginoides* within the West Indian Ocean sector are distinct between the various shelves and banks separated by such deep water (Williams et al., 2002). As a step towards understanding the stock structure of *D. eleginoides* within the West Indian Ocean sector, the authors undertook mtDNA and microsatellite analyses on *D. eleginoides* from Kerguelen, Crozet and PEMI and compared these data with previously collected genetic data from collections of *D. eleginoides* from HIMI (Appleyard et al., 2002).

Materials and methods

Sampling of *D. eleginoides*

National observers on board commercial vessels fishing legally for *D. eleginoides* around the Kerguelen, Crozet and PEMI collected samples between 2001 and 2003 (Table 1). Exact fishing locations are not given here due to commercial confidentiality restrictions (see Figure 1).

Samples consisted of pieces of white muscle dissected from whole fish and stored in alcohol preserving solutions at –80°C until DNA extraction. Genomic DNA was extracted from all samples using a modified CTAB extraction procedure and 1/10 dilutions of genomic DNA for polymerase chain reactions (PCR).

Genetic markers

Restriction fragment length polymorphisms (RFLPs) were used in two mtDNA fragments to examine haplotype variation in the collections of *D. eleginoides* (as in Appleyard et al., 2002). The first region consisted of the variable control fragment (BCL, containing the D-loop) (Gaffney, pers. comm., 2000) and was amplified using primers 12SAR-H (Palumbi et al., 1991) and L16498 (following Smith et al., 2001, without the GC clamp). This 1.3 kb fragment was then digested with *BstNI*. The second mtDNA fragment, ND2, contains the NADH dehydrogenase subunit 2 gene and was amplified using the t-Met primer of Park et al. (1993) and the Mt-76 primer of Smith et al. (2001). This fragment (approximately 1.1 kb, as in Smith et al. (2001)) was digested with *NlaIII*.

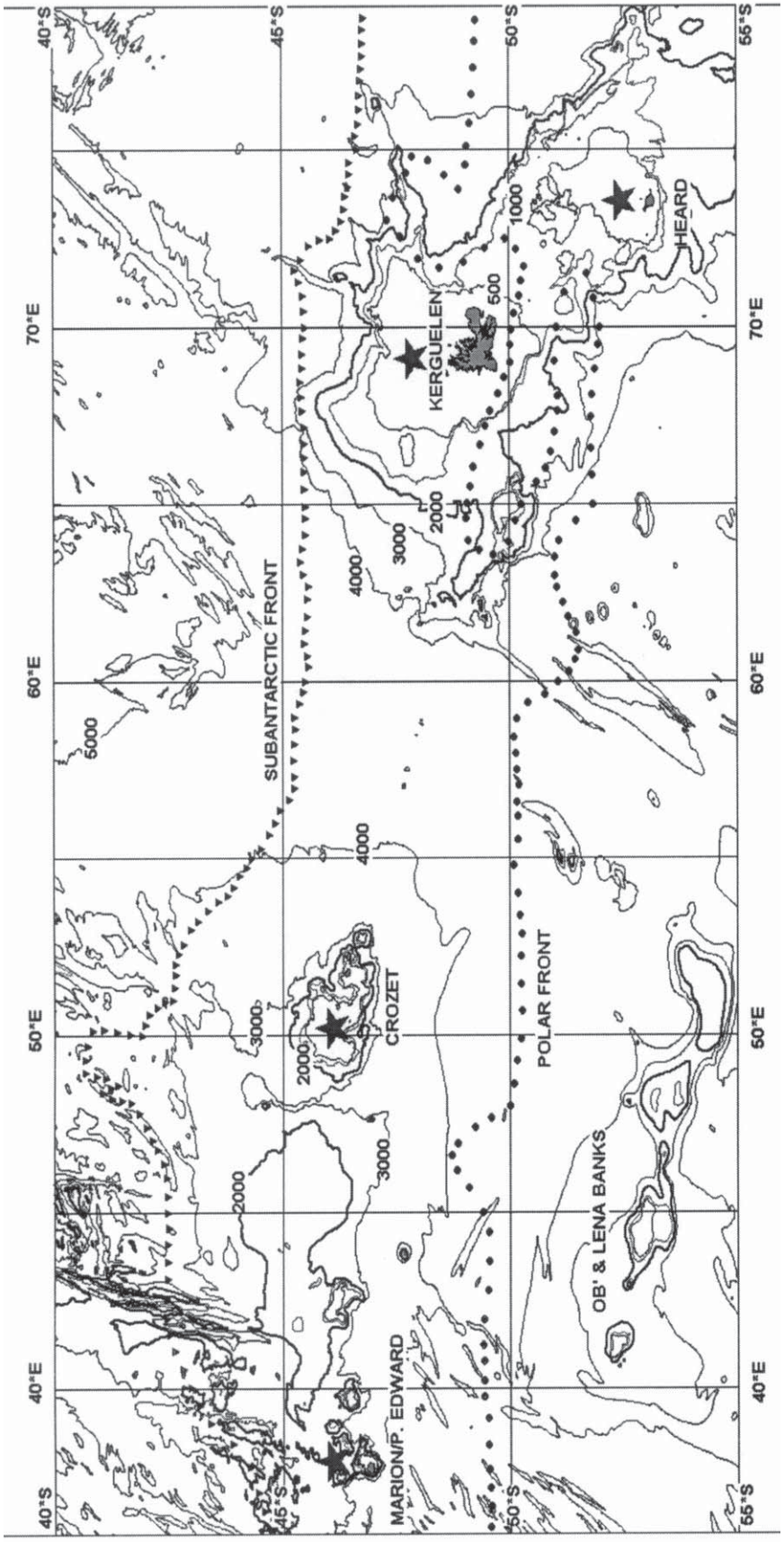

Figure 1: Map showing the relative positions of collections of *Dissostichus eleginoides* from Heard, Kerguelen, Crozet and Prince Edward and Marion Islands.

Seven DNA microsatellite loci were then used (*To5*, *To2*, *cmrDe30, cmrDe2, cmrDe13, cmrDe4* and *cmrDe9*) (Smith and Moon, unpublished; Reilly and Ward, 1999; Appleyard et al., 2002) to examine nuclear genetic variation within the collections.

PCR amplifications for both mtDNA and microsatellite loci were performed in a PE-Applied Biosystems 9600 thermocycler. Digested mtDNA fragments were electrophoresised in 2.5% agarose gels. Microsatellite PCR products were analysed on an ABI Prism 377 DNA sequencer (see Appleyard et al., 2002).

Statistical analysis of genetic data

MtDNA

For mtDNA, each fragment pattern from the two regions formed a composite haplotype. ARLEQUIN ver. 2.000 (Schneider et al., 2000) was used to estimate molecular diversity and haplotype frequencies (in each collection) from the combined data. Exact tests of mtDNA haplotype frequencies (to determine collection heterogeneity) were undertaken in ARLEQUIN ver. 2.000. Hierarchical Analysis of Molecular Variance (AMOVA) (see Excoffier et al., 1992) and *F*-statistics assessed subdivision among collections (in ARLEQUIN ver. 2.000).

Hierarchical AMOVA (resulting in ϕ_{ST} (and ϕ_{CT}), analogous to F_{ST} (and F_{CT})) was used to estimate the contribution of variance components (based on pairwise genetic/Euclidean distances) of gene frequencies in different population levels relative to the total variance (Excoffier et al., 1992; Taylor et al., 2000; see also Graves and McDowell, 2003). A lack of structure was inferred when the variance attributed to between-collection differences is not statistically significant.

F_{ST} values for all pairwise comparisons of collections and associated probabilities were also estimated in ARLEQUIN ver. 2.000. F_{ST} (an inbreeding coefficient) is an estimate of the proportion of genetic variation attributed to population differentiation. It ranges from little genetic differentiation among populations (0–0.05) to great genetic differentiation (>0.25) (Wright, 1978; Hartl, 1988; Graves and McDowell, 2003). Global tests of multi-locus differentiation were also undertaken in ARLEQUIN ver. 2.000.

Microsatellites

FSTAT ver. 2.9.3 (Goudet, 2001) was used to calculate microsatellite allele frequencies and number of alleles while Hardy-Weinberg Equilibrium (HWE) expectations within collections were assessed with ARLEQUIN ver. 2.000. GENEPOP ver. 3.2 (Raymond and Roussett, 2000) tested linkage disequilibrium between the microsatellite loci. Additionally, single-locus analyses and exact tests of microsatellite allele frequencies were undertaken in GENEPOP ver. 3.2 and ARLEQUIN ver. 2.000 respectively.

As in mtDNA analyses, multi-locus genetic differentiation and subdivision among collections, was assessed by hierarchical AMOVA and *F*-statistics in ARLEQUIN ver. 2.000.

For both mtDNA and microsatellites, the significance levels were adjusted in multiple tests using a sequential Bonferroni procedure (Rice, 1989).

Results

MtDNA

Digestion of the BCL fragment with *BstNI* resulted in five haplotypes (see haplotypes A, B, D, E and F in Appleyard et al., 2002). Amplification of the ND2 fragment and digestion with *NlaIII* resulted in two haplotypes (see haplotypes A and B in Appleyard et al., 2002). A total of six different composite haplotypes (Table 2) were identified among the 136 individuals from the three sampling locations. There was a single common haplotype (AA) in each of the collections.

Haplotype frequency differentiation among the four collections (three areas) was not evident (based on a global exact test, $P = 0.427$). Pairwise F_{ST} and exact tests demonstrated that the West Indian Ocean sector collections were not significantly different from each other (Table 3).

AMOVA analysis revealed a non-significant overall ϕ_{ST} value of –0.016 ($P = 0.884$). Genetic diversity was entirely due to within-collection variances; differentiation among collections was not evident. Hence, the null hypothesis of genetic homogeneity among the spatially separated collections could not be rejected.

Microsatellites

Genetic diversity statistics are summarised in Table 4. Numbers of alleles per locus per collection ranged from one (locus *To5*) to 21 (locus *cmrDe9*). After sequential Bonferroni correction per locus, five tests (out of a possible 25) were significantly different from Hardy-Weinberg expectations, with a lack of heterozygotes observed at *cmrDe2* (three instances) and *cmrDe9* (two instances). There was no significant linkage between any pairs of

Table 2: Composite haplotype frequencies at BCL and ND2 regions of mtDNA, sample sizes (*n*), number of haplotypes (*A*) and gene diversity (*h*) in *Dissostichus eleginoides* from the West Indian Ocean sector.

Haplotype	Crozet	Kerguelen	PEMI1	PEMI2
AA	0.814	0.807	0.800	0.807
AB	0.092	0.154	0.167	0.076
BA	0.019	0.000	0.000	0.000
DA	0.019	0.000	0.000	0.039
EA	0.037	0.000	0.000	0.039
FA	0.019	0.039	0.033	0.039
Parameter				
n	54	26	30	26
A	6	3	3	5
*h**	0.331 ± 0.080	0.335 ± 0.106	0.343 ± 0.097	0.351 ± 0.117

* Gene diversity, analogue for haplotype diversity according to Nei (1987), is the probability that two randomly chosen haplotypes are different in the same sample. Ranges from 0 (all individuals share a common haplotype) to 1 (all individuals have a unique haplotype).

Table 3: Pairwise estimates of differentiation for mtDNA haplotypes in collections of *Dissostichus eleginoides* from the West Indian Ocean sector. F_{ST} values below diagonal, (*P* values estimated after 10 100 Markov chain permutations are given in parentheses), *P* values for exact tests above diagonal (probabilities estimated after 10 100 Markov chain lengths).

Collection	Crozet	Kerguelen	PEMI1	PEMI2
Crozet	-	0.845	0.824	1.000
Kerguelen	–0.013 (0.707)	-	1.000	0.828
PEMI1	–0.006 (0.501)	–0.037 (0.999)	-	0.630
PEMI2	–0.026 (0.999)	–0.016 (0.788)	–0.007 (0.457)	-

Table 4: Summary of microsatellite variation in collections of *Dissostichus eleginoides* from the West Indian Ocean sector. N = total number of fish, N_{allele} = number of alleles, H_{obs} = observed heterozygosity, H_{exp} = expected heterozygosity.

Collection	Parameter	Loci						
		To5	*To2*	*cmrDe30*	*cmrDe2*	*cmrDe13*	*cmrDe4*	*cmrDe9*
Crozet	N	59	57	55	41	57	59	50
	N_{allele}	3	12	11	16	7	11	21
	H_{obs}	0.102	0.667	0.636	0.780*	0.684	0.814	0.740*
	H_{exp}	0.115	0.796	0.764	0.920	0.592	0.834	0.936
Kerguelen	N	29	28	29	23	30	30	13
	N_{allele}	1	10	9	14	6	11	14
	H_{obs}	-	0.643	0.793	0.739*	0.533	0.900	0.615*
	H_{exp}	-	0.810	0.699	0.932	0.564	0.853	0.966
PEMI1	N	36	32	35	27	21	33	15
	N_{allele}	1	9	9	15	4	10	14
	H_{obs}	-	0.750	0.571	0.741*	0.476	0.727	0.867
	H_{exp}	-	0.813	0.554	0.936	0.619	0.831	0.947
PEMI2	N	29	27	28	22	30	30	19
	N_{allele}	1	12	11	14	6	10	18
	H_{obs}	-	0.741	0.607	0.773	0.467	0.800	0.842
	H_{exp}	-	0.864	0.662	0.918	0.463	0.810	0.942
Mean	N	38.3	36	36.8	28.3	34.5	38	24.3
	N_{allele}	1.5	10.8	10	14.8	5.8	10.5	16.8
	H_{obs}	0.026	0.700	0.652	0.758	0.540	0.810	0.766
	H_{exp}	0.029	0.819	0.670	0.927	0.560	0.832	0.948

* Significant deviation from HWE after sequential Bonferroni correction per locus per collection.

Table 5: Pairwise estimates of differentiation for microsatellite loci in collections of *Dissostichus eleginoides* from the West Indian Ocean sector. F_{ST} values below diagonal (P values estimated after 10 100 Markov chain permutations are given in parentheses), P values for exact tests above diagonal (probabilities estimated after 10 100 Markov chain lengths).

Collection	Crozet	Kerguelen	PEMI1	PEMI2
Crozet	-	1.000	1.000	1.000
Kerguelen	–0.022 (1.000)	-	1.000	1.000
PEMI1	–0.018 (0.999)	–0.004 (0.644)	-	1.000
PEMI2	0.015 (0.002)	0.008 (0.076)	0.004 (0.179)	-

Table 6: Pairwise estimates of differentiation for mtDNA and microsatellite loci among collections of *Dissostichus eleginoides* from the West Indian Ocean sector and HIMI. MtDNA F_{ST} values below diagonal, microsatellite F_{ST} values above diagonal. Significant F_{ST} values (probabilities estimated after 10 100 permutations) after sequential Bonferroni correction are shown in bold.

Collection	Crozet	Kerguelen	PEMI1	PEMI2	H-B98	H-A98	H-C98	H-B99
Crozet	-	–0.022	–0.018	**0.015**	–0.041	0.004	–0.017	0.003
Kerguelen	–0.013	-	–0.004	0.008	–0.006	–0.031	–0.005	–0.008
PEMI1	–0.006	–0.037	-	0.005	0.003	–0.051	–0.003	–0.019
PEMI2	–0.026	–0.016	–0.007	-	–0.010	0.000	–0.001	0.010
H-B98	–0.007	0.012	0.022	–0.014	-	–0.074	–0.009	–0.029
H-A98	0.009	–0.024	–0.023	0.008	0.036	-	–0.035	–0.010
H-C98	–0.005	–0.009	–0.003	–0.011	0.003	0.004	-	–0.010
H-B99	0.005	–0.001	0.004	–0.005	0.022	0.009	–0.010	-

microsatellite loci (data not shown). Microsatellite allele frequencies for the Crozet, Kerguelen and PEMI locality 1 (PEMI1) and PEMI locality 2 (PEMI2) can be obtained from the authors on request.

Allele frequency differentiation among the four collections was not evident (based on a global exact test, $P = 1.000$). Pairwise exact tests of differentiation (considering microsatellite loci jointly) among the four collections showed no significant comparisons (Table 5). However, when microsatellite loci were considered individually, some instances of significant spatial differentiation after sequential Bonferroni correction were observed (*To2*: PEMI1 and PEMI2 ($P = 0.004$); *cmrDe30*: Crozet and PEMI2 ($P < 0.001$); *cmrDe4*: Crozet and PEMI2 ($P = 0.008$); Kerguelen and PEMI2 ($P = 0.005$)). The differences were, however, small and inconsistent across loci and across the spatially separated collections. Pairwise comparisons among the four collections based on F_{ST} values across the seven loci produced only one significant comparison between Crozet and PEMI2 ($F_{ST} = 0.015$, $P = 0.002$) (Table 5).

As with the mtDNA, AMOVA analysis among the four collections of *D. eleginoides* demonstrated no significant overall differentiation based on allele frequency variances ($\phi_{ST} = –0.005$, $P = 0.929$).

Collections of *D. eleginoides* from the West Indian Ocean and HIMI

The genetic data collected in the current study were compared with data obtained from the previous study of *D. eleginoides* from HIMI fishing locations. MtDNA haplotype frequencies and microsatellite allele frequencies from the four new collections were compared with existing data for collections H-B98, H-A98, H-C98 and H-B99 (HIMI) (see Appleyard et al., 2002).

Global exact tests for haplotype and allele frequency differentiation were not significant when all eight collections (Crozet, Kerguelen, PEMI1, PEMI2, H-B98, H-A98, H-C98 and H-B99) were considered jointly ($P = 0.451$ and $P = 1.000$ respectively). Pairwise exact tests (not shown) and F_{ST} estimates based on mtDNA haplotypes showed no significant values (after correction) among the eight collections with estimates ranging from –0.001 (H-B99 and Kerguelen, $P = 0.403$) to 0.036 (H-B98 and H-A98, $P = 0.015$) (Table 6). F_{ST} estimates based on microsatellite genotypes resulted in similar values ranging from a low of –0.008 (H-B99 and Kerguelen, $P = 0.963$) to a single significant high of 0.015 (Crozet and PEMI2, $P = 0.001$) (Table 6).

AMOVA analysis among the eight collections demonstrated that 99.7% of observed haplotype and 100% of observed genotype variances were attributed to within-collection differences ($P = 0.285$ and $P = 1.000$ respectively). When hierarchical AMOVAs based on likely biogeographical separation (group 1 = Kerguelen, H-B98, H-B99, H-A98 and H-C98; group 2 = Crozet, PEMI1 and PEMI2) were undertaken, non-significant ϕ_{CT} values of –0.003 ($P = 0.788$) (mtDNA) and 0.004 ($P = 0.400$) (microsatellites) were attributed to among-group differences. The majority of genetic variance observed among the West Indian Ocean sector collections was accounted for within samples. Thus, no significant genetic differences between these two bio-geographical areas in the West Indian Ocean sector of the Southern Ocean were apparent.

Discussion

Homogeneity of *D. eleginoides* in the West Indian Ocean sector

Composite mtDNA haplotype frequencies and microsatellite data among collections at Crozet, Kerguelen and PEMI revealed little evidence of population sub-structuring across this West Indian Ocean sector. The genetic data obtained in the current study were compared with previously collected data from HIMI fishing grounds (Appleyard et al., 2002). This extended genetic analysis also demonstrated no significant heterogeneity among *D. eleginoides* from these four fishing locations within the ocean basin.

From a molecular genetic viewpoint, there is no evidence that *D. eleginoides* populations of different fishing locations in the West Indian Ocean are distinct. Consequently, the null hypothesis of a single panmictic stock within this fishing area of the Southern Ocean cannot be rejected.

Similarly to this study, a lack of significant genetic heterogeneity was found in icefish (*Champsocephalus gunnari*) around the Kerguelen Plateau (Williams et al., 1994). Analysis of mtDNA of fish from several locations at HIMI (Heard Island shelf, Shell Bank, Discovery Bank) and the Kerguelen Island shelf did not support the hypothesis of the existence of separate populations of icefish (Williams et al., 1994). In contrast, on a more local scale, Reilly and Ward (1999) documented small genetic differences in collections of *D. eleginoides* from Macquarie Island. Their data were, however, preliminary and their sample sizes were smaller than those in the current study (an issue when dealing with highly variable markers like microsatellites).

As stated above, the null hypothesis for *D. eleginoides* in the West Indian Ocean sector could not be rejected. The caveat on this statement is that the failure to disprove the null hypothesis does not mean that stock structuring does not exist within this sector, only that it was not detected in the current study (see also Graves and McDowell, 2003 and references within). The inability to reject the null hypothesis may be related to the power of the genetic markers employed (Graves and McDowell, 2003) and the relatively small sample sizes examined (particularly for the hyper-variable microsatellite loci). Nonetheless, in a previous genetic study on *D. eleginoides* from HIMI and Macquarie Island, significant population structuring was detected with the same mtDNA markers and with broadly similar sample sizes (Appleyard et al., 2002).

Tagging and stock structure of *D. eleginoides* in the West Indian Ocean sector

Tagging data from HIMI suggest that *D. eleginoides* rarely move more than 15 n miles from their point of release and do not often move between grounds (Williams et al., 2002). However, the recent recapture of several *D. eleginoides* tagged at HIMI in neighbouring fishery areas suggests that some fish are capable of moving longer distances across deep-water basins (Williams et al., 2002). These fish may contribute to recruitment in the areas to which they have moved, therefore providing interchange of fish and genes among the areas (Williams et al., 2002).

Williams et al. (2002) indicated that although the numbers of fish tagged at HIMI and recaptured outside the HIMI fishery are low, and no recaptures have yet been found at PEMI, the limited tag data suggest that more fish than previously assumed are moving among locations. Further to this, Hartl and Clark (1989) state that genetic identity (homogeneity) can be maintained with limited exchange as relatively small amounts of gene flow can counteract the effects of genetic drift or selection.

Alternatively, if the *D. eleginoides* populations at the different fishing locations had only recently become isolated so that genetic differences had not yet accumulated (Graves and McDowell, 2003), similarly, the null hypothesis of genetic homogeneity could not be rejected.

The question remains as to how genetic homogeneity is maintained in the West Indian Ocean basin when there is genetic heterogeneity between ocean basins. In the previous genetic study on more global populations of *D. eleginoides* (Appleyard

et al., 2002), it was assumed that apparent homogeneity among grounds (separated by 40–200 n miles) within fishing locations (within ocean basins) was maintained by gene flow in young life stages (pelagic eggs and larval drift). Genetic homogeneity across fishing locations within the West Indian Ocean basin may also be provided by the migration of adult fish. In comparison, the striking genetic heterogeneity observed for mtDNA between fishing locations in different ocean basins of the Southern Ocean suggests that gene flow between these geographically isolated locations is likely to be very low (Appleyard et al., 2002).

This is in accord with the most recent tagging information. Adult fish movements between Macquarie and HIMI (about 3 000 n miles apart, water depth down to 4 000 m) have not been recorded. Movements between the HIMI and Kerguelen fishing areas that share the Kerguelen Plateau (approximately 200 n miles, maximum intervening water depth no greater than 600 m) have been recorded (Williams et al., 2002). The observation, at Crozet Island, of fish tagged at HIMI (Williams et al., 2002) (the distance from the northern tip of the Kerguelen Plateau to fishing grounds at Crozet is approximately 500 n miles, with water depth to approximately 4 000 m, but possible presence of refuges) (Appleyard, pers. comm., 2003), while unexpected, shows that *D. eleginoides* can swim large distances. Perhaps distance (without refuges) is a more limiting factor to *D. eleginoides* migration than depth alone. Researchers have not yet captured tagged fish from HIMI at PEMI, approximately 1 400 n miles from HIMI by the most direct route (Williams et al., 2002). If gene flow among the West Indian Ocean *D. eleginoides* populations is assumed, and distance (and refuges, see below) is considered as being the limiting factor in wide-distance migrations, plausible models to explain the genetic homogeneity in this ocean basin could be modifications of the island or stepping-stone population models.

In the island model, all populations are linked by equal levels of gene flow with an equal number of migrants per generation (Slatkin, 1993) but Hellberg et al. (2002) state that this model is only appropriate when two populations are considered (or analysis is based on equally spaced 'islands'). The fishing grounds considered in the current study are not equally spaced and eight collections of *D. eleginoides* were considered. Therefore, an alternative to the island model may be the stepping-stone model.

Stepping-stone gene flow occurs when migration occurs between neighbouring populations so that populations that are more distant are linked via intermediate 'stepping stones' (Hellberg et al., 2002 and references within). Under this model, pairwise differentiation estimates are lower for geographically closer populations than those for more distant populations (see Wright, 1943; Slatkin, 1993; Hutchison and Templeton, 1999; Hellberg et al., 2002). Adjacent populations exchange the largest number of migrants. This may be the case in the current study, as researchers have observed tagged *D. eleginoides* from HIMI at the Crozet Islands. These islands are adjacent to the Kerguelen Plateau (HIMI and Kerguelen are considered to be part of this area).

Therefore a Mantel test was performed to test for association between geographic and genetic distance measures (i.e. test of spatial differentiation). The overall mtDNA results did not provide any evidence for an association between geographic location and F_{ST} estimates (r^2 = –0.0272, P = 0.916), while the microsatellite data from the eight collections showed a weak but non-significant association (r^2 = 0.285, P = 0.065). The smallest individual pairwise F_{ST} value for the microsatellites was between HIMI and Kerguelen collections of *D. eleginoides*, while one of the largest values was between a collection from HIMI and one from PEMI (however, the F_{ST} estimate between a collection from Crozet and one from PEMI was also significant). Thus this data gives no clear support for the stepping-stone model.

If distance is considered to be a barrier to extended movements of *D. eleginoides*, it should be noted that none of the individual basins >2 000 m deep that separate the island groups between HIMI and PEMI is wider than 500 n miles. There are also extensive areas of relatively shallow water between Crozet and PEMI, so that the maximum distance between them of water deeper than 2 000 m is only 60 n miles. *D. eleginoides* refuges therefore exist within much of this region. In contrast, the area between Macquarie Island and HIMI is covered by water deeper than 4 000 m with no known refuges. This could account for the significant and striking lack of gene flow between *D. eleginoides* populations at Macquarie and HIMI (see Appleyard et al., 2002).

Conclusion

The confirmation of movements of *D. eleginoides* based on tagging data (Williams et al., 2002) coupled with the current genetic findings could have implications for the future management of *D. eleginoides* fisheries within this sector. At present, fisheries for *D. eleginoides* in the Crozet, Kerguelen

and PEMI areas are assessed and managed independently by various national governments (Williams et al., 2002).

In the current study, *D. eleginoides* sampled from HIMI, Kerguelen, Crozet and Prince Edward and Marion Island areas were not genetically different; hence, managers need to consider the possibility of managing *D. eleginoides* stocks across national boundaries and isolated submarine features. The genetic data should be used with available fish-tagging information and other assessment data to provide a background to an informed and sustainable management method for *D. eleginoides* in the West Indian Ocean sector.

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