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The two giant sister species of the Southern Ocean, *Dissostichus eleginoides* and *Dissostichus mawsoni*, differ in karyotype and chromosomal pattern of ribosomal RNA genes

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Abstract The two giant notothenioid species, the Patagonian toothfish *Dissostichus eleginoides* and the Antarctic toothfish *D. mawsoni*, are important components of the Antarctic ichthyofauna and heavily exploited commercially. They have similar appearance and size, both are piscivorous and bentho-pelagic, but differ in their geographic distribution and absence/presence of the antifreeze trait. We karyotyped these two sister species by analyzing specimens collected from multiple Antarctic and sub-Antarctic sites. Both species have a diploid number of 48, but differ in karyotypic formula, (2m + 2sm + 44a) for *D. eleginoides* and (2m + 4sm + 42a) for *D. mawsoni*, due to an extra pair of submetacentric chromosomes in the latter. Chromosomal fluorescence in situ hybridization with rDNA probes revealed unexpected species-specific organization of rRNA genes; *D. mawsoni* possesses two rDNA loci (versus one locus in *D. eleginoides*), with the second locus mapping to its additional submetacentric chromosome. The additional rRNA genes in *D. mawsoni* may be a cold-adaptive compensatory mechanism for growth and development of this large species in freezing seawater.

Keywords Antarctica · Chromosomes · *Dissostichus* · Ribosomal genes · Toothfish

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Introduction

The two species of toothfish, the Patagonian toothfish, *Dissostichus eleginoides* Smitt 1898, and the Antarctic toothfish, *D. mawsoni* Norman 1937, are important components of the Southern Ocean ichthyofauna (Gon and Heemstra 1990). They are similar in appearance, body size (~100 kg), piscivory and bentho-pelagic life style (Eastman and DeVries 2000; Horn 2002). Both species are usually caught between 200 and 2,000 m depth (Hanchet et al. 2003), but differ in geographic distribution. *D. eleginoides* is largely subantarctic and occurs in the Ross Sea, along the Antarctic Peninsula, around subantarctic islands in the Atlantic and Indian sectors of the Southern Ocean, and on the continental shelf off the coasts of Uruguay, Argentina and Chile (Brickle et al. 2005). Conversely, *D. mawsoni* occurs around mainland Antarctica, generally south of 60°S (DeWitt et al. 1990). The distribution of the two species overlaps north of the Ross Sea, in CCAMLR (Commission for the Conservation of Antarctic Marine Living Resources) Subarea 88.1 (Horn 2002) and on BANZARE Bank, i.e. the southern part of the Kerguelen Plateau in CCAMLR Division 58.4.3b (CCAMLR 2006). Confusion over species identity in some areas has sometimes been noticed, mainly off the

South Orkney/Antarctic Peninsula and the Southern Kerguelen Plateau (Fischer and Hureau 1985; Gon and Heemstra 1990).

The two *Dissostichus* species are the major fish resource exploited in the Southern Ocean by international longline and trawl fisheries, with significant international management efforts under CCAMLR jurisdiction (Eastman and DeVries 2000; Hanchet et al. 2003; Duhamel et al. 2005). Recent escalation in commercial fishing ventures, directed specifically towards the Antarctic toothfish, has increased concerns over the sustainability of the *D. mawsoni* fishery, due to the scant ecological and life history information available on this species (Parker et al. 2002; Hanchet et al. 2003).

Physiologically, the two species differ notably in their capability to live at subzero temperatures. *D. mawsoni* has a large family of antifreeze glycoprotein (AFGP) genes and produces high levels of circulatory AFGPs that prevent the freezing of its body fluids (Chen et al. 1997; DeVries and Cheng 2005). In contrast, *D. eleginoides* has barely detectable AFGP sequences in its DNA, and no antifreeze activity in its blood (Cheng and Detrich 2006). This distinct difference is consistent with the separate geographic distribution of the two toothfish in freezing versus non-freezing habitats. The evolutionary history of these two species in disparate thermal environments suggests other significant molecular and biological differences are likely to exist, despite their close phylogenetic kinship.

Taxonomically, the genus *Dissostichus* belongs to Nototheniidae, the most diversified family of the suborder Notothenioidei, and it is presently included in the subfamily Pleuragrammatinae (Balushkin 2000) together with the other pelagic genera *Pleuragramma*, *Aethotaxis* and *Gvozdarus*. According to phylogenetic analyses, *Dissostichus* is a basal notothenid lineage. Using penalized likelihood analysis combined with fossil calibration, the age of most recent common ancestor of the two *Dissostichus* species was estimated to be about 14.5 My (Near 2004).

Despite substantial scientific attention to the toothfish related to biodiversity conservation, fishery management (Parker et al. 2002; Hanchet et al. 2003) and molecular analyses of the antifreeze trait (Chen et al. 1997; Cheng and Detrich 2006), cytogenetic analyses of the two species are presently scanty. On the basis of a single juvenile caught off the Kerguelen Islands, Dousseau De Bazignan and Ozouf-Costaz (1985) provided preliminary data on the diploid number (48) and karyotypic formula (44a + 2sm + 2m) of *D. eleginoides*. For *D. mawsoni*, the only available karyotypic information is based on the analysis of a single female from the Weddell Sea (Ozouf-Costaz et al. 1991). The identification of the homologous chromosome pairs in this specimen was difficult due to the poor quality of the sample, but the diploid number and karyotypic formula could be determined and they are identical to those of the *D. eleginoides* (Ozouf-Costaz et al. 1991).

In the present work we define the specific chromosome set of each of the two toothfish species by using conventional karyotyping and molecular cytogenetics and provide for the first time an accurate description of the basic chromosomal features of these two important fish species. This was accomplished by examining the chromosome set of several specimens of both sexes, collected from multiple Antarctic and sub-Antarctic locations. A more in-depth characterization was obtained through cytogenetic mapping of ribosomal genes by fluorescence in situ hybridization (FISH), which revealed unexpected species-specific patterns in these two sister taxa.

In the broader context of comparative genomics, the cytogenetic data presented here provide the necessary basis for approaching further analyses of the genomes of these two important species through the visualization of gene loci at the chromosomal level. The use of specific DNA or gene sequences as probes in chromosomal FISH is a powerful tool that provides spatial landmarks and linkage information in a genome that can help resolve positional ambiguities in whole genome sequence assemblies, as well as facilitate the elucidation of gene family and genome organizations and evolution (Cheung et al. 2001; Jaillon et al. 2004).

Materials and methods

Animal sampling and chromosome preparation

Specimens of *D. eleginoides* were collected in the Indian and Atlantic sectors of the Southern Ocean. The specimens from the Indian sector (four females, three males and one undetermined juvenile) were caught by use of bottom trawls during the "Thirst" Cruise 1993 aboard the Australian R/V *Aurora Australis* (around 51°/52°S, 72°/75°E). The specimens from the Atlantic sector (six females) were collected by traps during the "ICEFISH" Cruise 2004 aboard the United States R/V *Nathaniel B. Palmer* (at 54°S, 55°W approximately).

Specimens of *D. mawsoni*, two males and two females, were collected in McMurdo Sound (78°S, 167°E) by winched cable and hook through large holes drilled through sea ice. Other specimens were caught by bottom trawls during the Western Ross Sea Voyage

2004 aboard the New Zealand R/V *Tangaroa*, one female in the Ross Sea (at 72°S, 173°E approximately) and two females around the Balleny Islands (67°S, 164°E). Six specimens (females) were caught by gill nets at the French Station Dumont D'Urville (Adélie Land) (64°S, 140°E).

Specimens were maintained in tanks or aquaria supplied with fresh, aerated seawater at local ambient temperature aboard the scientific vessels or at the Antarctic science stations. Specimens were injected with colchicine and mitotic cells were obtained from head kidney and spleen, following the method of Doussau de Bazignan and Ozouf-Costaz (1985) with slight modifications. Fixed cells were stored at −20°C for further analyses.

Karyotyping

Chromosome spreads on microscope slides were treated for conventional karyotyping and chromosome banding according to current protocols for notothenioid fishes (Ozouf-Costaz et al. 1997). Characterization of chromosomal morphology followed the standard nomenclature according to centromeric position and arm ratio (Levan et al. 1964; Klinkhardt et al. 1995), as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a). Metacentric and submetacentric chromosomes are bi-armed, whereas the acrocentric ones are one-armed. In the bi-armed elements the long arm is referred to as q (queue) and the short as p (petit), according to the International system for human cytogenetic nomenclature (Mitelman 1995). The total number of chromosome arms in a karyotype constitutes the fundamental number (FN). The chromosomes were arranged in the karyotypes according to morphology and size.

The karyotypes of the two *Dissostichus* species were established by examination of specimens collected from each of the different geographic areas. Multiple DAPI-stained metaphases from each specimen were analyzed with an Olympus BX61 equipped with a Sensys (Photometrics) CCD camera for digital imaging. Micrographs were processed either by the use of Genus Software (Applied Imaging) or by application of Adobe Photoshop and Corel Photopaint image analysis software.

Ribosomal DNA probes

28S rDNA probe

A partial sequence (762 bp) from the major ribosomal RNA genes of *D. mawsoni* was derived by PCR amplification of genomic DNA. On the basis of *Notothenia coriiceps* 28S ribosomal DNA partial sequences (GenBank accession numbers AY141673 and AY141674), a pair of primers, Noto_28S_C1 For (5′-CTA ACC AGG ATT CCC TCA GTA GCG G-3′) and Noto_28S_D3 Rev (5′-CAG GCA TAG TTC ACC ATC TTT CGG GTC-3′) were designed for amplification. The PCR product was cloned in the pGEM-T Easy vector (Promega) and sequenced with BigDye v.3 chemistry (Applied Biosystems). The sequence of clone 28S_C1D3 has been deposited under GenBank accession number AY926497. The 28S_C1D3 clone was used as a probe for FISH after nick translation labeling with biotin−16-dUTP (Roche Diagnostics) according to standard procedures.

5S rDNA probe

The DNA fragment used as probe for locating the 5S ribosomal genes is a partial sequence (86 bp) from the highly conserved 5S rRNA coding region. It was obtained by PCR amplification of *D. mawsoni* genomic DNA using the primers: CB-5S-R1 For (5′-CAC CTG GTA TTC CCA GGC-3′) and CB-5S-F2 Rev (5′-TAC GCC CGA TCT CGT CCG ATC-3′). The PCR product was sequenced in an automated DNA sequencer (Applied Biosystems). The sequence has been deposited under GenBank accession number DQ478734. The probe was labeled with digoxigenin-11-dUTP (Roche Diagnostics) by nick translation according to standard procedures.

Fluorescence in situ hybridization

The labeled probes were purified by ethanol precipitation and dissolved individually or together in the hybridization buffer (50% formamide/2 × SSC, 40 mM KH_2PO_4, 10% dextran sulfate) to yield final concentrations of 10 ng/µl (28S rDNA) and 20 ng/µl (5S rDNA).

One-color FISH and two-color FISH were performed as described previously (Mazzei et al. 2004). Briefly, the chromosomes were denatured by heating at 70°C for 1 min in 70% (v/v) formamide/2 × SSC (pH 7), dehydrated in a cold ethanol series, and air-dried. The probes were applied to chromosomal spreads (20 µl per slide) and incubated overnight in a moist chamber at 37°C. High-stringency post-hybridization washing was performed in 2 × SSC at 72°C (5 min) followed by 2 min in PBD buffer (4 × SSC, 0.07% Tween20) at room temperature. Hybridized 28S rDNA probe was detected by incubation of chromosomal spreads with streptavidin-FITC (MP Biomedicals), and 5S rDNA probe by rhodamine-anti-digoxigenin antibody

(Roche Diagnostics). Fluorescence signals of two-color FISH were visualized by simultaneous co-application of the two detection reagents. The chromosomes were counterstained in 0.3 μg/ml DAPI/2 × SSC and mounted in a standard anti-fade solution (Vector).

Chromosomal spreads were analyzed using a Zeiss Axiophot fluorescence microscope, and fluorescence signals were captured by use of a cooled CCD camera and processed with the software Genus for animal chromosomes (Applied Imaging).

Results

The karyotypes

Examination of multiple metaphase plates from the various specimens indicated that the two toothfish have a diploid number of 48 but differ in their FN, 52 for *D. eleginoides* versus 54 for *D. mawsoni*.

The karyotype of *D. eleginoides* is shown in Fig. 1a. Among the 24 chromosome pairs comprising the set, pair 1 is assigned to the smallest elements, which are metacentric and easily recognizable. Chromosome pair 2 is also recognizable in all the metaphase spreads as being the only pair of submetacentric chromosomes. Pair 2 shows a certain degree of heteromorphism in morphology in terms of length variability of the p (short) arms. This size polymorphism of the homologues of pair 2 does not occur in all specimens, such that homomorphic and heteromorphic combinations are observed in specimens regardless of their geographic location. All the other elements (44) of the karyotype are acrocentric chromosomes in decreasing size (Fig. 1a). The karyotypic formula for *D. eleginoides* is 2m + 2sm + 44a. No sex-related karyotypic differences were observed.

Similar to *D. eleginoides*, all specimens of *D. mawsoni* presented 48 chromosomes, arranged in 24 pairs as shown in the karyotype (Fig. 1b). However, the FN (54) and the karyotypic formula (2m + 4sm + 42a) are different, due to the presence of an additional pair of submetacentric chromosomes, and 42 instead of 44 acrocentric chromosomes. The metacentric elements of pair 1 are the smallest of the complement and likely corresponding to chromosome pair 1 of *D. eleginoides*. The submetacentric chromosomes comprising pair 2 appear very similar in morphology to the corresponding chromosomes of pair 2 in *D. eleginoides*, including frequent heteromorphism of the p arm size. The additional pair of submetacentric chromosomes in *D. mawsoni* (pair 3) does not correspond morphologically to any element in the karyotype of *D. eleginoides*. Similarly to pair 2, the two submetacentric homologs

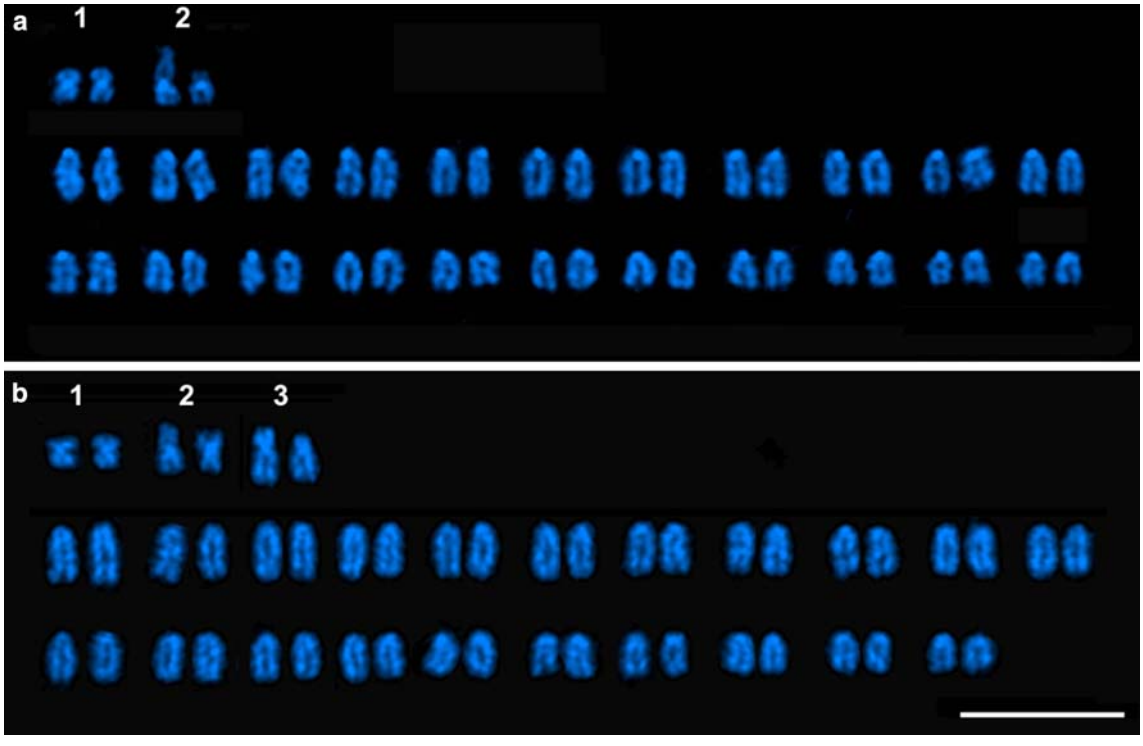

Fig. 1 The karyotypes of the Patagonian toothfish *Dissostichus eleginoides* (**a**) and Antarctic toothfish *D. mawsoni* (**b**). DAPI staining. *Bar* = 10 μm

comprising pair 3 in *D. mawsoni* are usually heteromorphic due to differences in size between their p arms. No sex-related karyotypic differences were observed.

Chromosomal location of the ribosomal RNA genes

In *D. eleginoides* the hybridization signal of the major ribosomal RNA gene probe 28S_C1D3 was localized on chromosome pair 2 (Fig. 2a). In all the specimens the clusters of major ribosomal genes occupy the entire p arms of the homologs regardless of heteromorphism or homomorphism of the arm length. The 5S ribosomal probe hybridizes to the same chromosome pairs and arm locations, indicating that the 5S ribosomal genes closely cluster with the major rRNA genes (Fig. 2b).

The FISH results for *D. mawsoni* unambiguously show that two pairs of chromosomes (pairs 2 and 3), instead of one, carry clusters of major ribosomal genes (Fig. 2c). In both pairs, the clusters of genes extend along the whole length of the chromosome p arms regardless of the heteromorphic length variations between specimens. The 5S ribosomal genes colocalized

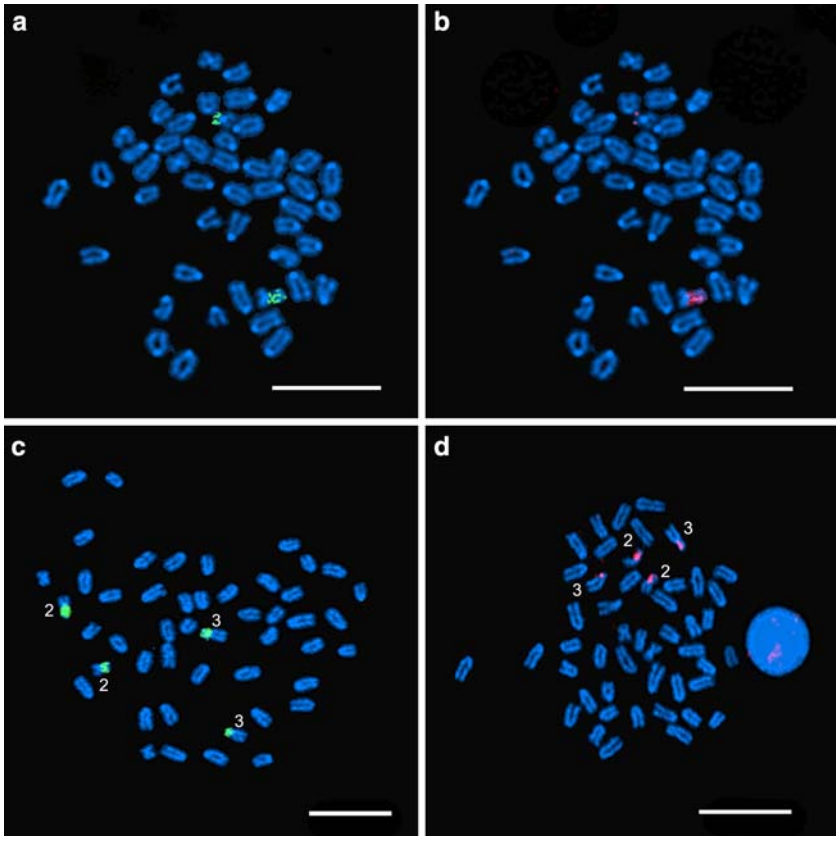

Fig. 2 Metaphase chromosomes from *Dissostichus eleginoides* (**a**, **b**) and *D. mawsoni* (**c**, **d**) after fluorescence in situ hybridization to map the location of major ribosomal genes (*green* signals in **a**, **c**) and 5S ribosomal genes (*red* signals in **b**, **d**). *Numbers* in **c** and **d** indicate the rDNAs bearing chromosomes of pairs 2 and 3 in *D. mawsoni*. *Bars* = 10 μm

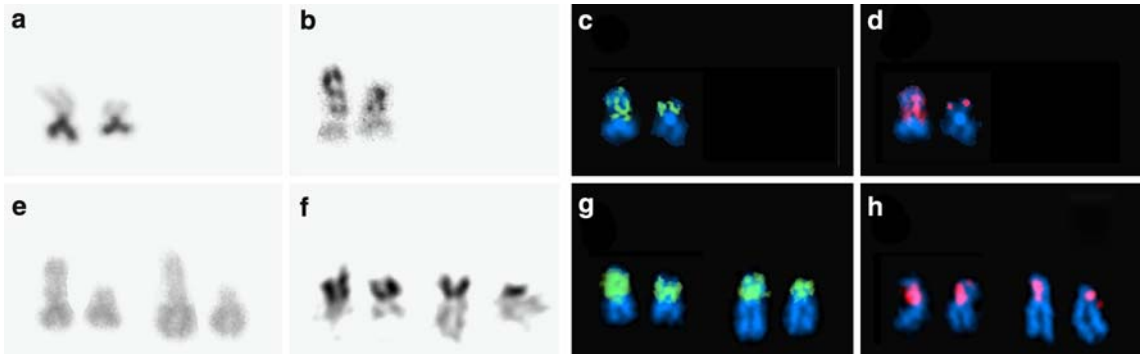

Fig. 3 Details of rDNAs bearing chromosome pairs, from different metaphases in *D. eleginoides* (**a**–**d**), and *D. mawsoni* (**e**–**h**) after banding and FISH. DAPI banding (**a**, **e**); Ag-NOR banding (**b**, **f**); FISH with the 28S rDNA probe (**c**, **g**); FISH with the 5S rDNA probe (**d**, **h**)

with the major rDNA clusters on the short (p) arms of the submetacentric chromosome pairs 2 and 3 (Fig. 2d).

The two rDNA-bearing chromosomal regions in *D. eleginoides* and the four in *D. mawsoni* share the common features of weak DAPI staining intensity (Fig. 3a, e), and comprise the Nucleolar Organizing Regions (NORs), as revealed by chromosomal banding with silver nitrate (Fig. 3b, f). Since the Ag-based techniques detect NORs by staining a complex of acidic protein associated with the fibrillar center of the nucleolus and nascent pre-RNA (Jordan 1987), the positivity to silver nitrate treatment indicates that ribosomal genes from all the chromosomal locations have been actively transcribed during the cell cycle.

Discussion

The toothfish karyotypes

Given the recognized monophyly of the Notothenioidei suborder (Near et al. 2004), cytogenetic evidence supports the hypothesis that the putative ancestor of the Notothenioidei had a karyotype close to that of the basal lineage Bovichtidae, consisting of 48 one-armed (acrocentric) chromosomes (Mazzei et al. 2006). By this reasoning, the karyotypes of the extant species of the more derived lineages should have arisen from rearrangements of the presumed ancestral set of 48 acrocentric chromosomes. The basal phylogenetic position of *Dissostichus* among Antarctic notothenioids (Near 2004) is consistent with our observed karyotypes of 48 chromosomes, the majority of which consists of acrocentric elements resembling the putative basal set of 48 one-armed chromosomes. The presence of two-armed chromosomes (pairs 1 and 2) in the karyotypes of the two *Dissostichus* species represents a derived karyotypic feature that could have arisen in their common ancestor by pericentric inversions in a pair of acrocentric chromosomes. Pericentric inversions lead to changes in chromosome formula but not in diploid number, and are among the most common modifications contributing to karyotypic rearrangement in fish, as well as in other vertebrates (White 1973; King 1993). This type of rearrangement, together with Robertsonian rearrangements (fusion or fission of chromosomes) and reciprocal translocations, have been recognized as responsible for some of the important changes leading to the present karyotypic diversity of Nototheniidae (Ozouf-Costaz et al. 1999; Pisano and Ozouf-Costaz 2003).

In both *Dissostichus* species, the metacentric chromosomes of pair 1 and the submetacentric elements of pair 2 are always recognizable in the metaphase plates by their similar shape, size and structure, supporting their common origin. The distinct difference between the karyotypes of the two *Dissostichus* species is the presence of an additional pair of bi-armed elements in *D. mawsoni* (chromosome pair 3) that has no morphological equivalent in the karyotype of *D. eleginoides*, leading to a different karyotypic formula. In addition, the FISH analysis demonstrated that ribosomal DNA occurs along the whole length of the p arms of chromosome pair 3, thus revealing a fundamental genomic/karyotypic difference between the two species: in *D. eleginoides* the multigenic units of rDNA are organized in a single locus on chromosome pair 2, whereas in *D. mawsoni* rDNA sequences are clustered in two separate loci on two separate chromosome pairs, namely pairs 2 and 3 (Figs. 2, 3).

The rDNA chromosomal loci, regardless of their number, share characteristics that indicate a common chromosomal organization. The nucleolar ribosomal genes, detected with the 28S rDNA probe, are located along the entire p heterochromatic arm, which also comprises the nucleolar organizer regions (NORs) as detected by $AgNO_3$ staining (Fig. 3). The extranucleolar rDNA 5S repeats, detected with the 5S rDNA probe, colocalize with the nucleolar ribosomal genes. Both *D. eleginoides* and *D. mawsoni* show size heteromorphism in the homologous chromosome bearing rDNA genes, indicating that various amounts of the ribosomal repeats can be present per haploid complement. The heteromorphic length variation of the chromosome regions bearing rDNA genes of the two *Dissostichus* species is a common organization found in all Antarctic notothenioids (Mazzei et al. 2004). The linked configuration of the 28S-5S ribosomal genes is also a common feature in cold-adapted notothenioids (Mazzei et al. 2006).

Among fishes in general, chromosomal organization of rRNA genes is highly variable. The 28S and 5S rRNA genes can occur separately or linked (reviewed in Martins and Galetti 2001; Martins 2006), and in single or multiple chromosomal locations (Sola et al. 2000; Almeida-Toledo et al. 2002) even within the same species (Almeida-Toledo et al. 2002; Boron et al. 2006). According to present information, a single chromosomal location of linked 5S and 28S rDNAs is the predominant condition among Antarctic notothenioids (Pisano and Ozouf-Costaz 2003; Mazzei et al. 2004). The dual rDNA loci in *D. mawsoni* is the only known occurrence beside the icefish *Pagetopsis macropterus* Boulenger, 1907, a member of the most derived notothenioid family Channichthyidae (Mazzei et al. 2004). Given the predominance of a single rDNA locus. In

notothenioids, we deduce that the extra locus in chromosome pair number 3 of *D. mawsoni* represents a locus gain, and that of the divergent *P. macropterus* a separate event. The extra rDNA locus in *D. mawsoni* extends along the entire p arms of chromosome pair 3 (Fig. 3e–h) while that of *P. macropterus* occupies a small region of a pair of submetacentric chromosomes (Mazzei et al. 2004), consistent with different mechanisms of locus evolution.

Evolution of dual rDNA loci in *D. mawsoni*

How did the additional rDNA chromosomal locus of *D. mawsoni* arise? A survey of the organizational variations of rDNA genes and proposed mechanisms may provide some insight. Both quantitative and qualitative variations of the ribosomal gene clusters occur between species as well as between individuals of the same species. Qualitative heterogeneity is mainly due to differences in the intergenic spacer regions (Long and Dawid 1980). Quantitative heterogeneity consists of variations in the extent of redundancy of rDNA copies, and in the number of ribosomal loci per genome (e.g., De Lucchini et al. 1997; Reed and Phillips 2000; Pedrosa-Harand et al. 2006). Interspecific and intraspecific variations in the number of rDNA loci have been described in several fish taxa (Zhuo et al. 1995; Rabova et al. 2001; Almeida-Toledo et al. 2002; Boron et al. 2006 among others). A number of mechanisms have been proposed to explain the changes in number and/or position of the rDNA chromosomal loci including translocations (Hayasaki et al. 2001), dispersion of rDNA repeats (Dubcovsky and Dvorak 1995; Schubert 1984; Raskina et al. 2004) and ectopic recombination (Pedrosa-Harand et al. 2006).

With regard to the two *Dissostichus species*, the morphological features of the two specific karyotypes suggest that the bi-armed chromosomes of pair 3 of *D. mawsoni* could have originated from a uni-armed element similar to one of the medium-sized acrocentric chromosomes in the karyotype of *D. eleginoides*. How this could have been accomplished would require detailed molecular analysis of the entire ribosomal locus. However, based on karyotypic evidence and general mechanisms of expansion of repeated genes or sequences, we suggest at least three possible mechanisms that could have led to the present double-rDNA chromosomal location in *D. mawsoni*.

1. In situ amplification of dispersed rDNA repeats—A first hypothesis supposes that in addition to the original rDNA locus on chromosome pair 2, a few dispersed repeats of ribosomal DNA were present in the antecedent of chromosome 3 of *D. mawsoni*. These underwent expansion through tandem duplication (Redi et al. 2001) in situ, possibly favored by their position close to the centromeric heterochromatin, giving rise to the additional recognizable rDNA site. Evidence of rDNA-related sequence elements dispersed throughout eukaryotic genomes has been documented (e.g., De Lucchini et al. 1997; Stupar et al. 2002 and reference herein; Martins 2006). It has also been suggested that the spread of such elements may occur frequently during evolution, with most events remaining undetected due to the divergence of the sequences (Falquet et al. 1997).
2. Translocation—A second and more parsimonious hypothesis is that a cluster of ribosomal genes could have been transferred from the original site of chromosome number 2 to the acrocentric predecessor of chromosomal pair number 3 through a translocation, since chromosomal translocation is a frequent structural rearrangement in fish karyotypes (White 1973; King 1993). Gene amplification and quantitative variations of heterochromatin could have preceded or followed such a translocation, contributing to the increase in the size of the rDNA bearing chromosomal regions.
3. Interchromosomal recombination—As a third possibility, we hypothesize that the presence of common repetitive peri-centromeric sequences could have enabled recombination to take place between the DNA region containing the rDNA site on chromosomes 2 and the centromeric region of the antecedent of chromosome 3, leading to a new rDNA locus on the latter. As in the previous hypothesis, gene amplification and quantitative variations of the associated heterochromatin could have contributed to further increase of the size of the rDNA bearing chromosome regions.

As an additional speculation, we cannot exclude that transposable elements could have been involved in the mechanisms leading to changes of rDNA loci in *Dissostichus* since several classes of transposable elements are abundant in fishes (Kidwell and Lisch 2000; Volff 2005) and possible relationships between transposable elements and rDNA have been recognized (e.g., Burke et al. 2003; Drouin 2000). In notothenioid fishes, Tc1-like DNA transposon sequence and two families of non-long terminal repeat retrotransposons, Rex 1 and Rex 3 have been found (Capriglione et al. 2000; Ozouf-Costaz et al. 2004), although no evidence of preferential accumulation of these transposable elements near the rDNA chromosomal sites has been confirmed.

Functional significance of the dual rDNA loci in *D. mawsoni*

Considering the very low frequency of dual rDNA loci among Antarctic notothenioids and the close phylogenetic relationship between the two toothfish, the distinct genomic difference in their rDNA loci was unexpected. Since the two species presumably diverged about 14 million years ago (Near 2004), the difference in rDNA loci may be associated with the evolutionary history of the two species in different thermal environments. Does the extra set of ribosomal genes in *D. mawsoni* serve a functional role in the freezing habitats of the Southern Ocean? Although the present structural analysis cannot provide an answer to this question, the dual rDNA loci in *D. mawsoni* quite clearly reflects a genetic gain (rather than a loss in *D. eleginoides*), conceivably brought on by natural selection. It is reasonable to suggest that the doubling of rDNA loci may increase ribosome production needed for extra protein (antifreeze proteins) synthesis in the toothfish living in freezing habitats, thus contributing to its survival. However, other Antarctic notothenioids (except for *P. macropterus*), living in similar cold environments and possessing similar high levels of antifreeze proteins, do not display dual rDNA chromosomal loci. We cannot exclude that quantitative increase of ribosomal genes did occur in other Antarctic notothenioids in their evolutionary past, but was perhaps not maintained, considering that no other Antarctic notothenioid species reaches the large body size of *D. mawsoni.* Low temperatures depress the rates of biochemical and physiological reactions (Hochachka and Somero 2002), and therefore the additional ribosome production by virtue of the extra rRNA genes may be a cold-adaptive compensatory mechanism for growth and development of this large species in freezing seawater. Consistent with this hypothesis is the observation that in *P. macropterus*, living in the same frigid waters but much smaller in body size, the rDNA in its additional locus is limited in content, occupying only a small chromosomal region (Mazzei et al. 2004).

Concluding remarks and perspectives

The difference in genomic patterns of ribosomal genes between *D. mawsoni* and *D. eleginoides* provides a new and unexpected discriminative character between the two toothfish. Such a difference can stimulate further molecular studies aiming at elaborating genomic markers based on these regions of nuclear DNA, with possible applications in toothfish stock assessment and fishery management (Smith et al. 2001).

In an evolutionary context, the data provided here can be a reference point for more extensive genomic studies of cold-adapted notothenioids. Genome mapping projects are completed or in progress for a number of fish species (reviewed in Phillips 2006), utilizing large DNA insert clones that can be used for chromosome mapping by FISH, so we can expect that the number of fish genomic studies will increase in the near future, including for Antarctic species. Such genomic studies should be approached by integrating sequence analysis and chromosomal analysis, according to cytogenomic methodology (Jaillon et al. 2004; Volff 2005; Pisano et al. 2006). Because *D. mawsoni* is a basal cold-adapted species among notothenioids, and genomic scale analyses of this species is currently in progress (Cheng and Detrich 2006), the Antarctic toothfish could be a promising species for a cytogenomic characterization.

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