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Chromosome change and the evolution in the Antarctic fish suborder Notothenioidei

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Abstract: The majority of species of the Antarctic coastal fish fauna is represented today by the perciform suborder Notothenioidei. The separation of basal lineages of notothenioids has been estimated to have occurred between 23 and 22 million years ago (m.y.a.), while a major diversification probably occurred 16–10 m.y.a. Cytogenetic approaches aim to study the genomic change that, at the cell level, accompanied the radiation of this group. The information available for 66 of 120–130 species makes possible the description of the main patterns of chromosome diversification within the suborder. Within some families (Channichthyidae, Artedidraconidae) the range of the chromosomal variability seems to be minimal whilst the high karyotypic diversity of Nototheniidae and Bathydraconidae is consistent with morphological and molecular data suggesting the paraphyletic nature of these two taxa. Molecular cytogenetics allows detailed chromosome characterization, including mapping of ribosomal genes and of telomeric sequences, thus providing information on processes of karyotypic rearrangement and direction of chromosomal change. Active process of genomic restructuring leads to intraspecific variability in several species, at different levels of chromosomal organization. The growing amount of information make it possible to use notothenioids as a model for testing hypotheses of evolutionary change in marine organisms, including chromosomal diversification.

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Introduction

The notothenioid fishes as a model for evolutionary studies in Antarctica

The perciform teleosts of the suborder Notothenioidei are a diversified fish group living in the coastal waters of the Southern Ocean. Most of them inhabit the shallow waters around the Antarctic continent where they dominate the fish fauna, in terms of both biomass and specific diversity and they successfully occupy a large variety of ecological niches (Andriashev 1987, Gon & Heemstra 1990, Kock 1992, Eastman 1993). Evolutionary biologists are interested in the Notothenioidei because of their unique morphological, physiological and biochemical features that make this suborder different from taxa in other large marine ecosystems (DeVries 1982, Eastman 1993, 1995, Clarke & Johnston 1996). Recently national and international research greatly improved knowledge of the biology and evolution of this group. The Network "Fishes of the Antarctic Ocean", launched in 1993 by the European Science Foundation (di Prisco & Clarke 1994) supported and facilitated exchanges and collaboration between scientists through workshops, visits, conferences, to encourage a multidisciplinary approach to Antarctic fish studies. The ICOTA Project (Ichtyologie côtière en Terre Adélie), is a multiyear international programme on Antarctic fish biology and ecology in which the specialist skills of researchers from different fields and countries are integrated in studies of the Antarctic coastal fish fauna (Hureau *et al.* 1998).

At present, key events in the adaptive evolution of notothenioids are being elucidated, such as the appearance of antifreeze glycoproteins (AFGPs), through molecular evidence of the origin of the AFGP gene, from an ancestral protease-encoding gene (Cheng & Chen 1999). Also Cocca *et al.* (1995) and di Prisco (1998) have postulated that the "white blood" condition of the Channichthyidae developed following the deletion of β-globin gene in these fishes.

Reasonable phylogenetic hypotheses, based on independent sets of morphological (Balushkin 1992), biochemical (Stam *et al.* 1998) and molecular data (Ritchie *et al.* 1997, Bargelloni & Lecointre 1998) have been proposed. Some areas of uncertainty still concern evidence of the ancestral stock from which the notothenioids were derived, intrafamilial relationships and the monophyly of the group (Lecointre *et al.* 1997).

Early evolution of Antarctic fish has been influenced by the tectonic and climatic events linked to the fragmentation of Gondwana. After the opening of the Drake Passage and the formation of a circumpolar hydrographic barrier, about 25–22 million years ago (m.y.a.), their diversification has

been facilitated by the thermal isolation of Antarctica, and by the particular ecological conditions of the surrounding Southern Ocean (Kennett 1977, Clarke 1990, Eastman 1993). Estimates of divergence time based on molecular data-sets indicate a separation of basal lineages of notothenioids between 23 and 22 m.y.a. while a major diversification probably occurred 16–10 m.y.a. At that time consistent sea ice formation, cooling water temperature and the expanding ice sheet all played an important role in creating the Antarctic ecological conditions to which notothenioid fish had to adapt. The rapid cooling of the Southern Ocean to subzero temperatures was a turning point in the evolutionary history of notothenioids and is correlated to their radiation (Eastman & Clarke 1998). The same authors also emphasize the characteristics of isolation of the Antarctic shelf and upper slope acting as an insular evolutionary site. In such an isolated habitat, the radiation of notothenioids and Antarctic liparids share common traits with the species flocks in ancient lakes. Therefore, the diversification of the notothenioids and the Antarctic liparids in the Southern Ocean are presently the only known examples of adaptive radiation in marine teleosts (Eastman & Clarke 1998).

The last 10 years have witnessed a considerable growth of biological information on the Notothenioidei. This large database and relevant knowledge especially gained from ecology, paleoceanography and geology, make it increasingly evident that this taxon represents a unique case in the evolution of marine organisms. The group can be used a model for testing hypotheses of evolutionary change in marine organisms, including chromosomal diversification.

Cytogenetics of notothenioids: significance for evolutionary studies

Cytogenetic studies can provide information related to the evolutionary history of notothenioids, independent from morphological, biochemical, behavioural and other characters that are used for comparative analysis between taxa. As with biochemical data, information on chromosomes can reveal differences and similarities that may not be obvious at the morphological level. The inherent appeal of cytogenetics is that, depending on the technology, it encompasses several levels of biological organization ranging from the morphological to the molecular. Chromosomes are usually studied as a morphological manifestation of the genome in terms of their microscopically visible size, shape, and number during mitosis, which provide species-specific characters for the karyotype. Banding studies reveal aspects of the general structural organization of chromatin along the lengths of individual chromosomes. Recent progress in molecular cytogenetics has allowed resolution of finer details of chromosome anatomy in terms of spatial arrangements, or presence/absence of particular kinds of sequences. In addition to species-specific information, cytogenetic data represent characters for phylogenetic analyses, especially if banding techniques and molecular cytogenetic techniques are used (Ozouf-Costaz *et al.* 1997).

One promising molecular cytogenetic method with broad application is fluorescence *in situ* hybridization (FISH). This technique has been widely used in human genetics to identify chromosomes and to locate genes on the chromosome arms (Trask 1991). Recent results using FISH have resolved the problem of species chromosome homology between many mammals and have identified the major translocations involved in the genome evolution of higher vertebrates, especially primates (Wienberg & Stanyon 1995). FISH can also provide valuable information in fishes (reviewed in Phillips & Reed 1996). For instance genomic specific sequences corresponding to a highly repetitive ribosomal genes (rDNAs) were obtained from Antarctic fish and then used as a probe to map the chromosome location of the 28S rDNA in notothenoid species (Ozouf-Costaz *et al.* 1996). The derived structural information has implications for general knowledge of the genome in teleosts and also provides information on change in position of the ribosomal cistrons during the diversification of the notothenioids. Moreover various molecular cytogenetic techniques are now available which permit the visualization of smaller DNA sequences directly on chromosomes or in interphase nuclei. These methods would allow the physical mapping of important genes in the diversification and adaptation of notothenioid species, such as globin or antifreeze protein genes. Such information would provide new understanding of how changes in genome organization and function are correlated with critical events in the evolution of this fish group.

Because of its relevance in phylogenetic and evolutionary studies, cytogenetics has been employed in research on Antarctic fish biology. Advances in this field, including discussions on the relevance and limits of karyological data for phylogenetic reconstruction, have been the subject of several recent reviews (Ozouf-Costaz *et al.* 1997, Prirodina 1997, Pisano *et al.* 1998). In this paper we report on the findings of recent studies undertaken as part of the ICOTA project, a joint venture of France and Italy. We focus on karyotypic diversification, direction of karyotypic change and aspects of intraspecific chromosomal variability.

Karyotypic diversification

If we look at the basic cytogenetic information which is number and shape of the chromosomes (karyotype) apart from a single work from a Brazilian team (Phan *et al.* 1986), all the available data are based on Antarctic studies carried out in the last 15 years by Russia, France and, more recently, by Italian teams (Pisano *et al.* 1998).

The main karyotypic features are known for 66 of 120–130 notothenioid species, representing all eight families of Notothenioidei living in Antarctic, sub-Antarctic and cold-temperate coastal waters (Table I).

The patterns of karyotypic diversification in the eight families can be summarized as follows:

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Table I. Main karyotypic features in notothenioid families.

	n	2*n*	NF	XY
Bovichtidae	3	48	48	–
Pseudaphritidae	1	48	52	–
Eleginopsidae	1	48	52	–
Nototheniidae	30	22–58	44–88	+
Harpagiferidae	2	48	50–54	–
Artedidraconidae	8	46	50–54	–
Bathydraconidae	8	20–48	40–58	+
Channichthyidae	13	47–48	52–62	+

n = number of studied species; 2*n* = diploid number, NF = fundamental number; XY = presence of sex-linked chromosomes.

In the Nototheniodei the most frequent diploid number is 48 (Prirodina 1994, Pisano *et al.* 1998).

With the exception of family Artedidraconidae, karyotypes of 48 chromosomes are present in species in all families, including both basal and derived. Since a diploid number of 48 chromosomes is also common in other perciform species, both Antarctic and non-Antarctic (Klinkhardt *et al.* 1995, Morescalchi *et al.* 1996), it is clear that effective comparative analysis in notothenioids cannot be based solely on this karyotypic feature (Ozouf-Costaz *et al.* 1997). However in two Antarctic taxa, Nototheniidae and Bathydraconidae, the range of the variability of the chromosomal parameters is very high, with chromosomal numbers ranging from 22 to 58 respectively and 20 to 48. Moreover various degrees of morphological diversification in both shape and size of the chromosomes are linked to change in their number, indicating active rearrangement events during the diversification within each of these two taxa. Figure 1 depicts metaphase chromosomes in *Cottoperca gobio* (a) and *Champsocephalus gunnari* (b) taken as representatives of two taxa separated by the greatest phylogenetic distance among Notothenioidei (family Bovichtidae and Channichthyidae). In both the species there are 48 chromosomes, with slight morphological differences. Figure 2 shows metaphase chromosomes in species taken as representatives of the high karyotypic diversification in the family Nototheniidae. *Trematomus hansoni* Boulenger (a) has 48 chromosomes, mostly acrocentric, *Trematomus pennellii* Regan (b) has a reduced number of chromosomes with some metacentric; *Notothenia angustata* Hutton (c) karyotypically very derived, shows a reduced diploid number (26) with 24 metacentric and only a pair of acrocentric chromosomes.

The high karyotypic diversity found in both the Nototheniidae and Bathydraconidae is consistent with molecular and morphological data suggesting the paraphyletic nature of these two notothenioid taxa and stressing the need for intrafamilial phylogenetic analyses (Bargelloni & Lecointre 1998).

Within other notothenioid families (Channichthyidae, Artedidraconidae) gross karyotypic change appears to have been minimal, thus making intrafamilial analysis difficult.

Moreover it has been stressed that some similar mechanisms of karyotype differentiation occurred separately in notothenioid families and genera, probably due to preferential sites of rearrangement, thus leading to similar chromosome numbers and formulae (Ozouf-Costaz *et al.* 1997, Prirodina 1997). Therefore conventional karyotypic characters (number and gross morphology) appear of limited use in comparative studies of Antarctic species, unless chromosomal banding and molecular cytogenetic methods are used (Ozouf-Costaz *et al.* 1997).

Direction of the chromosomal change

One critical point in any interpretation of chromosomal change in notothenioid fish, as well as in other fishes and vertebrates (White 1973, Klinkhardt *et al.* 1995, Sola *et al.* 1981), is the direction of chromosomal modifications leading to the morphology that we see in extant species.

The main patterns of karyotypic diversification, obtained from the analyses of the available information in notothenioids,

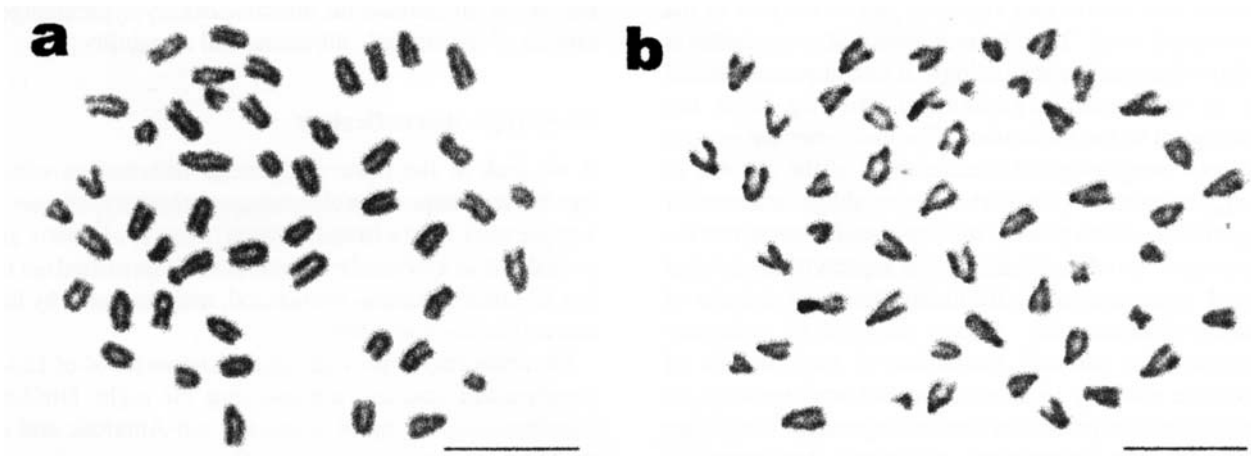

Fig. 1. Metaphase chromosomes in **a.** *Cottoperca gobio* (family Bovichtidae) and **b.** *Champsocephalus gunnari* (family Channichthyidae). (scale bar = 10 mm)

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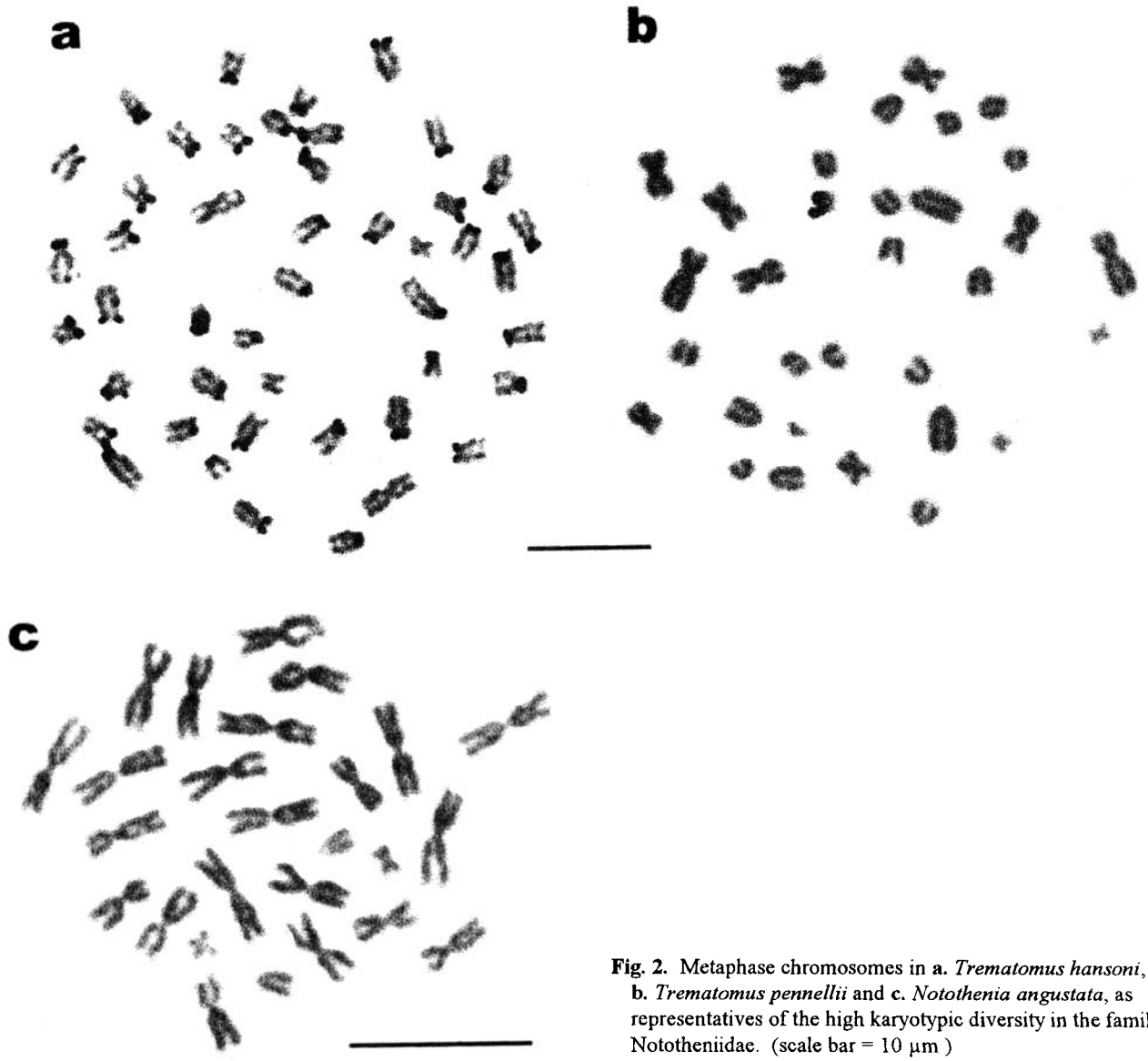

Fig. 2. Metaphase chromosomes in **a.** *Trematomus hansoni*, **b.** *Trematomus pennellii* and **c.** *Notothenia angustata*, as representatives of the high karyotypic diversity in the family Nototheniidae. (scale bar = 10 μm)

have been recently reviewed (Pisano *et al.* 1998, fig. 2) and compared with a recent phylogenetic hypothesis for the Notothenioidei (Lecointre *et al.* 1997). Assuming that the suborder is monophyletic, the result supports the idea that the ancestor of this lineage had a karyotype of 48 chromosomes, all acrocentric in shape. In fact such a karyotype has been found in three studied species of the family Bovichtidae, which represents the basic clade in the phylogeny of the suborder. Only one species of a phyletically derived taxon (*Psilodraco breviceps*, Bathydraconidae) has such a simple karyotype (Prirodina 1994).

Extant karyotypes could have evolved from the simple set of 48 acrocentic chromosomes, through chromosomal rearrangements similar to those observed in other vertebrates (White 1973). Among these, pericentric inversions, additions and amplifications of heterochromatin, fusion and fission of chromosomes have been hypothesized as the most important (Ozouf-Costaz *et al.* 1991, Morescalchi *et al.* 1992a, 1992b, Prirodina 1997, Pisano *et al.* 1998).

Progress in molecular cytogenetics allowed us to detect intrachromosomal telomeric remnants in chromosomes of some Antarctic fish species, providing experimental support for the hypothesis that centric fusion represents a major process of chromosomal change in notothenioids, leading from higher to lower chromosomal numbers. The process is described in the next paragraph.

From higher to lower chromosomal numbers

In order to obtain a more detailed characterization of chromosomes, the location of repetitive telomeric sequence (TTAGGG)n in a number of notothenioid species is currently a focus of our laboratories (Ozouf-Costaz *et al.* 1999a). Such repetitive DNA sequences are highly conserved in vertebrates

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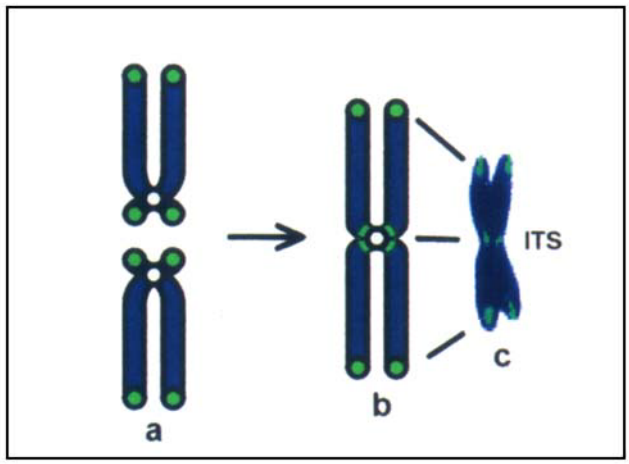

Fig. 3. Interstitial telomeric sequences (ITS) as evidence of Robertsonian rearrangements in karyotypic diversification of Notothenioidei. The metacentric chromosomes containing ITS signals (b, c) have originated through centric fusion of two acrocentric (a) chromosomes, without loss of telomeric sequences (in green). The chromosome (c) is taken from a metaphase spread of *Notothenia coriiceps* after fluorescence *in situ* hybridization with a telomeric probe.

and are located at the extreme termini of the chromosomes, where they are involved in maintaining chromosome stability and integrity (Zankian 1995). However sometimes the same sequences are found at non-telomeric positions along the chromosomes of mammals as well as in some fishes (Abuin *et al.* 1996) and they are therefore called Interstitial Telomeric Sequences (ITS). The non-telomeric location of telomeric sequences points to an intriguing evolutionary history. It can be related to the mechanisms of chromosomal rearrangement, especially end to end fusions, resulting in karyotype diversification (Slijepcevic 1998).

Among notothenioids, the species *Notothenia coriiceps* Richardson has a rearranged karyotype, made of 22 metacentric and submetacentric chromosomes (Ozouf-Costaz *et al.* 1999b). After *in situ* hybridization with a telomeric probe, all the chromosomes of *N. coriiceps* showed bright hybridization signals at the termini, as expected according to the telomeric functions. In addition, interstitial telomeric sites of the (TTAGGG)n sequence have been detected near the centromeres in some chromosome pairs (Fig. 3c). The location of telomeric sequences at paracentromeric positions may be considered as relicts of Robertsonian fusions i.e. the combination of two acrocentric chromosomes into a metacentric chromosome (Fig. 3a & b). This experimental evidence in *N. coriiceps* supports the hypothesis that chromosomal fusions plays a major role in the process of diploid number reduction, at least in some notothenioid lineages.

Change in ribosomal gene location

Fragments of 28S rDNA were extracted from the genomic DNA of channichthyid *Champsocephalus gunnari*, amplified by PCR and used as probe to locate ribosomal cistrons on the chromosomes of various species of the families of Bovichtidae, Nototheniidae and Channichthyidae (Ozouf-Costaz *et al.* 1999a). In the majority of the species studied a large quantity of rDNA genes are located in a single heterochromatic domain in a pair of chromosomes. Structural differences in the amount of rDNA per haploid set have been found in all the species which gives rise to an intraspecific size polymorphism of the two homologous chromosomes bearing such large DNA regions (Ozouf-Costaz *et al.* 1996).

Figure 4 depicts pairs of homologous chromosomes bearing the rDNA genes in three distantly related notothenioid species, *Bovichtus variegatus*, *Channichthys rhinoceratus* and *Notothenia coriiceps*. In *B. variegatus* rDNAs are located at paracentromeric position in a pair of medium sized acrocentric chromosomes (Fig. 4a). In most of the studied species rDNA has been mapped along the entire arm of a pair of submetacentric chromosomes, as here shown for *C. rhinoceratus* (Fig. 4b). In the karyotypically derived notothenid *N. coriiceps*, rDNA forms a large intercalar band along one arm of a large submetacentric chromosome (Fig. 4c). Relating the chromosomal location of rDNA genes of distantly related species to the phylogenetic position of these taxa corroborated some hypotheses concerning the direction and polarity of the rearrangements of these chromosomes (Ozouf-Costaz *et al.* 1997, Pisano *et al.* 1998).

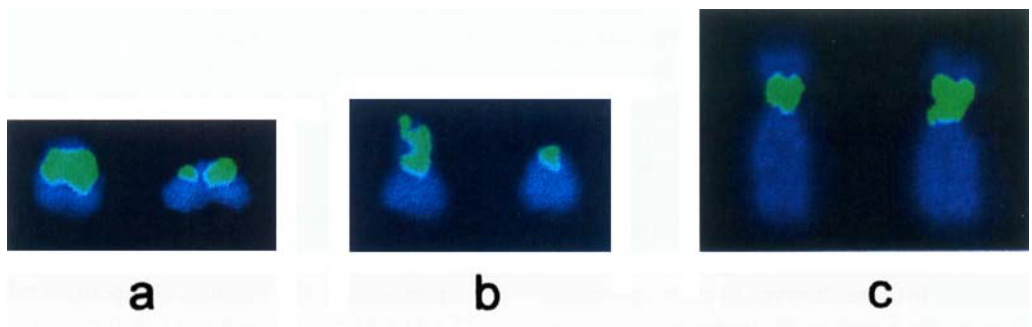

Fig. 4. Location of ribosomal genes on the homologous chromosomes in **a.** *Bovichtus variegatus*, **b.** *Channichthys rhinoceratus* and **c.** *Notothenia coriiceps* (after fluorescence *in situ* hybridization with a 28S DNA biotynilated probe, avidine-FITC detection).

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Intraspecific chromosomal variability

Intraspecific chromosomal polymorphism has probably been understimated in this suborder. For several species, as cytogenetic data increases and new methods are applied, more intraspecific polymorphism is detected at various levels of chromosomal organization. We discuss below the following forms of chromosomal polymorphism, a) presence or absence of heteromorphic sex-linked chromosomes and b) changes in chromosome macrostructure. For a more complete information refer to Ozouf-Costaz *et al.* (1999b).

a) Presence or absence of heteromorphic sex-linked chromosomes

Several species of channichthyids and nototheniids possess heteromorphic sex-linked chromosomes, with male heterogamety (Ozouf-Costaz *et al.* 1991, Morescalchi *et al.* 1992a, 1992b). In most cases a multiple Y and an odd (2n-1) number of chromosomes is present in the males. The Y chromosome in the metaphase chromosomes of the channichthyid *Chionodraco hamatus* Loennberg (Fig. 5) is an example of such sex-linked heteromorphism. The unpaired Y chromosome probably originated from the fusion between an autosome and an early acrocentric Y chromosome morphologically similar to the X. The multiple Y chromosome of different channichthyid species has different size and morphology. It is metacentric in some and submetacentric in others, depending on whether the primary supposed gonosome is joined with an acrocentric, or a submetacentric autosome (Fig. 6).

In one species of the family Bathydraconidae a different type of sex-linked heteromorphism has been described (Ozouf-Costaz *et al.* 1991). Since the rearrangement giving rise to sex-linked chromosomes seem different in the different taxa, even within the same genus (Morescalchi *et al.* 1992a, 1992b) this feature may have been independently evolved. Moreover, sex-linked chromosomal heteromorphism may have evolved recently and apparently is not yet fixed at the species level. This is evident from the observation that a minority of males in a given population do not possess heterochromosomes. Moreover in one species (*Trematomus hansoni*) frequencies differ among populations (Ozouf-Costaz *et al.* 1999b). The presence of the gonosome-like heterochromosomes has been considered a convergent apomorphic character in karyological differentiation of Notothenioidei (Ozouf-Costaz *et al.* 1999b).

Although sex chromosomes are present in non-Antarctic perciform species, the frequency of sex-linked heterochromosomes is higher among Antarctic fishes (Klinkhardt *et al.* 1995). For this reason it has been considered an adaptation of sex-determining mechanisms to the permanently cold Antarctic waters, where thermal invariability prevents a temperature-dependent sex determination (Morescalchi 1992). However evidence of a possible sex-specific DNA sequence in the heteromorphic sex-linked chromosomes of Antarctic fishes could not be established (Capriglione *et al.* 1994).

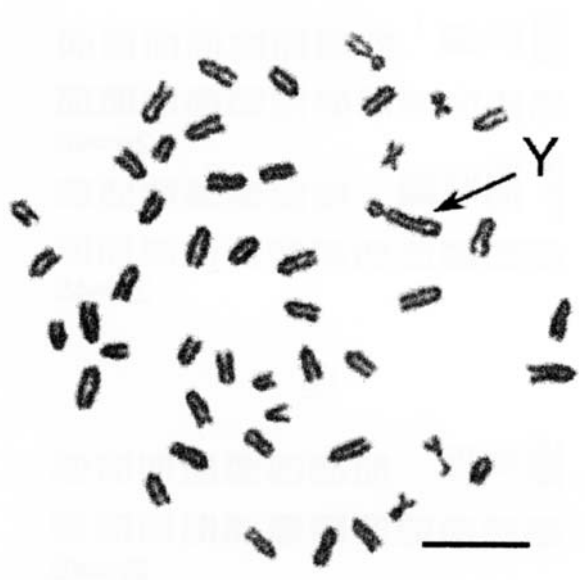

Fig. 5. Metaphase chromosomes of the channichthyid *Chionodraco hamatus* male ($2n = 47$). The arrow indicates the unpaired Y chromosome (Giemsa staining). (scale bar = 10 μm)

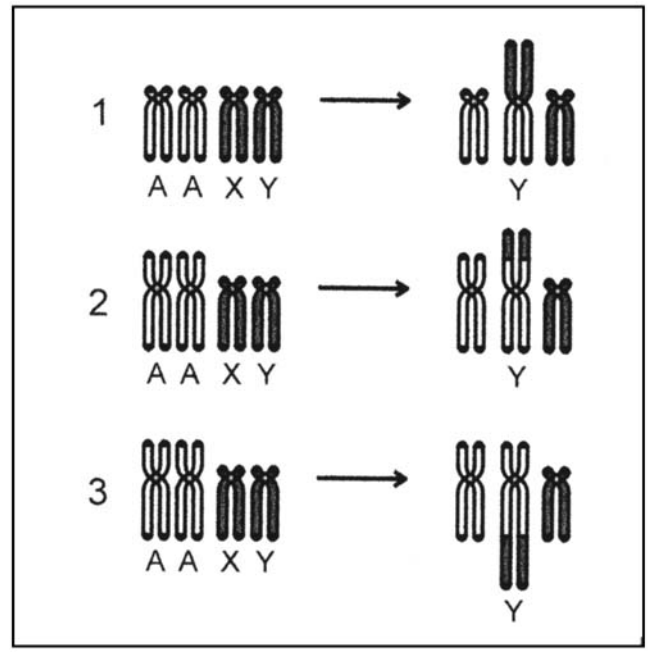

Fig. 6. Three possible rearrangements leading to heteromorphic sex-linked chromosomes in species of the family Channichthyidae. The large Y found in the males (right) could have differentiated through the translocation of an early homeomorphic Y (left) on a acrocentric (1) or on a submetacentric (2 and 3) autosome (A = autosome; X and Y = female and male sex-chromosomes, respectively).

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b) Changes in chromosome macrostructure

Chromosomal structural changes are due to various kinds of rearrangements and/ or variations of heterochromatinization, leading to different chromosomal morphologies and sometimes to a different diploid number. An intriguing case is the karyotypic variability of the nototheniid *Trematomus loennbergii* Regan. Populations in the Ross Sea show different karyotypes composed of 28 and 30, mainly of bi-armed, meta-or submetacentric chromosomes. One of the two is derived from the other, by a centric fusion (from 30 to 28) or by fission (from 28 to 30) (Morescalchi *et al.* 1992b). Specimens from Adélie Land have a different karyotype, made up of 48 chromosomes, mainly acrocentric (Ozouf-Costaz *et al.* 1999b). Since the chromosomes in the karyotypes described for the different populations have the same arm number (NF = 52), the three karyomorphs may represent a case of balanced intraspecific heteromorphism and could be linked to each other through Robertsonian relationships (White 1973). However, given the small number of specimens studied, it is not possible to get a clear explanation of these striking intraspecific differences, but the results point out the necessity of large-scale studies at the population level.

Another example of extensive intraspecific polymorphism involves the circum-Antarctic nototheniid *Trematomus hansoni* (Fig. 7). Specimens from the Atlantic Sector have 48 chromosomes (C) the population of the Ross Sea has 45/46 chromosomes and heterogonosomes (A) while the population at Adélie Land has 46 chromosomes, no heterogonosomes, and a very unusual pattern of silver staining-positive heterochromatin (B).

The chromosomal variability in *T. hansoni* could indicate an active process of intraspecific genomic restructuring. It is therefore possible that similar diversification is occurring in populations of other species, especially those with a wide geographic distribution. Moreover these results point again to the need for more extensive study, possibly through integrated and complementary approaches, in order to evaluate degrees of genetic exchange between populations and possible intraspecific reproductive barriers. If the populations are not reproductively isolated, the observed chromosomal differences must not present difficulties for meiotic segregation. In this case the balanced polymorphism can be viewed as a kind of karyological biodiversity of Antarctic notothenioids. On the other hand, if chromosomal variants become fixed within a population, this population could be partially or totally isolated from other conspecific populations, and the chromosomal findings could be evidence of sibling species (Knowlton 1993, Ozouf-Costaz *et al.* 1999a). Whether certain chromosomal rearrangements play any role in promoting speciation or whether such changes occur incidentally during or after species formation, is matter of controversy (Sites & Moritz 1987, King 1993). The growing amount of information for the different taxonomic levels of the Notothenioidei, could make it possible to use this group as a model to test, in future work, some general assumptions on the role of chromosomes change in speciation processes in fishes.

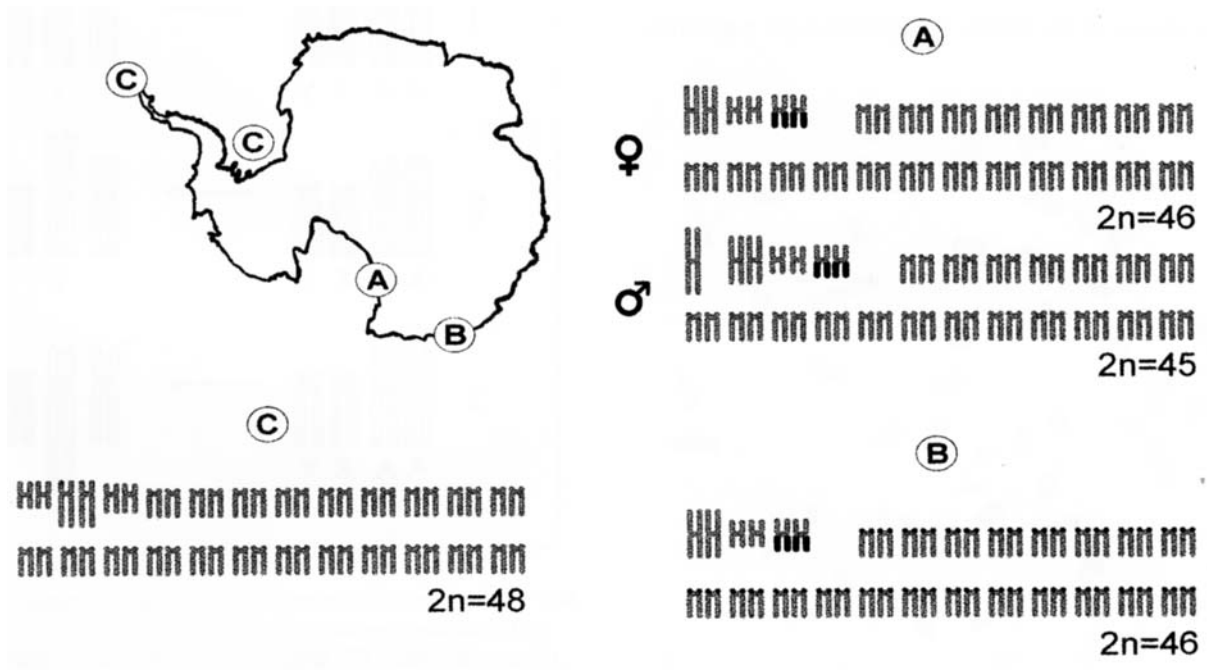

Fig. 7. Intraspecific polymorphism in the circum-Antarctic nototheniid species *Trematomus hansoni*. Specimens studied in populations of the Atlantic Sector have 48 chromosomes (C); the population of the Ross Sea has 45/46 chromosomes and heterogonosomes (A); the population studied at Adélie Land has 46 chromosomes, no heterogonosomes, and an unusual pattern of silver staining-positive heterochromatin (B). Dark regions in chromosome ideograms correspond to silver staining-positive heterochromatin.

Conclusions

Cytogenetic research has been carried out on representatives of all the notothenioid families in High Antarctic, sub-Antarctic and cold peri-Antartic regions. More extensive data have been collected near the Italian Antarctic station Terra Nova Bay and the French station Dumont d'Urville, within the European Science Foundation Network "Fishes of the Antarctic Ocean" and, more recently, during the French-Italy multidisciplinary ICOTA (Ichtyologie côtière en Terre Adélie) project.

Recent reviews of cytogenetic data, including the present, stress the following:

1) Assuming that the Notothenioidei are a monophyletic group, the broad pattern of their karyotypic diversification, based on the chromosomal features of about 50% of the known species, supports the idea that the ancestor of this lineage, had a karyotype of 48 acrocentric chromosomes. However, this ancestral chromosome set and any other phylogenetic hypothesis, remains speculative if not confirmed by a resolution of unambiguous chromosomal homologies. Therefore future work will address detailed resolution of chromosomal structure, based not only on conventional characters (diploid number and gross chromosomal morphology) but on chromosomal banding and mapping of DNA sequences and genes.

2) The active process of genomic change at different levels of chromosomal organisation, leads to strong intraspecific variability in several species, as described for populations of the nototheniids *Trematomus hansoni* and *T. loennbergii*. These findings point to the need for more extensive research, especially on those species with wide geographic distribution, possibly through integrated and complementary approaches, in order to evaluate degrees of genetic exchange between populations and possible intraspecific reproductive barriers. In this respect intraspecific cytogenetic polymorphism can have implications for resolving such questions as stock identity and species affinity among Antarctic fishes. Moreover, the growing amount of information for the different taxonomic levels of the Notothenioidei, could make it possible to use this group as a model to test some general (and controversial) assumptions on the role of chromosomes change in speciation processes in fishes.

3) Various molecular cytogenetic techniques are now available which permit the visualisation of smaller DNA sequences directly on chromosomes. Our recent results on the chromosomal location of ribosomal genes and the detection of intrachromosomal telomeric remnants in some species, through fluorescence *in situ* hybridization, demonstrates the power of molecular cytogenetics in answering some important questions such as the direction of chromosomal change during the diversification of the Notothenioidei. The physical mapping of DNA regions and genes important in the adaptive radiation of notothenioid species, such as the globin genes, would provide valuable structural information on how changes in genome organisation are correlated with critical events in the evolution of this group of fishes.

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