

Proposal for the construction of BAC libraries for Antarctic notothenioid fishes

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Unplanned natural experiments create ecological communities that we would never have dreamed of creating.... (Diamond, 2001)

1. The importance of notothenioid fishes to biomedical or biological research

Polar biology stands on the threshold of a revolution: the application of genome science to investigate the evolution, biodiversity, physiology, and biochemistry of the exotic organisms and communities of polar ecosystems (NRC, 2003). The rapid onset of extremely cold (-1.86°C , the freezing point of seawater), thermally stable, and oxygen-rich conditions in the isolated Antarctic marine ecosystem over the past 15 million years has certainly driven the evolution of its biota. Among polar organisms, the phylogenetic history of the teleostean suborder Notothenioidei, which is largely endemic to the Antarctic, is the most completely understood (Ritchie *et al.*, 1996; Chen *et al.*, 1998; Eastman, 2000; Eastman & McCune, 2000). The notothenioid radiation (Fig. 1) has produced different life history or ecological types similar in magnitude to those displayed by taxonomically unrelated shelf fishes elsewhere in the world. On the basis of habitat dominance and ecological diversification, notothenioids are one of the few examples of a “species flock” of marine fishes (Eastman, 2000; Eastman & McCune, 2000). Thus, this group provides a compelling model for understanding the rapid evolution of vertebrates under harsh, polar conditions.

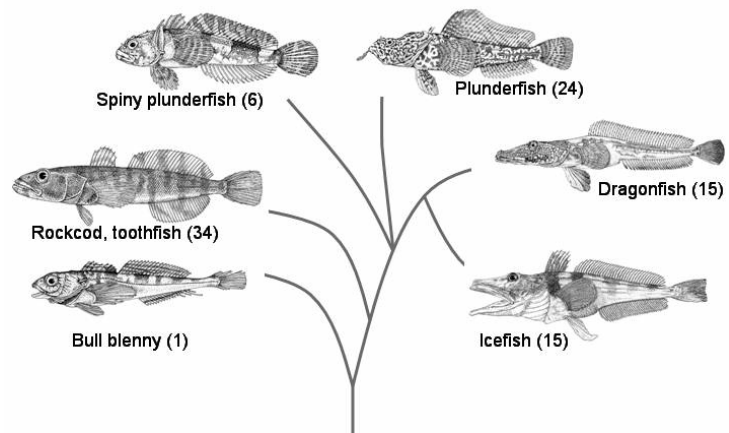


Figure 1. Relationships of the suborder Notothenioidei. The notothenioids are thought to comprise six families whose relationships are given in the tree. The numbers in parentheses indicate number of taxa in the Southern Ocean. The origin of the group as well as its interrelationships to the rest of the teleosts (order Perciformes) are not clear (Eastman, 2000).

Diversification within the Notothenioidei occurred during the mid-Miocene, only ~5-14 million years ago (Bargelloni *et al.*, 1994), with an average time for speciation of 0.76-2.1 million years (Eastman & McCune, 2000). The key evolutionary adaptation of the Antarctic notothenioids was the *de novo* acquisition of genes that encode the antifreeze glycopeptides (AFGPs) (Hsiao *et al.*, 1990; Chen *et al.*, 1997a,b; Cheng, 1998; Cheng & Chen, 1999). The AFGPs prevent the lethal formation of intracellular ice by resisting the propagation of ice from external, ice-containing tissues (the integument, gills, and intestinal tract) into other fluid compartments. Other evolutionary changes are equally unique. The Antarctic icefishes (family Channichthyidae), for example, lack the red blood cells (Ruud, 1954; Hureau *et al.*, 1977; Barber *et al.*, 1981) and the oxygen transporter hemoglobin (Ruud, 1954) that are a virtual *sine qua non* of vertebrates. Recent evidence from the Detrich lab (Cocca *et al.*, 1995; Zhao *et al.*, 1998; di Prisco *et al.*, 2002; H. W. Detrich,

unpublished results) indicates that four distinct deletional events are required to explain the patterns of loss of the adult α/β -globin gene complex among the 16 species of the family. Yet little is known about the genomic changes that have produced the erythrocyte-null “knockout” phenotype. Given their long histories in highly stable environments, it is also not surprising that the Antarctic fishes are among the most stenothermal animals in the biosphere; they live within a very narrow temperature range, die of heat stress at temperatures above 4° C (Somero & DeVries, 1967), cannot acclimate long-term to elevated temperatures (Hofmann *et al.*, 2000), and fail to activate the protective heat-shock response (Hofmann *et al.*, 2000). Finally, although notothenioids lack swim bladders, many species have evolved secondary pelagicism via the alteration of buoyancy through pedomorphism, or the retention of larval characters in adulthood. The mechanism that underlies pedomorphism is thought to be the slowing of somatic development relative to gonadal maturation (Montgomery & Clements, 2000) and is likely to involve major changes in genetic regulatory networks (e.g., the *Hox* genes). The examination, for example, of their *Hox* clusters should be revealing both in terms of adaptive axial patterning but also in terms of the utilization and regulation of *Hox* gene duplicates for generation of evolutionary novelties or innovations (Chiu *et al.*, 2002; Martinez and Amemiya, 2002).

Important as these examples are, they constitute but the “tip of the iceberg” in terms of the complexity of genomic change likely to be encountered in the Antarctic notothenioid fishes. The availability of BAC libraries for two key notothenioid species will enable us to explore systematically the totality of genomic change that enables these fishes to survive and indeed thrive in their extreme environment. These investigations are likely to be guided by three overriding questions, which are quoted from the National Research Council report, *Frontiers in Polar Biology in the Genomic Era* (NRC, 2003; abridged version in Appendix):

- *First, how does the repertoire of genetic information change during evolution in highly stable environments, compared to evolution in environments that confront organisms with wide and often rapid changes in key variables such as temperature and oxygen availability?*
- *Second, what new types of genetic information are needed to permit organisms to cope with polar conditions—and how is this new information generated from pre-existing ‘raw material’ in the genome?*
- *Third, what are the genetic mechanisms that cause the genomic changes that lead to rapid evolution in polar environments?*

To address these questions, we propose that the following two notothenioid species be considered for construction of BAC libraries:

Family	Species	C Value (pg)	Thermal Phenotype	HMW DNA
Nototheniidae	<i>Notothenia coriiceps</i>	1.01 ± 0.05	Psychrophilic	Yes
Channichthyidae	<i>Chaenocephalus aceratus</i>	1.86 ± 0.12	Psychrophilic	Yes

The rationale for selection of these species is as follows. *N. coriiceps* (the bullhead notothen or rockcod) is a basal, psychrophilic, red-blooded species of the nototheniid family. The icefish *C. aceratus* represents an advanced member of the icefish clade. These fishes are readily obtainable by trawling in the Antarctic Peninsula and are amenable to experimental study.

2. Uses to which the notothenioid BAC libraries would be put, in addition to genomic sequencing

P.I. Amemiya’s major research interests are: (1) molecular evolution and development of the vertebrate immune system; and (2) evolution and functional elaboration of genomic regulatory networks. P.I.

Detrich focuses his research program on: (1) the adaptation of protein function (exemplified by microtubules and their motor proteins) and gene expression (i.e., globin genes) to psychrophilic conditions; (2) the evolution of the erythrocyte-null phenotype of Antarctic icefishes; and (3) the use of comparative genomics and the icefish erythroid knockout system to discover new genes involved in erythropoiesis. The P.I.s have already collaborated on a study of the molecular evolution and adaptation of the immunoglobulin heavy chain in Antarctic notothenioids (Ota *et al.*, 2003). All of these projects would make extensive use of the proposed BAC resources. Beyond our interests, numerous other laboratories (see letters of support and section 3) would also benefit enormously.

The biotechnological potential of enzymes and proteins from extremophiles for use in food production, chemical production, and medical applications has long been recognized. Although most attention has been focused on enzymes from thermophiles, a compelling case can be made for the energetic advantages of psychrophilic enzymes, which generally do not require heating for activity at mesophilic (i.e., room) temperatures (Herbert, 1992). We envision the systematic prospecting of the genomes of Antarctic fishes for enzymes and proteins with desirable biomedical or commercial properties (see box 1 in abridged NAS report in Appendix). Furthermore, scanning the genome and transcriptome of the red-blooded *N. coriiceps* for genes that are no longer expressed by *C. aceratus*, an icefish “erythroid knockout” model, will help to elucidate the genetic program of erythropoiesis in all vertebrates, including humans. The discovery of novel erythroid genes via this comparative genomics approach may ultimately be exploited to produce new treatments for blood-related diseases and syndromes, such as anemias associated with kidney dialysis treatment and cancer chemotherapy.

3. The size and interest of the research community that could potentially use notothenioid BAC libraries

Solely based on the polar fish community, there are more than 50 major laboratories worldwide that are likely to make use of these resources. However, it is our opinion that many “nonpolar” laboratories will embrace the notothenioid system as its benefits as a comparative model of vertebrate evolution become apparent (see section 2 and abridged NAS report in Appendix). Letters of support from both polar and nonpolar biologists are appended.

4. Whether the notothenioids will be, or have been, proposed to NHGRI or another publicly funded agency for BAC-based genomic sequencing and the status of that request

BAC libraries do not currently exist for any notothenioid species.

5. Other genomic resources that are available that will complement this resource

P.I. Detrich’s laboratory has generated numerous genomic DNA libraries (in phage λ dm β) from Antarctic (*N. coriiceps*, *C. aceratus*, *C. gunnari*, etc.) and temperate (*N. angustata*) notothenioids as well as cDNA libraries from brain, kidney, and spleen for most of these species. These resources would be made available to the community at large without restriction to assist the functional studies that will surely grow out of BAC cloning and sequencing projects.

6. Strain of the proposed organisms and rationale for their selection -- N/A

7. Genome sizes

We recently determined nuclear DNA contents via flow cytometry for eight notothenioid species collected from the Antarctic Peninsula in April 2003 (see Table 1 for the two species under consideration). We used erythrocytes for measurements for all taxa except the channichthyids (icefish), where lymphocytes were used. Chicken erythrocyte nuclei (2.33 pg/2C) were used as internal controls. The small genome sizes of the notothenioids are advantageous for genome-level investigations, notably construction of good-representation BAC libraries.

8. The availability and quality of the source DNAs

High molecular weight DNAs were embedded in InCert™ agarose from erythrocytes or lymphocytes of selected notothenioid specimens recently collected from the Antarctic Peninsula (Table 1). These DNAs were found to be of good quality based on pulsed field gel electrophoresis.

9. Specifications of the libraries and supporting rationale

Libraries should be of equivalent genomic coverage and quality as mandated by the NHGRI (10x coverage, 150 kb average insert size), although any BAC libraries would be extremely useful to the community at this point.

10. Time frame in which the libraries are needed

No libraries exist for the notothenioid fishes. Here we propose to generate libraries from two critical species that are widely disparate in terms of physiology and life history strategies: *Notothenia coriiceps* (red-blooded rockcod) and *Chaenocephalus aceratus* (white-blooded icefish). These libraries would be extremely useful for demonstration studies in many areas where comparisons are made between red-blooded vs. erythrocyte-less conditions (e.g., research in comparative physiology, development of the hematopoietic and immune systems). Subsequently, libraries might be constructed for *Dissostichus mawsoni* (Antarctic), *D. eleginoides* (temperate) and *Notothenia angustata* (temperate) to enable comparative studies on psychrophilic versus mesophilic life history characteristics. Ultimately, for phylogenetic considerations as well as investigation into the loss of oxygen-carrying molecules (hemoglobin, myoglobin), we would like to see libraries generated from the dragonfish *Parachaenichthys charcoti* (the Bathydraconidae is the sister family to the icefishes) and two additional icefishes *Champscephalus gunnari* (basal in the icefish clade) and *Pseudochaenichthys georgianus* (intermediate in the clade).

11. Other support that is available or has been requested for construction of desired libraries -- N/A

12. The need for an additional BAC library if one or more already exists -- N/A

13. Any other relevant information

It is important to note that the Antarctic specimens are exceedingly difficult to procure, i.e., one essentially must collect them from Antarctic waters and it is not possible to keep the specimens in captivity for any length of time. As such, whole animal experiments utilizing these species must be performed “on the ice” at one of the field stations in Antarctica. Thus it becomes all the more important to the community to archive their genomes as BAC libraries. Moreover, despite the logistical difficulties of performing certain experiments on these organisms, BAC libraries alleviate some of these problems by allowing biological inferences to be drawn via both targeted comparative sequencing and empirical interrogation using BAC-transgenic analysis (in an exogenous system such as cell culture, zebrafish or mouse). Finally, we emphasize that the notothenioids should be considered a biological entity *in toto*, a “system” that has undergone notable genomic evolution to facilitate myriad adaptive changes in order for these organisms to thrive in their extreme environment. Thus, the entire group serves as a model system for understanding the genetic and genomic bases for adaptive evolution.

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